Chapter 1
Life on Earth

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Life on Earth was initiated some 10 billion years after the Universe was created. Life was created on the basis of, and has to obey, the laws of physics. At the same time, physical laws are useless for understanding living processes because the combination of atoms into molecules and molecules into cells and organisms is based on emergent properties that only arise through the interactions between the components, the cells, the organisms, the ecosystems, and the whole biosphere of the little blue-green planet we live on.

Our powerful modern biotechnologies undoubtedly do have the potential to change life on Earth. The fundamental question arising is then: Do we really know what we are changing, and the risks that are involved?

This chapter is intended to give a brief overview of the evolution and constituents of life. Hence, it presents basic concepts related to the issues treated more comprehensively in the more specialized parts of this book. The chapter is organized according to the following outline:

1. Origins of Life

1.1 Tellus, our common spaceship
The Milky Way is a medium-sized galaxy. The Sun, located in one of its spiral arms, is a medium-sized star formed by atoms released from a nearby supernova. The Sun evolved approximately 4.5 billion years ago. It has enough hydrogen fuel to burn for another 5 billion years.

During the birth process of the Sun, some of the surrounding material assembled into small aggregates that grew and collided and merged with one another to eventually stabilize as its orbiting planets, moons and comets. Importantly, some of these orbiting aggregates contained iron and radioactive elements that are now the Earth's broiling core, the silicon that forms its crust. Yet most important was the presence of carbon, oxygen, nitrogen, and other elements that are essential for life.

Comets colliding with the developing Earth contributed even more atoms from distant supernovae, and also brought in a great deal of water in the form of ice. Gases from the Earth's interior were released through fissures and volcanoes, and were trapped by gravity to form the early atmosphere. The floating surface settled into large masses that drift and crash into each other, creating continuous geological activity that defines and changes the continents and ocean basins. It took half a billion years before the physical conditions on Earth became such that life could originate and continue.

1.2 The chemical prerequisites
Life depends on atoms that form bonds with one another and hence associate into molecules, and also on smaller molecules to associate into larger molecules. Such events are defined within chemistry, which again may be reduced to physics. Chemical binding and association of molecules can only take place under certain conditions. For chemical reactions to proceed there are three main compulsory conditions. First, an available flow of energy, from source to sink must be available. The Earth has two important energy sources: The Sun and the planet's own molten core. Second, temperatures must be such that atoms and molecules can coexist in solid, liquid and gaseous forms. Third, the atoms that are more likely to engage in early biochemical reactions – carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulphur – must be present. These are called 'the Big Six' of living systems. They can form bonds with one another under conditions of energy flow, e.g. hydrogen combines with oxygen to form water, carbon combines with oxygen to form carbon dioxide, carbon starts to combine with all the others and forms more complex molecules.

1.3. The early biochemicals and building blocks
In order for life to start, the so-called building blocks of life – water, carbon dioxide and small molecules such as formaldehyde, methane and hydrogen sulphide – had to be generated, and consecutively these had to associate into larger assemblies, the early biochemicals. Small but complex building blocks may have accumulated in the waters of the Earth from the time of its birth, approximately 4.5 billion years ago. This so-called 'primal soup', contained three groups of small molecules called sugars, amino acids and nucleotides. The latter comprised two kinds, ribonucleotides and deoxyribonucleotides. These are the starting materials for all forms of life on Earth. Approximately 4 billion years ago the formation of biomolecules from the primal soup building blocks was initiated. Recently, it has become common to speak of the first stages of life as having developed in a 'RNA world'. There are good reasons to believe that relatively simple cells with self-replicating RNA were the first to inhabit the earth. The first cells may simply have

been a lipid membrane-enclosed self-replicating RNA that had taken on the ability to direct synthesis of ribonucleotides and membrane lipids. This might make self-replication possible.

2. Cells

The cells of the 'RNA world' evolved into cells whose genes are encoded in DNA molecules, and later they vanished. Hence, we are now living in a DNA world. DNA uses deoxyribonucleotides instead of ribonucleotides as precursors, and is more stable than RNA. The basic concept is, however, the same: a long chain of deoxyribonucleotides carries genes that code for molecular products making replication of the chain possible.

The genes encoded in DNA came to specify large molecules called *proteins*. Some proteins are responsible for ensuring that the biochemical processes inside the cell proceed accurately and efficiently. These proteins are called *enzymes*.

Life depends on the ability of cells to construct new copies of itself, remember how to do it and pass the instructions on to the daughter cells. The key role of DNA is to encode readable instructions for how to make proteins and pass these instructions along during replication. Along the way cells acquired the ability to extract energy from small molecules such as hydrogen and hydrogen sulphide. At some point, they also invented the capacity to carry out photosynthesis, i.e. to capture energy from sunlight and transfer it into chemical bonds. Most of the living creatures are single celled, but some, e.g. humans and plants, are made up of many different kinds of cells that cooperate to form a single organism. Each cell has a membrane around it; a thin film of lipid keeping the outside out and the inside in, and each cell contains the DNA instructions for its various activities.

2.1. Proteins

The activities in the cells are executed by proteins, and protein functions are all about *shape*. Proteins have protuberances and pockets and long, straight as well as tightly coiled parts. Each part is called a *domain*. Domains are the interactive sites of proteins.

When it is made, a protein starts out as a long chain of amino acids. There are twenty different kinds of amino acids. Each of them has its own properties. Some are greasy, some are bulky, while others are long and slender. Some have negative charges, others positive charges. The DNA sequence in a given gene dictates the sequence of amino acids in a given protein chain. Once a protein chain is made, it folds up. Amino acids that prefer to be next to each other, such as a group of greasy ones, may associate to form one domain. Amino acids with negative charges might line up next to some with positive charges to form a second domain. A bulky amino acid might cause a protuberant domain to stick farther out. The result is a protein with a distinctive overall size and shape that displays a collection of very specific domains. A second chain with a different sequence of amino acids will self-assemble into a protein with a different size, shape and set of domains. Protuberances and pockets are important for proteins to form, as in a jigsaw puzzle, *multi-protein complexes* that perform many important functions in the cell. Furthermore, pockets are crucial to the functions of proteins that are called *enzymes*.

2.1.1 Enzymes

The pockets made by the folding of an enzyme are not destined to interact, e.g. make complexes, with other proteins. Instead, they are shaped to cater for interactions with small molecules that the cell must handle chemically. The enzyme will have one pocket exactly shaped for each of the two sugar molecules, e.g. glucose and galactose. When both pockets are filled the enzyme changes its shape and brings the sugars close enough together for a chemical bond to be established between

them. The combined glucose-galactose molecule then pops out, the enzyme resumes its original shape, and the process may start all over again. The enzyme is said to *catalyze* the chemical reaction. If many sugar molecules are joined together in this way the end result is a *polysaccharide*. Such sugar polymers are important in many cellular functions.

Every cell is packed with thousands of different kinds of enzymes. Each enzyme displays a distinctive surface combination of protuberants and pockets, and is able to catalyze one or several chemical reactions. Some enzymes catalyze the formation of chemical bonds, as in the sugar-sugar example. Others catalyze the disruption of chemical bonds to generate smaller molecules from bigger ones. Some enzymes catalyze long chains of amino acids (proteins), nucleotides (DNA or RNA) or glycerides (lipids). All these polymers are key cellular components.

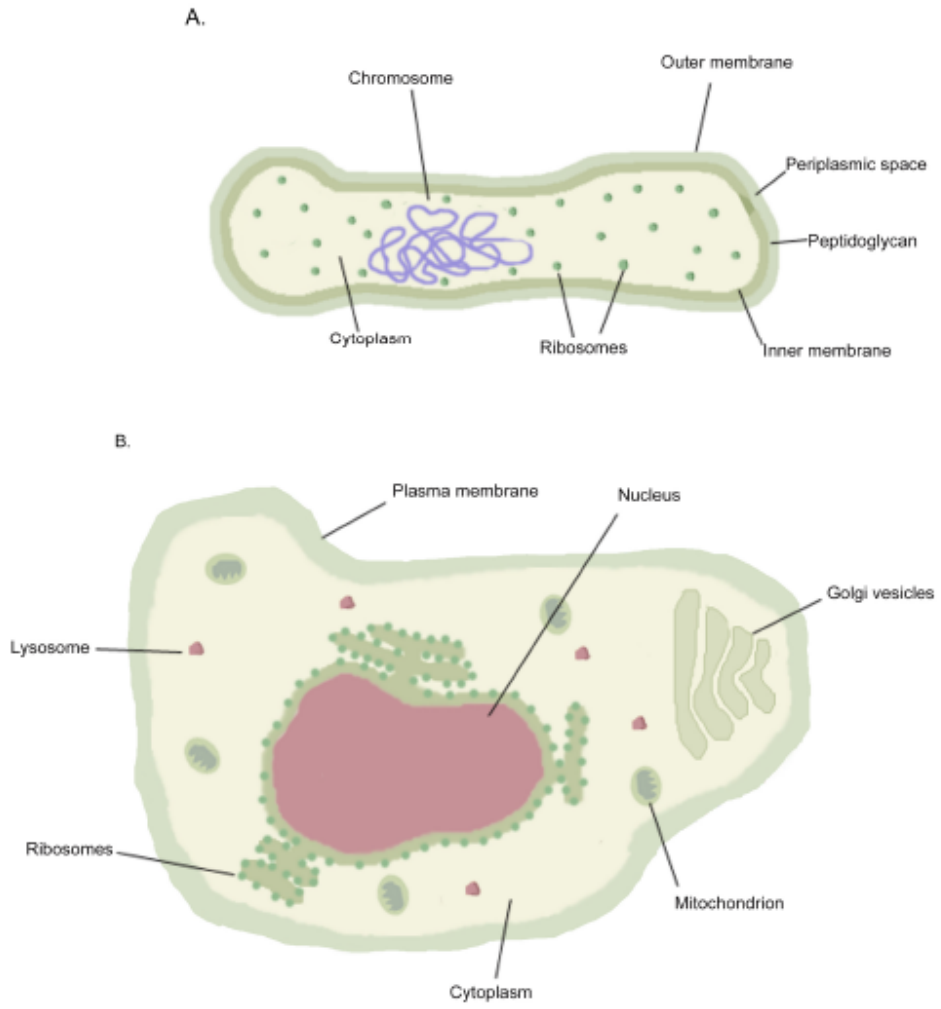

Figure 1.1. Outline of a generic prokaryotic (a) cell and eukaryotic (b) cell.

2.2. Channels and pumps
Such protein complexes space the cell membrane and determine which electrically charged ions, e.g. calcium, potassium and chloride, are allowed to cross the membrane and at what rate. Some of these ions are positively charged while others are negatively charged. Their net distribution generates ion gradients. For example, the inside of the cell is rendered more negative than the outside, and contains more potassium and less sodium and calcium than the outside. If such electrochemical gradients do not function properly cells will quickly disrupt and die.

2.3. Cascades and receptors
Life proceeds as *cascades of shape changes*. Three proteins may fit together into a complex that associates with some lipids in the cell membrane to form a sodium channel. When the channel changes its shape, an influx of sodium changes the shape of an internal enzyme so that pockets hidden in its interior become exposed. The pockets are then engaged in chemical reactions that induce another protein to change its shape, and so on. A sequence of such events is called a *cascade*.

Cascades are important for how a cell perceives the world, and for how organisms adjust to changing environmental conditions. All cell membranes carry *receptors* composed of three domains. One domain faces outwards, towards the environment, the second bridges the membrane, and a third faces the cell interior. The outer face carries a pocket exactly shaped to fit some molecule that may be present in the world outside. Such molecules may be hormones, growth factors, odorants, or other signal substances. When a cognate molecule (ligand) has filled its pocket the receptor changes its shape, and the change propagates through the membrane-spanning domain and induces a new conformation in the interior domain. This may, sometimes through intermediary steps, lend enzyme functions to the interior domain. It may now catalyze a shape change in a protein in the interior of the cell, and so on, one shape change catalyzing the next until the 'message' is brought into the cell nucleus to become interpreted. The signal, e.g. the presence of a specific hormone, sets off a *signal transduction cascade* whereby the receptor transduces the external signal into appropriate biochemical reactions.

The inside of the cell is designed to optimize the flowing of cascades. Proteins predetermined to interact with each other have domains, called 'addresses', that target them to the same subcellular location. Each location is optimal for particular biochemical reactions, and is delimitated by a defined boundary, often an intracellular membrane.

2.4. The genes and the genome
Each cell contains a complete set of instructions for how to make all its proteins, and these instructions can be copied so that more cells can be produced. The instructions are stored in DNA, which uses a universal code to specify different amino acid sequences which self-assemble as structural units or three-dimensional enzymes or receptors or channels. Each sector of the DNA that encodes a protein is called a *gene*.

The collection of all genes necessary to specify an organism is called its *genome*. The entire genome must be replicated and transmitted to the next generation for a lineage to continue.

The human genome contains some 25,000 genes. There are approximately 250 different cell types in the human organism, and they all contain exactly the same genome. This immediately informs that the genome is differently expressed in different cells.

There are several hundred thousand proteins expressed in the human organism. This tells us that each gene may give instructions for more than one protein to be made. Different cells express a different assortment of proteins, and the same proteins expressed in different cell types may be present in different relative amounts.

A gene is an instruction for making a protein, and a cell has the option to express that gene and hence contain the protein, or not express that gene and hence lack the protein. It also has the option to express the gene often, and hence have a lot of the protein, or express it rarely and hence have little. These decisions are mediated through domains of DNA that are hooked up to the protein coding sectors, and are called *promoters*.

2.5. Internal clocks: The cell cycle

Cells can switch genes on and off in response to changes in the environment, e.g. through specific signal transduction cascades. In addition, important sets of genes are regulated internally, a good example being the genes that govern what is called the cell cycle.

A cell is made to copy its entire genome and perform DNA replication by an elaborate enzymatic process. Once replication is finished, a second decision is made that allows the cell to divide into two by mitosis. One of the genome copies goes to each of the daughter cells. Then the cell cycle starts over again. The process is bracketed by a large number of sub-decisions, and all are dictated by changing patterns of gene expression, coordinated up- and down-regulated expression of proteins that regulate the different stages of the cell cycle.

The time it takes for a cell cycle to elapse may be influenced by the environment, but cell cycles have an inherent timescale of their own.

3. Multicellular Organisms

The human body contains more than a trillion cells that remain together to form an organism. Each cell possesses the full set of genetic instructions for making a human being, but only some of the instructions are read in a given cell type.

Red blood cells switch on the genes encoding haemoglobin, but never express the genes encoding the hair protein keratin. Hair-follicle cells, on the other hand, switch on keratin, but never haemoglobin genes. Each cell thus recognizes its position and fulfils its specific role.

Each cell type in the body goes through a cell cycle following its own cell-specific rate. Surface cells in the intestines divide twice a day. Liver cells divide only once a year. Some nerve cells do not divide at all. All the diverse cell-specific patterns still generate an organism with a controlled size and shape.

Organisms are characterized by a remarkably complex organization which endows them with the capacity to respond to external stimuli. They have a metabolism that binds or releases energy. They are able to grow, to differentiate and replicate.

Organisms have the remarkable property that they are open systems, maintaining a steady-state balance in spite of much input and output. This *homeostasis* is made possible by elaborate feedback processes, unknown in their precision in any inanimate system. Even the simplest living organisms we know of depend on *c.*550 linked biochemical processes.

Such complexity has often been put forward as the most characteristic feature of living systems. However, complexity is not a fundamental difference between organisms and inorganic systems.

The weather systems on Earth or in any galaxy are also highly complex systems. In general, however, organic systems are more complex by several orders of magnitude than those of inanimate objects.

The complexity of organisms is evident at every hierarchical level, from the nucleus, to the cell, to the organ systems, to the individual, to the species, the ecosystem, and to society.
On each hierarchical level, two clearly recognizable properties are observed: i) units act as wholes, as though they were a single entity, and ii) their characteristics cannot be deduced even from the most complete knowledge of its components. When an organism is assembled from its components, new characteristics of the whole emerge. *Emergent properties* occur also through the inanimate world, but only organisms show such dramatic emergence of new characteristics at every hierarchical level of the system.

3.1. Genotype and phenotype
The presence of the genetic 'programme' gives organisms a peculiar duality, consisting of a *genotype* and a *phenotype*. The genotype is handed over largely unchanged from generation to generation. Occasional mutations, horizontal gene transfer events and recombination introduce some new variability all the time. The genotype interacts with the environment to produce the visible phenotype that we observe.
The genotype dates back to the origin of life. It endows all organisms with their remarkable capability for goal-directed processes, leading to diversification and evolutionary development, a capacity totally absent in the inanimate world.

Since each genome is a unique combination of thousands of different genes, the differences among them cannot be expressed in quantitative terms, but only in qualitative terms. Thus, quality becomes one of the dominant aspects of living organisms and their characteristics. This becomes particularly obvious when comparing properties and activities of different species, e.g. with regard to their courtship displays, pheromones, niche occupation, or whatever else may characterize a particular species.

3.2. Genomic evolution
Evolution can, in a simplistic way, be defined as changes in the frequencies of different sets of instructions for making organisms. Thus, we need to understand how the instructions become different, which happens by mutation. We also need to know how the frequencies of those instructions are changed, and that happens by *natural selection*.

A mutation is a change in the sequence of nucleotides in a genome. A mutation may arise as an uncorrected error during DNA replication. Yet it may also be due to physical or chemical damage if the genome is exposed to environmental agents. Furthermore, both naturally occurring *horizontal gene transfer* and *transgenic engineering* are, by definition, mutations, changing the genome by inserting foreign pieces of DNA into it. Mutations in protein-coding parts of a gene may lead to a change in the amino acid sequence. The new product may have deleterious, beneficial or neutral effects. Mutations in promoters will also have deleterious, beneficial or neutral consequences depending on which nucleotides are altered. Activator or repressor proteins may recognize and bind to a mutated promoter sequence less well, either better or at the same level as the unmodified promoter. Each new gene and promoter is subject to very discriminating and purposeful acts of selection.

3.3. Natural selection

A deleterious gene is likely to be lethal and the new gene will fail to spread, while a beneficial mutation may give the cell or organism an advantage, and hence the new gene may become more prevalent than the previous version.

Mutations change the quality of genes and natural selection changes the frequency of genes. The end results are strongly influenced by context. Evolution is hence contingent on the environmental circumstances in which it is occurring. The traits that define an organism, its motility, its mating behaviour, its perception of odours, its metabolism, or its embryology, are *not* determined by single genes, but by sets of interacting genes and gene products, which again interact with the physical environment and other organisms, in space and time. These complex interactive traits or 'units' are hence the true substrates of evolution.

The general principle is that evolution produces cumulative change. New protein versions do not leap into existence fully formed. Rather, they appear as slightly modified versions of the previous molecules, only a little more efficient, serving an additional function or serving the same function(s) under different conditions. Increasing complexity entails selections of selections of selections.

At the gene level, evolution seems to be remarkably conservative, in spite of all the novelty that emerges. Once a gene sequence encoding a particularly useful protein domain appears, that sequence shows up again and again, in different contexts, in different genes, lineages and species.

As a result, a great deal of homology exists between the genes of all modern organisms. This reflects the fact that all species evolved from the same common ancestor. We have moved through evolution while the same basic sets of protein domains were manipulated.

Most of our genes are akin to most chimpanzee genes, but are also like many of the genes in a fruit fly. The important lesson here is our intimate interrelatedness and close genetic homology with our co-inhabitants of this little blue-green planet. We all came from a singe-celled organism from which the three major branches of life, *bacteria*, the *archea* and the *eukaryotes* developed.

Archea are single-celled organisms that are now confined to hot sulphurous springs and other extreme niches, but their ancestors were probably major parts of life in earlier times when the Earth was very hot and salty. Bacteria are by far the most abundant organisms on the Earth. It has been stated that there are as many bacteria in our gastrointestinal tracts or in a spadeful of soil as there has ever been humans on the planet. Further, the body cells are outnumbered by the bacterial cells the body is hosting.

Eukaryotes are organisms that contain their genome in a separate organelle called the *nucleus*. They also possess an internal cytoskeleton that allows them to move about. More than two billions of years ago, eukaryotic cells engulfed bacteria that became permanent occupants and gave rise to the energy generating organelles called mitochondria and chloroplasts.

Some 600 million years ago, during the Cambrian explosion, numerous eukaryote lineages appeared. Some remained unicellular, while others adopted a multicellular body plan and gave rise to the present day fungi, plants and animals.

Much of the biological evolution entails the development of what organisms are aware of, attracted to or repelled by. Once a sufficient number of species and organisms came into existence, their awareness of each other as prey, predators or symbionts was developed. Further,

when eukaryotic 'sex' was invented, systems were developed to recognize a mate of the correct species, gender, age and quality. In addition, the neuron, a cell type specialized for awareness was invented. This made possible the avenue of awareness called consciousness through more or less elaborated nervous systems.

4. Germline versus Soma

In eukaryotes, the genome is not encoded in a single DNA molecule. The genome is divided into a number of DNA pieces called *chromosomes*. The genome of each species is portioned into a distinctive number of chromosomes. Humans, for instance, have 23 chromosomes, while maize has 10.

Sex entails making two kinds of cells. The *haploid* cell contains one full set of chromosomes while the *diploid* cell has two complete sets. Diploid cells arise when two haploid cells fuse together. Haploid cells are formed when diploid cells give one each of their chromosome sets to two daughter cells.
Formation of a diploid cell occurs during *fertilization*. A haploid sperm or pollen cell from the male fuses with the haploid egg from the female to form a single diploid cell called the *zygote*.

Having two versions of each chromosome confers distinct advantages: if a serious error is present in a gene, a 'healthy' version of the gene will be present on the other member of that particular chromosome pair. For humans, this holds true for 22 of the chromosome pairs. The 23rd 'pair' is the *sex chromosomes* X and Y. Since girls (XX) have two X chromosomes, a mutation in a gene on one X chromosome can be compensated for by a healthy gene on the other. In boys (XY) having just one X chromosome, mutations in the same gene may have deleterious effects.

For making haploid *gamete* cells the task is to transfer one exact set of chromosomes into each of the daughters of a diploid cell. This takes place by the marvellous process called *meiosis*. One member of each chromosome pair is carefully segregated and assorted so that new complete sets are generated. However, the chromosomes are reassorted, and each haploid cell may, for example, receive chromosome 1, 4, 6, 7, etc. from one of the original sets, and chromosomes 2, 3, 5, 8, etc. from the other set. When a haploid sperm cell is fertilizing an egg, the egg nucleus will contain a full set of chromosomes, but these have also been shuffled during meiosis. Therefore, while the resultant diploid human zygote will have 46 chromosomes, the two full sets will be very different from the sets that were present in the parents. The consequences of all this are profound. Through evolution a number of non-lethal mutations have been collected. Hence, there may be many versions of any given gene.

The protein products of these genes may carry out their intended 'job' somewhat better or worse than average. Different versions of a gene are called *alleles*. The shuffling of chromosomes that carry genes, present as many different alleles, is the basis for the diversity of different traits, characteristics and behaviours within any given species.

Meiosis provides each gene allele with a fair chance of being transmitted to the next generation. That allele will then be expressed together with, and influenced by, all the other genes that have found their way into the nucleus of the same zygote. Then *natural selection* works on the particular combinations obtained. Surviving alleles are then reshuffled by meiosis and distributed into new zygotes. These processes allow a given species to keep and display its full range of variation and possibilities for each new generation. Certain alleles may become more prevalent under certain conditions, but this can be changed to yet another assortment, or reversed, should the niche or ecosystem conditions again change.

Each new zygote is, in fact, a unique experiment. A given gene allele is placed in a nucleus with other genes (in the human case 24,999), many of which it has probably never coexisted with before. Even subtle differences in the time of expression, amounts produced, shape, or resistance to degradation of the encoded protein may generate subtle differences in the abilities of the individual, for better or for worse. This gives the species the fundamental property of variation: on the whole, the capacity to adapt to new ecological niches or to dramatic changes in the total environment.

The overall goal, transmission of genomes from one generation to the next, is the same for asexual and sexual organisms, though for the latter the genomes are handed over to immature offspring. Hence, the *nurture* of offspring becomes important for the survival of the offspring, up to their reproductive age. Plants secure their fertilized ovules with hardy seed coats and fruit tissues. Social insects produce classes of non-reproducing 'workers' to protect and feed a reproducing queen; others carry their larvae in their mouths to save them from destroyed nests. Vertebrates have also developed an amazing array of behaviours to assure the survival and maturation of their progeny.

4.1. Eternal or mortal?

The matter of sex was omitted from our account of how multicellular organisms evolve all kinds of specialization by expressing different sets of genes in different sets of cells. It may, however, be argued that sex was a prerequisite for multicellularity to evolve.

The animal zygote proceeds to cleave into two cells, and then four and then eight. Each cleavage generates daughter cells that stay together as a developing embryo. Thereafter, they start to specialize. If we focus on one of the cells in the eight-celled embryo, we see a cell that switches on a certain set of genes. In the sixteen-celled embryo, the focused cell becomes two daughter cells containing the protein products of the switched-on genes, and these products switch on a second subset of genes. In the thirty-two celled embryo, the proteins of the second subset initiate a signal transduction cascade that induces the by now four daughter cells of the same lineage to move together to a common location. Following this, the lineage may, after additional cleavages, move into the interior of the embryo by a process called gastrulation. Following gastrulation, the lineage contains 512 daughter cells, and they have different fates. Sixty-four of the cells at one end of the embryo activate a set of genes that tells their daughters to differentiate into gut cells. Eight cells near the midline activate genes that start the development of a heart, and so on.

Early on during this *embryogenesis*, some cells switch on sets of genes that order them to become *germ line cells*, precursors of the sperm and egg cells that are uniquely capable of carrying out meiosis. They migrate into what will become the animal's *gonads*, and remain dormant there until sexual maturation of the individual. Then they begin to carry out meiosis in order to produce haploid gametes.

The germ line cells and the remaining, *somatic*, cells have split the job of staying alive and becoming a permanent part of evolution. The germ line transmits the genome to the next generation, while the somatic cells negotiate between the individual and the ecosystem for optimizing the chances of the germ cells to be transmitted: The germ line is protected in the gonads and is released only at appropriate times. The somatic cells are the ones that pump blood, grow muscles, sprout feathers, are aware of dangers, find a good sex mate, and release the sex cells, after which a life cycle is completed. Some organisms die shortly after reproduction (e.g. annual plants, many insects, salmon) and some do not (e.g. humans).

Once there is a life cycle with a germ line and a soma, immortality is handed over to the germ line. This liberates the soma, the individual, to focus on strategies and evolve behaviours for getting the gametes transmitted. Since *morphogenesis* is the key strategy for negotiations with the environment, multi-cellular eukaryotes have evolved all the beautiful and marvellously complex morphological structures we can observe. All the parts of an organism contain cells than retain two full copies of the genome. All the parts work together to ensure the transmission and the nurture of the germ line, and then they vanish, i.e. die.
Death is a part of life already from early embryogenesis. Some cells have been programmed to die. The limbs of human embryos initially terminate as blunt stubs. Then sets of cells die in order to create separate fingers and toes. In every deciduous tree, each autumn the cells at the base of each leaf obey the determination that they should die to cut off the flow of nutrients, and the leaves themselves die. These events are governed by *apoptosis*, a sort of very precisely coordinated cell suicide.

The more general fate of the organism is that the whole soma dies. Natural death may occur after only a few days of life, as with dragonflies. However, death may also be postponed for hundreds of years, e.g. as with sequoia trees. If we do not die by accident, infection or cancer, we die because of age. Our somatic cells die after a certain number of cleavings. Cancer cells, however, are characterized as 'immortalized'. They carry somatic mutations in key cell cycle regulating genes so that they do not stop dividing, either in the body or in laboratory cell cultivation trays.

5. Speciation and Biodiversity

New biological species arise through the process of *speciation*. Organisms segregate into groups that will or will not mate with one another. Segregation leads to the use of new resources, habitats and niches. Traits adapt and evolve under natural selection in order to improve conditions for the organism to live in, e.g. a new forest habitat. This new habitat, however, consists mainly of other organisms (trees) that also evolve to improve *their* conditions. Hence, organisms interact and coevolve. On one hand, segregation leads to *expansion of niches*, and to development and refinement of traits. Any successful development is picked up by natural selection and not diluted after reproductive isolation. On the other hand, competition for limited resources leads to a *compression of niches*, i.e. *specialization.* Specialization reduces competition and lets more species coexist. The outcome of the natural evolutionary processes is the unfolding of more and more complex organisms, and also the generation of *biodiversity* (Figure 1.2).

The origin of *new species* is far from being fully understood, but the outcome is known. Members of a new species fail (by definition) to generate fertile offspring when placed in contact with related species. Why? Because an important barrier is created: sexual behaviours have changed, because the sperm can no longer fertilize the egg, or because the embryos fail to develop properly, and die.

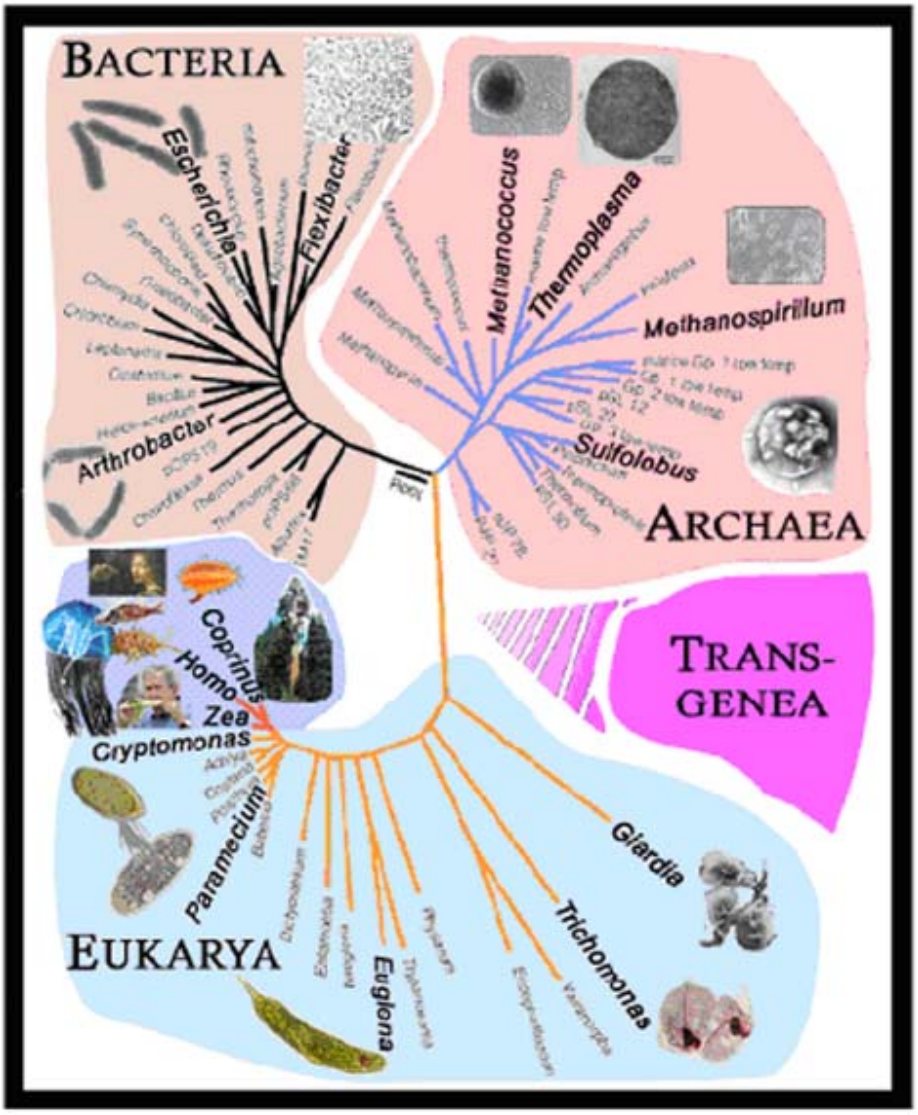

Figure 1.2. Domains of Life as viewed from the dimension of DNA relatedness. In this image, all forms of life existing on planet Earth are shown in their mutual relationship. Longer lines connect more distantly-related organisms, and each of the known domains of life is included in a different colour (Archaea, Bacteria, Eukarya, and Transgenea, representing the new domain created by transgenic manipulation of living organisms). The largest majority of living organisms are invisible (light blue and three purple domains). Only a fraction (red lines, darker blue) represent organisms that are visible, and therefore included in human economic, political and cultural affairs. The purpose of this image is to develop a device and method to visualize all domains of life, including those invisible to most humans, over large geographical dimensions. Of particular interest is the visualization of the novel domain formed by transgenic organisms (GMOs), which have several different ancestries. (Reproduced with the kind permission of Dr. Ignacio Chapela, UC Berkeley)

6. Concluding Remarks

On a larger scale, the outcome of evolutionary development, the incredible biodiversity of more than 1.5 million named species, is known to some degree, but the underlying processes, including the origin of the first organisms and the evolutionary diversification, are more or less a complete mystery to us. Even with the organisms that we study today with all the methodology available,

including the '-omics' techniques (see Chapter 8), we have to admit: the central core of the living is not at all well understood. We cannot explain how gene regulation starts; we cannot explain the differentiation in multicellular organisms, nor the coordinated timing of gene expressions that secure the homeostasis of organisms. In the last few years it has become evident that horizontal gene transfer (HGT) has been much more important for the evolution of life on Earth than earlier realized. Transgenesis-based genetic engineering represents enforced HGT, insertional mutagenesis, possible epigenetic changes and unpredictable chromatin aberrations (see Chapters 1–5, 9, 12–14). The only thing we know is that we do not know. If we realize and accept this, how can we dare to interfere in fundamental and unpredictable ways with ecosystems that have evolved by laws largely unknown to us during the course of 4.5 billion years?

7. Resources

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Chapter 2

Introduction to some basic features of genetic information: From DNA to proteins

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Molecular biology is the study of *biology* at a *molecular* level, with the aim of understanding the interactions between the various systems of a cell, including the interrelationship and regulation of *DNA*, *RNA* and *protein* synthesis. In general terms, DNA (deoxyribonucleic acid) is the basic genetic information macromolecule of the cell. It provides the rudimentary instructions for all kinds of biochemical functions, from making proteins to regulatory functions. DNA is found in every cell and every cell type and organism, from single-celled organisms (*prokaryotes*, e.g. bacteria), to larger multicellular organisms (*eukaryotes*, e.g. seaweeds, fungi, plant, animals) that can have many different cell and tissue types.[1] DNA contains the genetic 'code' of information that makes each species unique. Smaller variations in the DNA can lead to minor differences among individuals of the same species. The combination of specific DNA composition, epigenetic changes (see Chapter 8) and environmental influences determine an organism's appearance and development. In this book, we discuss how the main carrier of heritable information (DNA) and the environment interact, with particular emphasis on how genetic engineering may intentionally or unintentionally affect this interaction. This chapter focuses on DNA, RNA and the concept of genes. It is structured as follows:

1. **Structure and replication of DNA**
2. **Genes as specific nucleotide compositions within DNA**
3. **RNA molecules**
4. **Genes and protein synthesis**

1. Structure and replication of DNA

The primary feature that makes DNA unique lies within its chemical structure. The information-containing properties of the nucleic acids arise from unique combinations of individual nucleotides that form long polynucleotide chains; this macromolecule is collectively called DNA. Each nucleotide consists of three parts: a nitrogen base, a pentose sugar, and a phosphate group (see Figure 2.1). DNA consists of four different base nucleotides: adenine, thymine, guanine, and cytosine (A, T, G, and C, respectively).[2] The phosphate group of one nucleotide is attached to the sugar of the adjacent nucleotide that is next in line in the chain. This results in a 'backbone' structure of alternating phosphate groups and sugar groups, from which the nucleotide bases project outward. Yet, how can so much genetic diversity come from only four basic units (nucleotides) of genetic information? This is possible because the DNA is a long strand of information, like letters in a sentence. There is almost an infinite number of combinations of nucleotides possible in a DNA macromolecule. For instance, even a short DNA molecule 10 base pairs (bp) long has 4^{10} or 1,485,576 possible combinations of bases. A bacterial gene is often 1000 bp long.

[1]Viruses form their own class of life. They may have single-stranded or double-stranded DNA or RNA as their genetic material, using the replication machinery of the organisms they infect to multiply.
[2]Note that RNA, which we will discuss later, also has four nucleotides but replaces Thymine with a Uracil (U) base.

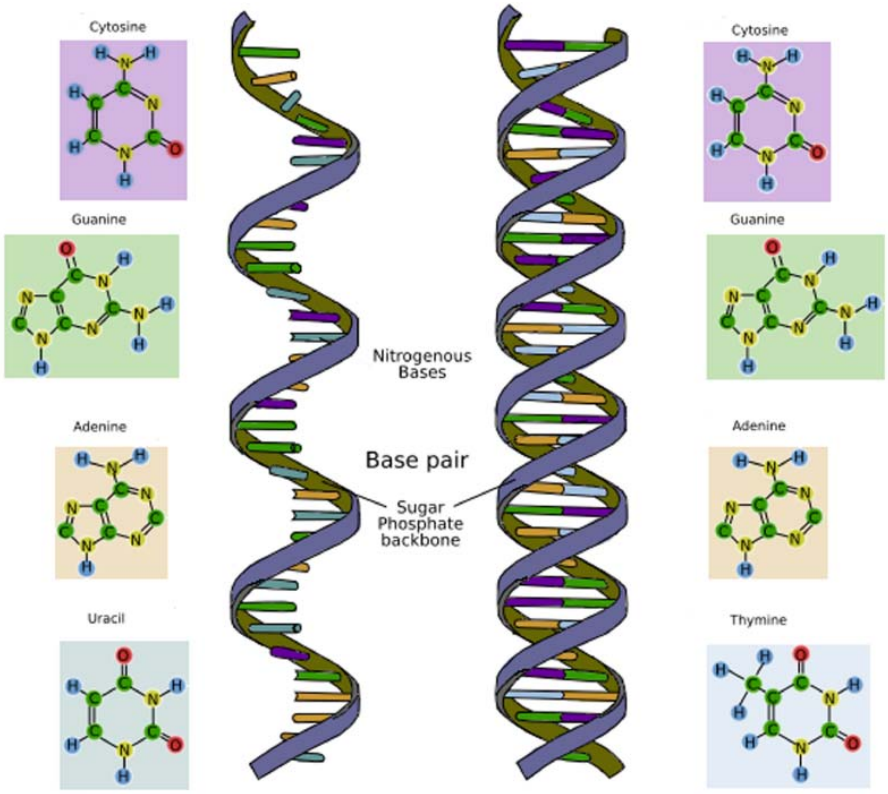

Figure 2.1. The chemical composition and structure of the DNA double helix.

DNA is a double-stranded molecule whose primary features are its *complementarity* and its *base pairing* with its sister DNA strand, forming *the double helix*. The complementarities of the nucleotide bases also facilitate replication, or copying of the genetic material. How does an organism pass this DNA to daughter cells and offspring? Inheritance, the passing of genetic information (genes) from one generation to the next, involves either i) sexual recombination (mixing of genetic information from parents via the combination of sperm and egg), or ii) through cell division that results in the inheritance of the same genetic information from the parent to the daughter cells. This is achieved by DNA replication (Figure 2.2). So each DNA strand is complementary to the other in their base pairing of nucleotides: T always pairs with A and G always pairs with C. These two complementary polynucleotide chains make a very stable spiralling structure, and form the DNA's well-described double helix.

DNA replication produces two molecules by semi-conservative replication, that is, each DNA molecule is made up of one of the original two parental strands (that make up the double helix) and one completely new synthesized strand (Figure 2.2). During replication, the DNA is unwound by enzymes, called helicases, that open up the double helix, allowing DNA replication enzymes, called DNA polymerases, to come in and synthesize a new strand of DNA. The polymerase is like a DNA copier, requiring the template (original), DNA, and the individual A, T, C, and G nucleotide units paired to its complementary base (A to T, and G to C), all one nucleotide at a time.[3] This process is thus almost identical[4] to a polymerase chain reaction (PCR) that will be described further in Chapter 33.

[3]Note that this is essentially the same biological machinery used in the laboratory to produce a Polymerase Chain Reaction (PCR), a laboratory technique that has many applications in genomic research, and is widely used as a means to detect the presence of genetically modified DNA (as described in later chapters).

[4]In PCR amplifications of DNA, a termostable polymerase is used, that allows the reaction to be repeated after heat-mediated separation of the two DNA strands.

2. Genes as specific nucleotide compositions within DNA

A gene is classically understood as a short region of DNA that encodes, for example, for the production of a particular protein product or trait. Genes are commonly described as a physical unit.[5] In such a physical conception, genes, in essence, are functional units of inheritance of DNA.

The sum of an organism's genetic information is what is generally referred to as its *genome*. Understanding the function of genes and other parts of the genome is known as *functional genomics*. The genome of an organism consists of very long strands of DNA molecules, usually packaged with specific proteins into *chromosomes.*

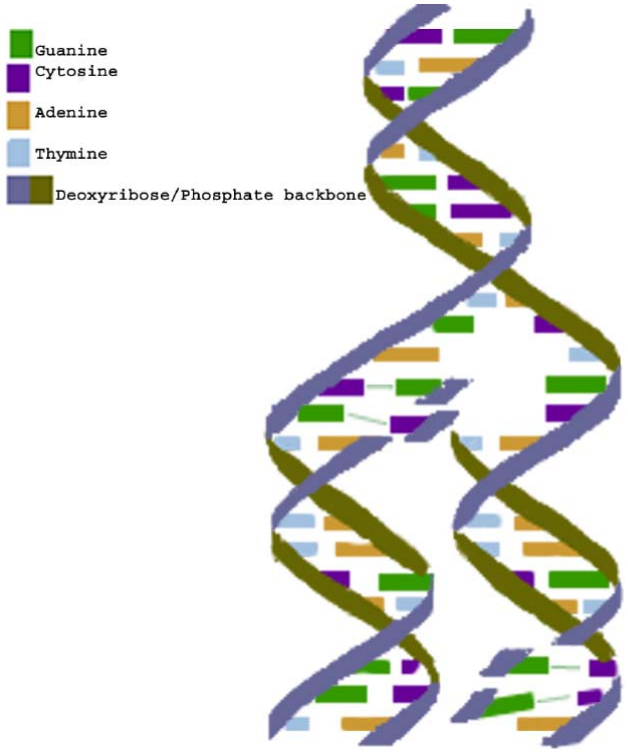

Figure 2.2. DNA replication is semi-conservative, with one of each parental strand serving as template for each newly synthesized complement.

Different organisms have different sized genomes (see Figure 2.3), though the size of an organism's genome does not necessarily correlate with its complexity. It has been demonstrated that only a very small percentage of the DNA in the whole genome actually encodes for a protein (only *c.*5% in humans, for example). Thus, the remaining DNA may have important genome stability, and developmental and regulatory functions. The large regions of DNA not encoding proteins were earlier termed 'junk DNA'.[6]
The DNA is tightly wound around a series of proteins (e.g. histones) that have both DNA packaging and regulatory functions.[7] These protein complexes are further wound to produce

[5]While this can be true in a most reduced sense, genes and genomes are really much more than that, as they participate in interactive layered biological networks of metabolic regulation with the cell, tissue and organism. The concept of a gene, and the genome itself, is therefore not as straightforward as it may seem at first.
[6]In later sections, we will see that this 'junk DNA' is now known to have important regulatory functions.
[7]The nucleus of a single diploid human cell contains approximately 6 x 109 bp of DNA. This enormous degree of packaging is achieved by wrapping up the DNA with proteins called histones. In vertebrates, there are five

chromosomes (in eukaryotes). Chromosomes are amazingly long (stretched out, the DNA of just one human cell would be almost two metres long) and hence need to be compacted within the cell. In the case of humans, we have 23 chromosomes, with two copies per cell (one from each of the sexes). In eukaryotes, the majority of the genetic information is compartmentalized in the cell's nucleus (mitochondria, and chloroplasts in plants, also contain functional DNA from their former lives as free living organisms). In prokaryotes, genetic information is more loosely compacted in a single circular chromosome within the organism.

3. RNA molecules

RNA molecules, like DNA, are made up of nucleotides, except that the thymine (T) nucleotide is replaced with a uracil (U) nucleotide, which is not found in DNA. Due to this small but important difference, a double helix structure does not form easily, but instead, RNA remains single stranded (ss).[8] SsRNA serves various functions in the cell, such as messenger RNA (mRNA) and transfer RNA (tRNA), two types of RNA that are required for protein synthesis. Other RNAs serve regulatory functions. The role of RNA within the cell is explained in greater detail in Chapter 3.

histones, H1, H2A, H2B, H3, and H4. The basic packaging unit, or nucleosome, is an octamer composed of two molecules of each of the histones H2A, H2B, H3, and H4, forming a disc-shaped structure. Exactly 146 bp of DNA are wound around the disc, like a thread on a spool, making slightly less than two complete turns. The gap between neighbouring nucleosides is approximately 50 bp in length, and one molecule of histone HI binds in this linker region. In transcriptionally inactive chromatin there is a further order of packaging to form a structure known as the solenoid, comprising nucleosomes wrapped around a multimeric rod of H1 subunits. The solenoid is 30 nm in diameter and each turn contains six nucleosomes and six H1 molecules.

[8]Double-stranded (ds) RNAs do, however, make up the genomes of some virus families (e.g. Reoviridae), and are also important in the regulation of gene expression (see Chapter 3).

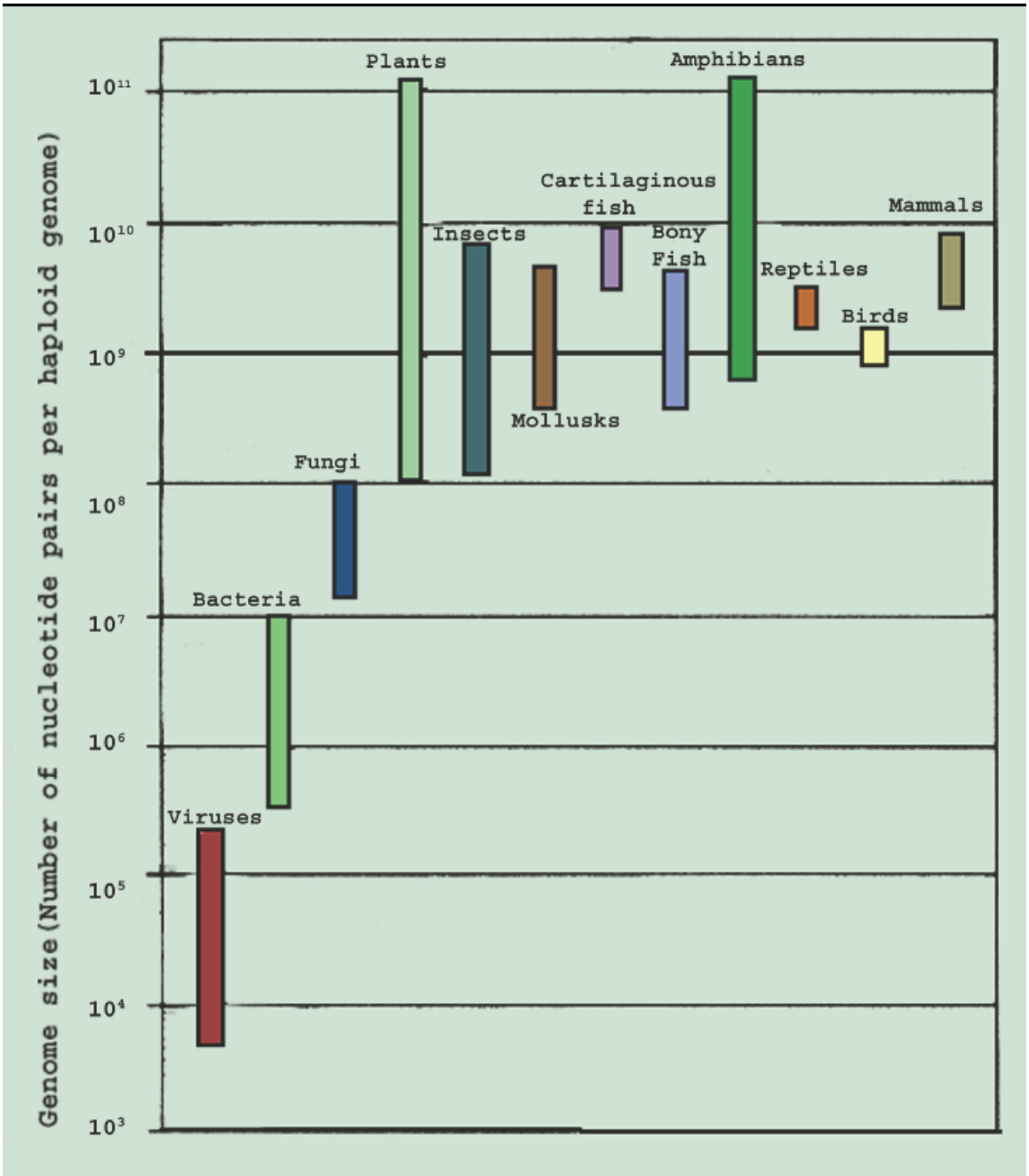

Figure 2.3. Genome size variations in different categories of organisms.

4. Genes and protein synthesis

One theory in molecular biology has, for the last half century, been the guiding principle for understanding how genetic information is processed in the cell. This theory, called The Central Dogma of Molecular Biology, states that genetic information that instructs protein synthesis flows in one direction, from DNA (via transcription) to RNA to protein (via translation) (see Figure 2.4). This dogma is the guiding principle of genetic engineering, suggesting that genes are independent modules that can function equally well in different organisms where the gene is in command regardless of its cellular (biological) and environmental context.

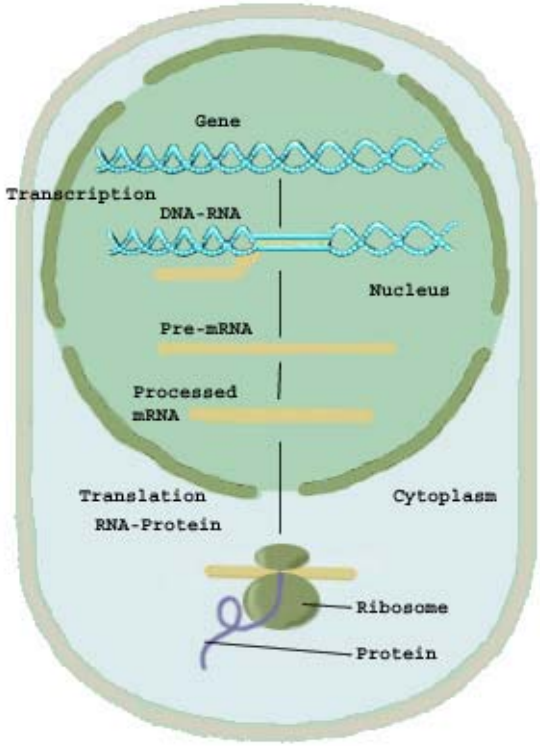

Figure 2.4. Simplified illustration of the Central Dogma assumed information flow from gene to protein in a prokaryotic cell.

The next chapter (Chapter 3) examines in more detail how genetic information contributes to the synthesis of a particular gene (protein) product and discusses how DNA is only part of a two-way regulatory network influenced by both abiotic and biotic factors at complex levels of organization within an organism.

Further Reading

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Chapter 3

The complex and interactive pathway from (trans)genes to proteins

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Multicellular organisms, such as animals and plants, consist of hundreds of different cell types. Each cell type (e.g. the various types found in liver, heart and lung organs) contributes a specific function to the organism. Yet, all of these cells contain the same number of identical genes (i.e. 20,000–50,000). The high cell diversity is achieved not through gene content, but through the tightly controlled regulation of expression of a subset of the genes in each cell type. This chapter introduces the different steps on the pathway from the gene to functional protein(s), and shows that a single gene can give rise to a high number of more or less related yet functionally distinct proteins. Knowledge of the broad range of factors governing gene expression in various cellular and environmental conditions is necessary to understand how genetic engineering may introduce novel risk aspects of genetically modified organisms (GMOs). From a basic science standpoint, genetic engineering has been an important development in science to uncover the inherent complexity of factors regulating gene expression. Hence, the limited understanding of how these factors relate within a biosafety context is an important source of uncertainty in the risk assessment of GMOs.

The pathway from genes to proteins in higher (eukaryotic) organisms (outlined in Chapter 2) involve a complex series of pathways divided into the following steps:

1. Regulation of gene transcription
- 1.1. Promoter recognition
- 1.2. RNA transcript modifications
- 1.3. Stability of RNA transcript
- 1.4. mRNA transport to the cytoplasm

2. Regulation of mRNA translation

3. Regulation of protein activity and stability
- 3.1. Protein folding, cleavage and chemical modification
- 3.2. Higher order protein interactions
- 3.3. Regulated protein degradation

1. Regulation of gene transcription

1.1. Promoter recognition

The first step from gene to protein involves the transcription of a gene's DNA code into messenger RNA (mRNA). The start site (switch) of mRNA production is called

the promoter.[1] The various genes in a cell have different promoters ensuring gene expression is «on» or «off» in response to specific developmental and environmental conditions. A variety of proteins, known as *transcription factors* (TFs), bind to DNA in a sequence-specific manner, that initiate and regulate transcription.[2] The transcription factors bind to DNA either in the promoter or further upstream of the gene.[3] A given promoter is composed of a variety of partly overlapping binding areas for different TFs. The occurrence of relevant TFs in a given cell type will determine whether, and to what extent, a particular gene is transcribed and proteins are produced. Figure 3.1 presents an outline of a generic eukaryotic promoter.

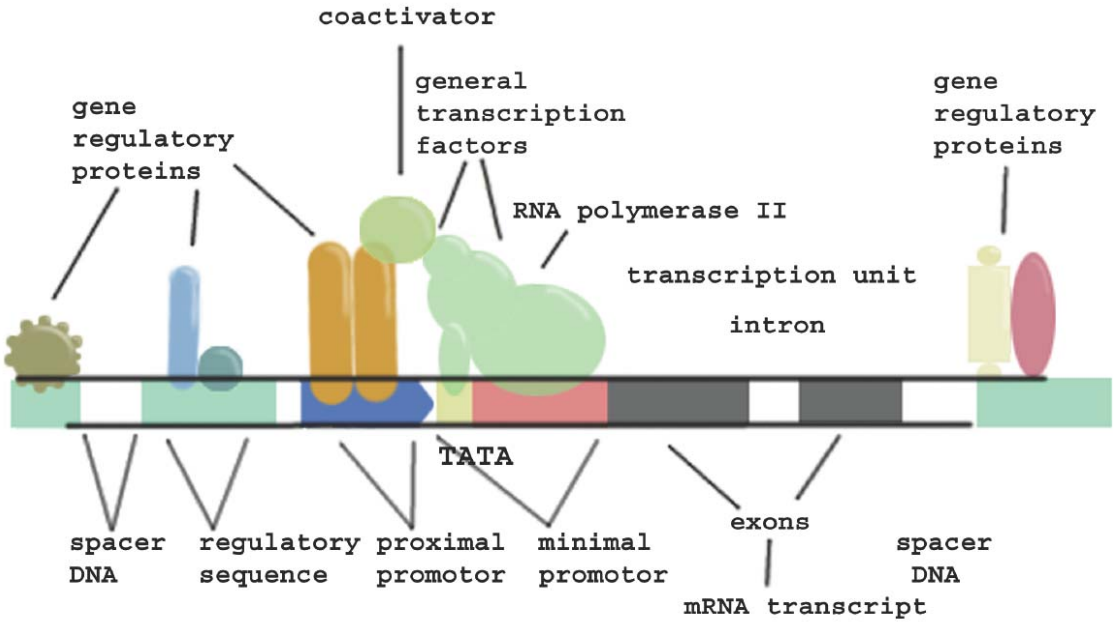

Figure 3.1. Outline of a generic eukaryotic promoter.

In addition to the promoter, three other types of DNA elements bind transcription factors and regulate cell type specific gene expression:

– *Enhancers* are DNA sequences that serve as specific binding sites for transcription factors to up-regulate the rate of transcription initiation. Enhancer regions are usually relatively short (30–500 base pairs), and have several binding sites for TFs.

– *Silencers* are DNA sequences that serve as specific binding sites for transcription factors that upon binding will down-regulate transcription initiation.

[1]A promoter is defined as a segment of DNA to which the RNA polymerase II enzyme attaches. The promoter binds general and specific transcription factors (proteins) that guide the polymerase to the initiation site and regulate the rate of transcription. The minimal promoter is the DNA sequence at which the general transcription factors and RNA polymerase II assemble.

[2]Each TF recognizes and binds to a specific 5–20 bp long DNA region. One important member of this class of proteins is TFIID. This protein binds to a short AT-rich sequence, the 'TATA box', found approximately 30 nucleotides upstream of the transcription initiation site in many eukaryotic genes. The primary function of TFIID is to direct RNA polymerase II to the initiation site.

[3]The fact that 5% of an organism's genes encode TFs underscores the importance of this protein family in biology.

– *Insulators* are DNA sequence elements that prevent inappropriate interactions between adjacent chromatin domains.

These DNA motifs, called functional elements, may all be located upstream or downstream of the gene, or within an *intron* (non-coding DNA sequence). While promoters have defined sequence orientations, enhancers and silencers can be turned around, and still exert their biological functions. The combination of these regulatory elements and their locations relative to the promoter are different for each gene. In a GMO context, it is important to realize that all the mentioned regulatory elements may influence the transcription of more than one gene, including non-target native genes. Furthermore, the transgenesis process inserts new promoters and/or other functional elements at unpredictable sites in an established genome. Finally, small parts of the inserted transgenic construct (e.g. plasmid backbone sequences) may contain functional regulatory elements.

1.2 RNA transcript modifications

Once appropriate TFs are bound to the promoter, enzymes called RNA polymerases[4] will produce single-stranded mRNA (transcripts). This RNA transcript undergoes a series of modifications in the cell nucleus before it is translocated to the cytoplasm for subsequent translation into a protein strand. Both ends of the primary mRNA transcript are modified.[5] Moreover, non-protein coding RNA regions (introns) are removed from the RNA strand, leaving only the regions that contain information left to be transcribed (exons). This intron removal is called *splicing*. The DNA signals, which direct the splicing, flank the intron.[6] Most genes in higher eukaryotes contain one or more introns, which are generally longer than the exons. Hence, the major part of the primary RNA transcript is removed in order to generate a functional mRNA ready to be translated in the cytoplasm. This can occur in a number of combinations (Figure 3.2), leading to the production of different mRNAs, and hence protein products, from the same initial DNA sequence[7]. The combination of introns, which are removed by splicing, varies between cell types, thus allowing a single gene to produce transcripts coding different protein sizes. This form of post-transcriptional regulation is called *differential splicing* or *alternative splicing.*

Because alternative splicing allows individual genes to produce multiple protein types with variable post-transcriptional RNA modifications, stability and function, the 'one gene, one protein' rule of the Central Dogma is erroneous. The genetic composition of an organism cannot therefore be used to predict *a priori* the actual protein composition (*proteome*) of a cell at a given life stage or under different sets of ecological or biological conditions. Alternative splicing is the most important process

[4]In eukaryotes there are three separate types of RNA polymerases (enzymes) which are responsible for the production of different kinds of RNA. Messenger RNA (mRNA) is synthesized by RNA polymerase II, while RNA polymerases I and III synthesize structural RNAs.

[5]The earliest processing step in the formation of mRNA is the enzymatic addition of a cap, which occurs almost simultaneously with the initiation of transcription. The site in the genomic DNA at which transcription starts is commonly known as the cap site. Close to the cap site in the DNA are recognition sites for DNA binding transcription factors which cause RNA polymerase II to initiate transcription. While transcription initiation may be reasonably well understood, the termination process has been less well defined. Transcription proceeds beyond the eventual 3' end of the mature mRNA, and the resultant primary transcript is then cleaved internally to generate the mRNA precursor. Cleavage takes place 10–20 nucleotides downstream of a specific AU-rich sequence, AAUAAA, which is highly conserved in all eukaryotic mRNAs. An enzyme called poly (A) polymerase then synthesizes the poly (A) tail at the 3' terminus of the mRNA.

[6]Almost all introns have a GU dinucleotide pair at their 5' boundary, and an AG dinucleotide pair at their 3' boundary. These dinucleotides form part of a larger consensus DNA sequence that overlaps the intron-exon boundaries. Pre-mRNA splicing operates towards at least 95% of the primary transcript pool.

[7]This is only one of several ways in which the same 'gene' or region of DNA can lead to the production of many different protein products in the same organism.

that generates a large number of mRNA and protein types from the surprisingly low number of genes. Unlike variable promoter activity, alternative splicing changes the structure of transcripts and their encoded proteins, thereby also affecting the protein binding properties, intracellular localization, enzymatic activity, stability, and post-translational modifications. The magnitude of the effects of alternative splicing ranges from a complete loss of protein function, acquisition of a new function, to very subtle modifications in function. Evidence is now accumulating that alternative splicing coordinates physiologically meaningful changes in protein expression and is a key mechanism to generate the complex proteome of multicellular organisms (Stamm et al., 2005). In the most extreme case of alternative splicing described to date, the Down's syndrome cell adhesion molecule (Dscam) gene alone could potentially encode more than 38,000 different protein isoforms (Zipursky et al., 2006).

Additionally, less understood processes act on the RNA transcript prior to translation. These are collectively called *RNA editing* and result in sequence modifications of the original RNA molecule. Alterations can include substitutions, insertions or removal of nucleotides and bases. RNA editing can be regulated in a developmental stage or tissue-specific manner.

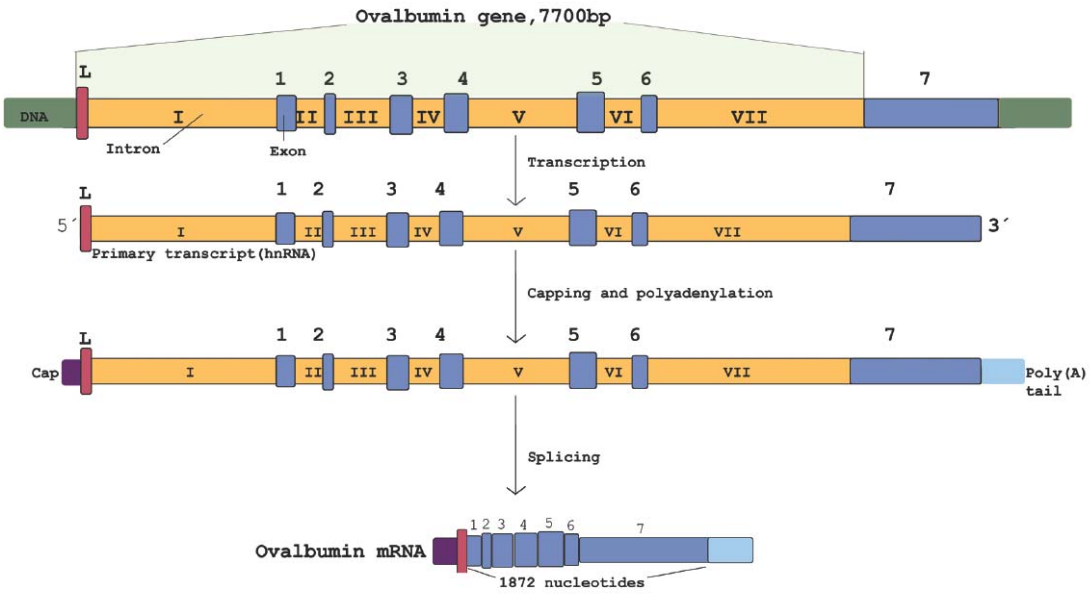

Figure 3.2. Processing of the primary RNA transcript for the gene encoding the protein ovalbumin. The intron regions (yellow) are spliced out, leaving only the exon regions (blue), which code for the protein in the final transcript.

1.3 Stability of mRNA transcript

The initial mRNA transcript needs to survive enzymatic degradation during modification, transport and translation. The limited lifetime of RNA transcripts allows a cell to change its pattern of protein synthesis continuously to changing physiological needs. Several types of molecules affect the stability of the RNA transcript. A particularly interesting group of regulatory molecules are small (19–28 nucleotides long), *non-protein encoding RNA molecules*. Such molecules are derived from cleavage of double-stranded RNAs (dsRNAs). Small RNAs can induce *gene silencing* through specific base pairing (binding) with the targeted mRNA transcript, thereby preventing protein expression. Small RNA-mediated gene silencing has been

observed in a number of eukaryotes for almost two decades, but the fundamental role of small RNA molecules in regulating gene expression has been unravelled only recently (Mattick 2003).

Degradation rates of mRNAs are important determinants of transcript availability for protein synthesis. The degradation rates of mRNAs differ in different cell types: In the gut bacterium *E. coli*, typical mRNAs' half-lives are *c.*15 minutes. In mammalian cells, unstable mRNA has about the same half-life as the *E. coli* mRNA, while stable mRNA such as the transcript of the β-globin gene has a half-life that exceeds one day. The control of mRNA degradation is an important component of the regulation of gene expression since the concentration of mRNA is determined both by the rates of transcription and rates of decay.[8]

1.4. mRNA transport to the cytoplasm

Once RNA processing is complete, mature mRNAs are exported to the cell's cytoplasm, where they serve as the blueprints for protein synthesis by ribosomes. Specific mRNAs may be directed to and anchored at specific subcellular locations, where they may be temporarily withheld from the translation apparatus and have their 3' ends trimmed or extended. From there the modified RNA may associate with other mRNAs encoding proteins of related function, and be scrutinized by protein complexes that serve as 'the quality-control police'. Hence, mRNAs in multiple cell types are subject to a diverse array of regulatory activities affecting essentially every aspect of their short lives and contribution to protein synthesis.

Throughout their existence, mRNAs are escorted by a complement of proteins and small non-protein coding RNAs (e.g. miRNAs), some of which remain stably bound while others are subject to dynamic association. Together with mRNA, these constitute *the messenger ribonucleoprotein particle* (mRNP). Individual mRNP components can be thought of as adaptors mediating the mRNAs' activity. Some adaptors make positive interactions and thereby serve as activators of a particular process, whereas others disrupt the positive interactions and act as repressors. By containing binding sites for diverse adaptors, individual mRNAs can respond to a myriad of regulatory inputs, allowing their expression to be selectively fine-tuned in response to changing conditions. The result is an elaborate web of regulatory networks of equal, if not greater, complexity to those controlling initial mRNA synthesis.

Box 3.1 Examples showing how the complex characteristics of transcription affect the understanding of the biology of GMOs

a. *Promoters*. A lack of in-depth understanding of promoter regulation and activities has led to the frequent insertion of strong promoters from pathogenic microorganisms and viruses into genetically modified (GM) plants. For instance, the use of the 35S CaMV plant virus promoter leads to a continual expression of the transgenes in the

[8]Two general pathways of mRNA decay have been described in eukaryotes. Both pathways share the exonucleolytic removal of the poly(A) tail (deadenylation) as the first step. In one pathway, deadenylation is followed by the hydrolysis of the cap and processive degradation of the mRNA by a 5' exonuclease. In the second pathway, the mRNA is degraded by a complex of 3' exonucleases before the remaining cap structure is hydrolyzed.

GM plant; the promoter can be active in a range of other organisms (Myhre et al. 2006).

b. *Enhancers*. The introduction of viral DNA sequences containing an enhancer into a GM plant can lead to unexpected results such as a change in the transcription of other unrelated genes. Recent studies provide evidence that the CMV enhancer may activate other unrelated promoters. Introduced genetic material may thus produce unexpected changes in expression of various genes localized far away from the transgene insert site (D'Aiuto et al. 2006).

c. *Transcript length variability*. Inefficient termination of transcription in a GM soybean variety led to the presence of various unexpected transcripts, and potentially also proteins (Rang et al. 2005).

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2. Regulation of mRNA translation

After the processing of the mRNA, the translation machinery localized in the *ribosomes* converts the RNA information into the specified protein. The proteins are produced by ribosomes reading the *codon triplets* of the mRNA strand. The codon triplet is a sequence of three bases in the RNA that gives instructions to ribosomes to produce a specific individual amino acid to be assembled into a linear amino acid strand that makes up the protein. The individual amino acids are transported to the ribosome by *transfer RNAs*, small RNAs that are specialized in providing each of the 20 naturally occurring amino acids. The genetic codes of these triplets are universal for all organisms (Figure 3.3) although species-specific preferences on codon usage exist when there is more than one codon specifying a given amino acid (redundancy).

	U		C		A		G		
U	UUU UUC	Phenyla-lanine	UCU UCC UCA UCG	Serine	UAU UAC	Tyrosine	UGU UGC	Cysteine	U C A G
	UUA UUG	Leucine			UAA UAG	Stop codon Stop codon	UGA	Stop codon	
							UGG	Tryptophan	
C	CUU CUC CUA CUG	Leucine	CCU CCC CCA CCG	Proline	CAU CAC	Histidine	CGU CGC CGA CGG	Arginine	U C A G
					CAA CAG	Glutamine			
A	AUU AUC AUA	Iso-leucine	ACU ACC ACA ACG	Threonine	AAU AAC	Asparagine	AGU AGC	Serine	U C A G
	AUG	Methionine; start codon			AAA AAG	Lysine	AGA AGG	Arginine	
G	GUU GUC GUA GUG	Valine	CGU GCC GCA GCG	Alanine	GAU GAC	Aspartic acid	GGU GGC GGA GGG	Glycine	U C A G
					GAA GAG	Glutamic acid			

Figure 3.3. The codons specifying the amino acid compositions of proteins. Genetic information in genes becomes encoded into mRNA in three-letter units known as codons, comprised of the bases uracil (U), cytosine (C), adenine (A), and guanine (G).

The regulation of gene expression at the level of translation is an important, but still not completely understood process. Several recent studies using comparative proteomic profiling of cells have documented a lack of correlation between the mRNA level and composition and the corresponding protein levels of numerous genes. This indicates that post-transcriptional control is more important in the regulation of the protein content of a cell than often assumed. Regulation at this level allows for an immediate and rapid response to changes in environmental, physiological or pathological conditions (for example, heat shock, oxygen deprivation, pollution with *endocrine disrupters*, nutrient deprivation). In eukaryotes, translation is divided into three distinct phases *initiation, elongation* and *termination*. Although all three phases are subject to regulatory mechanisms, under most circumstances the rate limiting step is initiation.

- *Initiation.* A single mRNA transcript can have several translation initiation codons, thus several lengths of a protein can potentially be translated from a single mRNA transcript. A small, yet growing number of mammalian mRNAs have been shown to initiate translation from other sites than the standard AUG nucleotide start codon (Figure 3.3). These start codons may be downstream in frame or out of frame AUG or CUG codons. Translation initiation on such mRNAs results in the synthesis of proteins with different sizes (i.e. harbouring different amino terminal domains), potentially conferring distinct protein functions.[9]

- *Elongation.* The straightforward codon-by-codon translation of an mRNA is looked upon as the standard way in which proteins are synthesized. An increasing number of unusual elongation events are, however, being discovered. One of them is *frame shifting*, a process occurring when a ribosome pauses in the middle of an mRNA, moves back one nucleotide or, less frequently, forward one nucleotide, and then continues translation. The result is that the codons that are read after the pause are not contiguous with the preceding set of codons: they lie in a different triplet codon reading frame.

Spontaneous frame shifts occur randomly, are commonly non-functional and perhaps deleterious, because the protein synthesized after the frame shift has the incorrect amino acid sequence. However, not all frame shifts are not spontaneous. Some mRNAs utilize *programmed frame shifting* to induce the ribosome to change to a specific point within the transcript. Programmed frame shifting occurs in all types of organisms, from bacteria through to humans, as well as during expression of a number of viral genomes.

[9]The biological significance of the non-AUG alternative initiation is demonstrated by the different subcellular localizations and/or distinct biological functions of the protein isoforms translated from a single mRNA. Use of non-AUG codons appears to be governed by several features, including the sequence context and the secondary mRNA structure surrounding the codon (Touriol et al., 2003).

– *Termination*. Termination signals of protein synthesis are encoded in the gene and are also present in the mRNA transcript in the form of three different base triplets referred to as termination, stop or nonsense codons[10]. Inefficient translation termination can lead to variation in the size of translated protein products, which in turn may result in new proteins with unexpected biological functions.

Box 3.2 Examples showing how the complex characteristics of translation can affect the biological understanding of GMOs

a. Given the unique combinations of factors regulating protein production from each mRNA transcript, it is clear that changing the cellular environment of a given mRNA transcript (as done in GMOs) will affect its stability and translational properties.
b. Alternative translation start codons that are normally not recognized in one organism may become active when the gene is modified and inserted into another organism. The result is that translation of certain gene products might be turned on, off, or up- or down-regulated abnormally within the GM recipient cell.

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3. Regulation of protein activity and stability

3.1 Protein folding, cleavage and chemical modification

After the protein is produced by the cell, the protein undergoes a series of modifications to its structure to ensure that it functions properly and that it is directed to the correct region of the eukaryotic cell. Such *post-translational modifications* are essential processes in the regulation of eukaryotic protein functions. The types of modifications that occur can have dramatic effects on the bioactivity, specificity and stability of the modified protein. Four types of post-translational processing are common:

– *Protein folding*. The protein emerging from the ribosome machinery may require the assistance of specialized proteins called *chaperones* to become folded into its functional 3-dimensional structure.

– *Proteolytic cleavage*. Some proteins are cleaved by enzymes called *proteases* that may remove segments from one or both ends of the polypeptide chain, resulting in a shortened active form of the protein. Alternatively, proteases may cut the polypeptide into a number of different segments, all or some of which are biologically active.

[10]When a stop codon has been translocated into the ribosomal A-site by the action of elongation factors, it is decoded at the small ribosomal subunit. The chemical reaction that is triggered by a stop signal leads to cleavage of the ester bond between the peptidyl and tRNA moieties of the peptidyl-tRNA complex. This occurs within the large ribosomal subunit at the peptidyl transferase centre (PTC) of the ribosome. How a stop signal can be transduced from the small to the large ribosomal subunit and trigger hydrolysis of peptidyl-tRNA remains unknown, and alternative hypotheses are still being discussed in the literature (Mitkevitch et al., 2006).

– *Intein splicing.* Inteins are intervening sequences in some proteins, similar to introns in mRNAs. They have to be removed, and the *exteins* (similar to exons) ligated in order for the protein to become functional.

– *Chemical modification.* Individual amino acids in the polypeptide chain may be modified by attachment of new chemical groups. The modifications may influence the folding of the proteins and their interactions with other proteins.

Chemical modifications are often introduced on the surface of the proteins at different amino acid sites. Modifications at single or multiple sites occur in different ways, by inserting additional side chains. Some examples include glycosylation, phosphorylation, acetylation, methylation, ubiquitination, sumoylation, and citrullination. Multi-site modification on a protein constitutes a complex regulatory programme that resembles a dynamic 'molecular barcode'. The chemical modification patterns hence encode 'loss-of-function' and 'gain-of-function' processes that affect bioactivity and protein stability. Recruitment of these modifying groups on proteins is often modulated by chemical modifications occurring at neighbouring and distant sites on the affected molecule. Multi-site modifications thus coordinate intra- and inter-molecular signalling for the qualitative and quantitative control of protein function.[11]

One of the most common and least understood post-translational chemical modifications of proteins is *glycosylation.* Proteins may be glycosylated with a bewilderingly array of complex N- and O-linked sugar molecules.[12] Glycosylation of proteins is highly regulated and changes during differentiation, development, under different physiological and cell culture conditions, and in disease. When a given transgene is expressed in different organisms, the glycosylation patterns may be very different, and this may add or retract biochemical and biological activities from the proteins. This may be the case even for the same gene expressed in different crop plants (see example in Box 3.3).

3.2 Higher order protein interactions

Many proteins have multiple functions that may be exerted by discrete parts of the proteins called *active domains* or *active sites.* Most proteins are conceived as globular beads on a string, where the 'beads' represent domains that range in length from 50 to 250 amino acid residues. Each domain may perform a specific biochemical function. Some protein activities, however, are performed at the interface between two or more domains situated on two different protein molecules. The structure is called a *homodimer* if the two molecules are the same, otherwise it is a *heterodimer*. There may be multiple, different proteins in the active complex. The self-association of proteins to form dimers and higher-order oligomers (the formation of protein chains consisting of many shorter proteins linked together) is a common phenomenon. Dimerization and oligomerization requirements for protein function allow regulation to occur by interfering in the assembly process. Whether and to what extent transgenic proteins engage in such interactions are unknown.

[11]Post-translational modifications are often modulating and coordinating the activities of transcription factors (discussed earlier). Chemical modifications can rapidly and reversibly regulate virtually all transcription factors, including subcellular localization, stability, interactions with co-factors and transcriptional activities, and thus have important regulatory function on protein production as well, illustrating the circular and multi-dimensional regulation of gene expression.

[12]Originating from the regulated activity of enzymes within the endoplasmatic reticulum and Golgi apparatus of eukaryotic cells.

3.3. Regulated protein degradation

Protein degradation (*proteolysis*) is a means to remove obsolete or damaged proteins. Proteolysis is mediated by specific enzymes called proteases, which vary from small proteins such as extracellular trypsin and the intracellular caspases to large, ATP-dependent, multifunctional proteases called *proteasomes*. Protein degradation is mediated by conjugation of the protein to the signal molecule *ubiquitin* that is regulated by specific degradation signals (*degrons*) in short-lived proteins. Regulated ubiquitin-dependent degradation processes are thought to play a major role in controlling the levels and menus of intracellular proteins, a function previously thought to be mediated almost exclusively at the transcription or translation stages.

Box 3.3 Examples showing how the complex regulation of protein activity and stability affects the biological understanding of GMOs

The effects of variable extent and type of post-translational processing are important when considering the biological properties of GMOs.

a. Different host organisms of a particular gene may process the resulting protein in non-similar ways; that can affect protein activity, stability and composition. For example, recombinant human insulin, for the treatment of diabetes, is produced in GM bacteria and yeasts. However, because the insulin protein does not fold to the active conformation when produced in a microorganism, an extra enzyme must be added to re-fold the protein before it can be administered to humans.

b. The changed glycosylation patterns that can occur in the recombinant proteins produced by GMOs are of critical importance. Glycosylation profoundly affects the protein's biological activity, function, clearance from circulation, and antigenicity. The cells of non-human species, particularly plants, do not glycosylate their proteins in the same way as human cells do. Different plants may even glycosylate the same protein in different ways. In many cases, the differences are profound. Furthermore, there may be important differences in the processing and degradation of glycosylated proteins between mammalian and plant cells. Thus, expressing recombinant proteins in novel cell contexts may substantially alter the biological properties of the proteins produced by the transgene (Prescott et al. 2005).

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4. Genome-scale factors affecting gene expression

4.1 Genome structure

Chromatin (Fig. 3.4) is one of the hallmarks of eukaryotic life. DNA in eukaryotes is tightly associated with a group of proteins called *histones*. Two molecules each of the four different core histones (H2A, H2B, H3, and H4) form a histone octamer, around which 146 bp of DNA are wrapped to form a nucleosomal core particle. A linker histone (H1, H5 and a number of histone-like proteins) binds to the free ('linker') DNA between two nucleosomal core particles, and this finally makes up the *nucleosome*. Given the length of the haploid human genome (3.3×10^9 base pairs), every diploid cell nucleus contains roughly 5×10^7 nucleosomes. Any molecular process entailing genomic DNA or the nucleus by default provokes or depends on

chromatin structural dynamics on various space and timescales. Chromatin dynamics are a result of changes in the properties of the chromatin constituents themselves or in the nuclear environment (Benecke, 2006). The transgenic process itself, with integration of foreign DNA and insertional mutagenesis as a key element, may change chromatin dynamics, and hence influence the expression of the endogenous genes profoundly (Recillas-Targa, 2006).

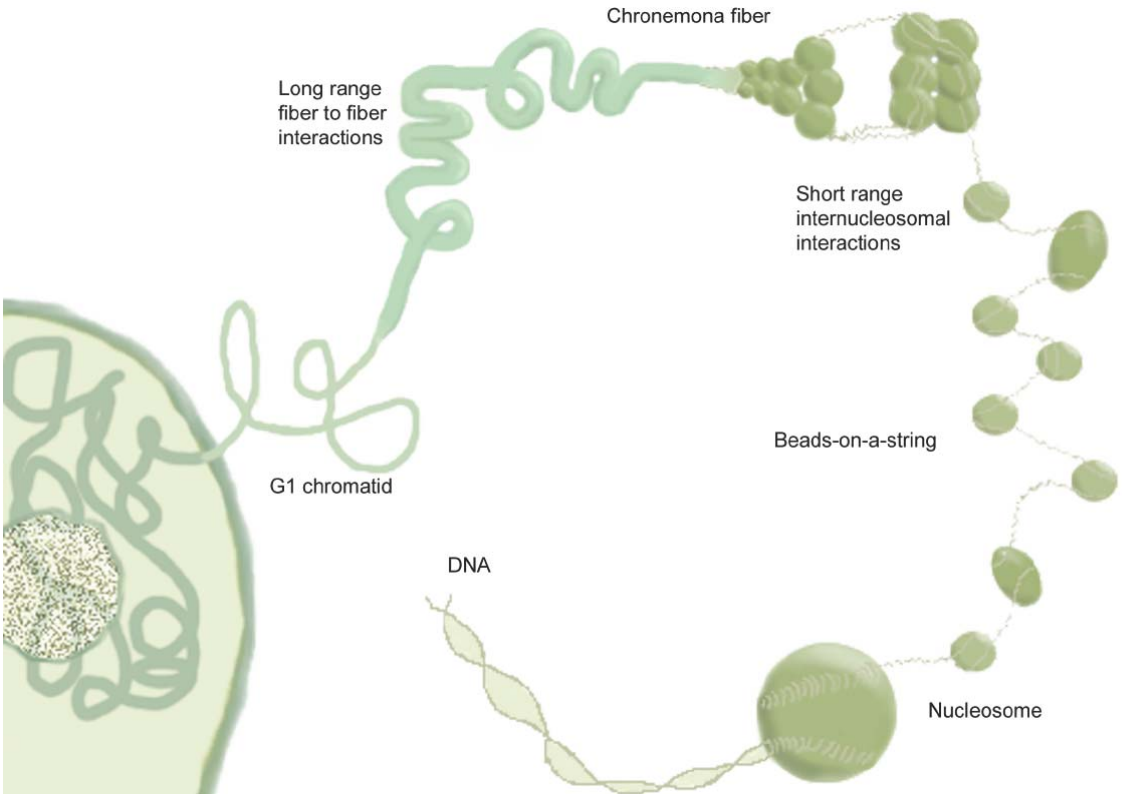

Figure 3.4. A schematic presentation of chromatin components and topology.

Upon transcriptional activation, the DNA strand with the activated gene can loop so that the gene will be present in nuclear locations enriched in the enzyme RNA polymerase and the larger transcriptional machinery, known as *transcription factories*. Gene promoters have been observed to be in close physical contact with enhancer elements upon transcription. Thus, intricate networks of DNA strands, their regulatory elements and the transcription factories will form during gene transcription. Thus, positional effects of genes in a genome can be conceived of as important for gene regulation and function.

Chromatin is also subject to a diverse array of chemical modifications that can regulate access and transcriptional activity of the underlying DNA.[13] Introduction of methyl-groups (methylation) is very common at specific DNA locations in most organisms. Methylation causes two major effects: 1) to displace transcription factors that normally bind to the DNA, and 2) to attract methyl binding proteins that are

[13]The complete DNA strand in a chromosome, called the chromatin fiber, is composed of multiple specialized domains, each of which contains a distinct subset of proteins such as nucleosomes, linker histone variants and non-histone proteins.

functionally associated with gene silencing. DNA methylation is discussed in more detail in Chapter 5.

5. Synthesis and conclusions

What can we surmise from the information on complex regulatory networks governing gene expression presented here? The dynamic changes taking place within a eukaryotic genome, and the dynamic interplay between the genome and its outside world, is slowly coming to light (Leitch, 2007). Clearly, contemporary science is evolving a picture of the genome and its regulation that is much different from the reductionist paradigm (DNA-RNA-protein) that has guided the biological understanding of DNA function over the last half century (including the foundational basis of genetic engineering). It is important to recognize that much of what is presented in this chapter is a conceptually and mechanistically framed understanding of genes and genome developed independently of the environmental or biological *context*. By placing the genes (DNA and downstream regulatory processes) in context, new layers of information coming from both within and outside the cell, influence these fundamental processes of gene activity. As a result, the prevailing paradigm of a mechanistically and deterministically defined gene regulation that forms the conceptual basis of genetic engineering is now widely understood to be invalid. Such a static view of transgenes as inert to their genomic and regulatory context needs to be revised not only in theory, but also in practical terms. This is particularly germane to developing scientifically sound GMO products and policies. Currently, significant levels of uncertainty and gaps in knowledge in the behaviour of transgene expression in the GMO itself require greater investments into biosafety research in order to assure their safe use.

We have yet to develop models, concepts and metaphors that can inform us about how this molecular orchestrating comes about. If the organism is not caused by its molecular parts, but these parts themselves are orchestrated in concert by the organism and ecosystem as a whole, there are a lot of concepts and thinking habits that have to be reconstructed. The field of ‘systems biology’ attempts to take a more holistic approach to understanding organismal gene expression and development. A revised view of the factors governing gene expression and, hence, organismal properties will also impact the fundamental rationale of genetic engineering; namely the Central Dogma inspired idea that the organism can be precisely controlled by the engineer (e.g. adding one gene for the addition of one single trait, without further genomic effects).

The Central Dogma represents the guiding idea underlying genetic engineering. This idea was conceptualized some 35 years ago when understanding of gene expression and function was in its infancy. The Central Dogma does not deal with the complex interactions leading to protein production as we observe them today. *It seems now more relevant to think of genes as the tools of the organism, rather than as the cause of the organism.*

We observe from genetic engineering that the introduction of a new gene into a new host or into a new location in the genome of the same hosts can:

- Significantly alter the phenotype of the host organism beyond what is expected from the inserted/moved trait. This can, for instance, occur by up-regulation or down-regulation of production and chemical composition of unrelated gene products.

- Result in one or more proteins different from the protein produced in the original organism (from where the gene was found).

These changes can occur in GMOs without the genetic engineers being able to *a priori* predict the outcome. Thus, the multiple levels of environmental and cellular interactions guiding gene expression and protein functionality are not represented in the narrow interpretation of the Central Dogma. The relevant questions guiding further development, and investigation, of the safety and monitoring of GM crops in the environment require an adoption of a more holistic concept of (trans)genes in their new contexts. A critical analysis of the concepts, methods and paradigmatic models of thinking that have predominated the field of transgene biology is required. The emerging new holistic methods and models in transgene biology will not replace the reductionistic approaches, but will complement them with the multidimensional interactions between genomes and their environments. With this broader understanding, we can start asking important biosafety questions that include context and changing conditions.

How are the GMO, transgene expression and recombinant protein compositions affected when an organism is put into new organismal and environmental contexts? How do these multi-scale changes interact? These basic questions require a methodological approach that considers all levels of biological organization and ecological interactions.

What this chapter aims to illustrate is that the simplistic, unidirectional and deterministic cause-and-effect understandings gene expression, which forms the basis for genetic engineering, has become scientifically invalid. The connection between genotype and phenotype is not solely determined by DNA, but is dependent on a multilayered informational network of, for example, proteins, RNA, genomes, and environmental stimuli, all of which are context dependent, and their outcome cannot yet be predicted *a priori*. With this in mind, it can be seen that *a single gene delivers only part of the identity and function of a protein*. In this connection it has to be remembered that the development of the first generation of GM plants was based on the knowledge of the 1970s and 1980s.

Lastly, the random insertion of foreign genes into an organism during transgenesis does not comprehend the importance or effect of the organizational placement and interacting factors upon the inserted gene, nor its long-term implications for the host cell, environment, or interacting species. Genes, including transgenes, are not autonomous units and should not be treated as such in a scientific or even regulatory sense. For example, the complex pathways to protein synthesis discussed mean that a number of different recombinant proteins may be produced from the same transgene, leading to changes in allergenic potential, target bioactivity, or influence on host biochemical composition, function and survival. Currently, there is little data and research that shed light on these important processes as they occur in GMOs. The

research field of gene ecology seeks to address these fundamental knowledge gaps to improve the biological understanding of, and hence, the safety of GMOs.

Some biosafety issues related to transgenic organisms are further discussed and exemplified in Chapters 8–14.

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Chapter 4
Genetic Engineering of Living Cells and Organisms

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Genetic engineering (GE) by *transgenesis* has three main application areas: medicine, agriculture and bioremediation of the environment. In all three areas, transgenic crop plants, livestock, microorganisms and viruses are used. In the future we will increasingly be confronted also with transgenic trees, insects, fish species, and viruses. A development towards multi-transgenic, 'stacked', constructs is anticipated. Finally, multi-transgenic organisms based on nanobiotechnology, RNAi technology and 'synthetic biology', used separately or in combinations, may become realities.

This chapter provides a broad overview of strategies and techniques that are being used, or will be used in the near future, to produce transgenic organisms. This chapter is structured according to the following outline:

1. The processes involved in making a genetically modified organism
- 1.1 General strategies for making a GMO
- 1.2 Sources of transgenes
- 1.3 Vector construction for gene transfer into higher organisms: General aspects

2. Insertion of genes into plants

3. Insertion of genes into animals

4. Insertion of genes into microorganisms

5. Location of the inserted genes

6. Future prospects of gene transfer methodologies

1. The processes involved in making a genetically modified organism

1.1 General strategies for making a GMO

A number of strategies for physical transfer of DNA into cells are available. Some of these are generally applicable, while others are only feasible for cells from specific sources. The strategies and approaches used are often collectively termed *recombinant DNA technology.* The term comprises an arsenal of laboratory methods used to identify and isolate a DNA fragment from one organism, insert it into a vector and transfer the vector-insert combination into a host cell. The vector is often a bacterial *plasmid*. The process would not be possible without «biological scissors», i.e. *enzymes (restriction endonucleases)* that reproducibly cleave DNA molecules into fragments of defined sizes. Furthermore, the process requires 'biological glue', i.e. enzymes called *ligases*, to join the insert and vector together. A generic gene cloning process may be divided into some general steps as illustrated in Figure 4.1.

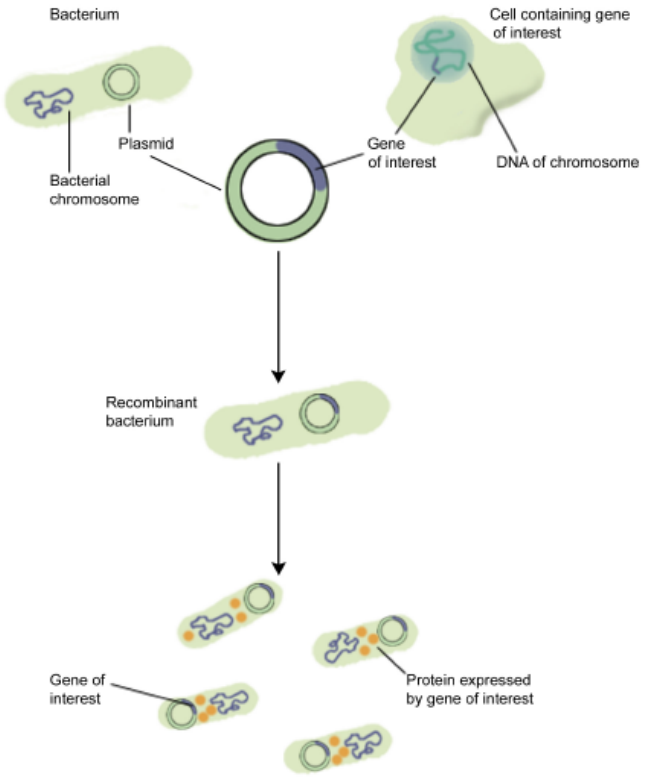

Figure 4.1. Simplified outline of DNA cloning and gene expression in bacteria.

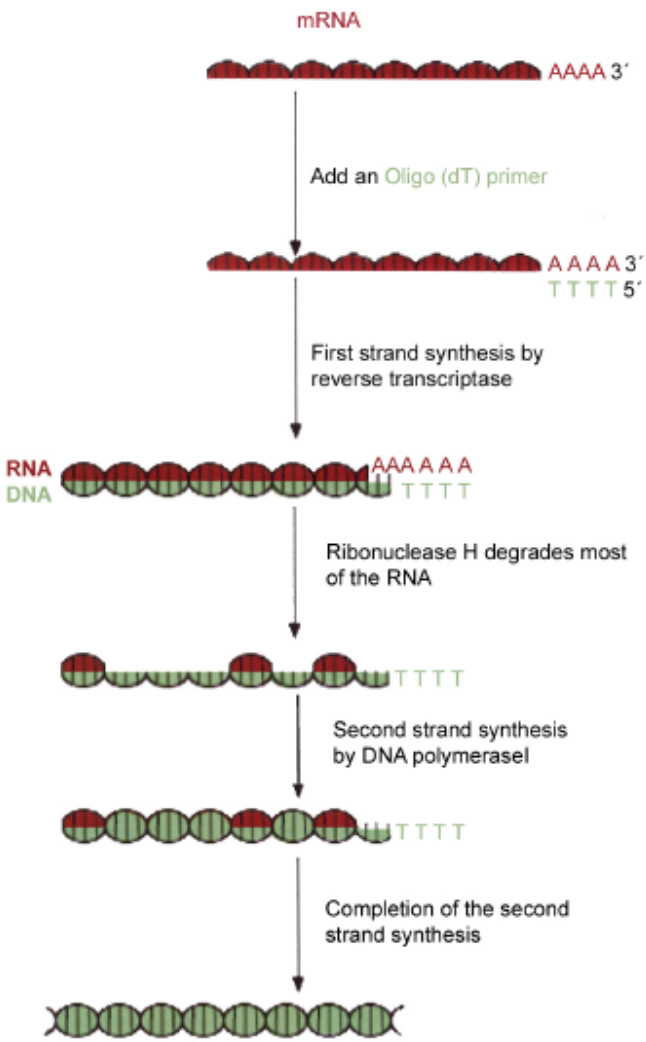

Figure 4.2. Outline of a reverse transcription process.

It is important to acknowledge that, due to the presence of introns, many eukaryotic genes are too large for direct cloning into bacterial vectors. Hence, cloning of eukaryotic genes is commonly initiated by isolation of the corresponding already processed mRNAs. The mRNA is then reversely transcribed into a double-stranded *cDNA* (complementary DNA). This is achieved by a *reverse transcriptase enzyme* making the first strand and a *DNA polymerase* making the second strand. The process is outlined in Fig. 4.2. The completed cDNA contains the open reading frame necessary for expressing the protein originally encoded in the genomic DNA (with the introns).

Table 4.1 The general steps involved in a generic gene cloning process

Gene isolation and excision	The DNA or mRNA is isolated from an organism that contains the target gene (e.g. a Bt toxin (*cry*) gene from *Bacillus thuringiensis*). In the case of DNA it is cut with a restriction endonuclease.
Vector preparation	The chosen DNA cloning vector is cut with the same restriction endonuclease.
Ligation	The two DNA samples are pasted together by a *DNA ligase* to produce *recombined molecules*.
Transformation with the vector	*E. coli* cells are transformed with the combined DNA molecules from the ligase reaction to produce cells that carry the target gene-vector recombinant molecules. The vector contains a DNA sequence, *origin of replication (ori)*, that enables it to be replicated in *E. coli*, and hence the recombinant molecules may be replicated into a high number of copies. Uptake of DNA in *E. coli* may be facilitated by a number of procedures, e.g. $CaCl_2$ – heat shock treatment or electroporation.
Marker and target gene expression	*E. coli* cells containing the vector, and hence the target gene, are selected on the basis of an *antibiotic resistance (AR) gene* which is an integral part of the vector. When the corresponding antibiotic (e.g. ampicillin, kanamycin or neomycin) is added, only cells containing the recombinant vector molecules will survive the treatment.

1.2 Sources of transgenes

Any kind of organism may be a source of useful transgenes. Transgenes already in commercial use have been taken from viruses, bacteria, plants, and animals of various kinds.
The arrival of *Synthetic Biology* (see Section 6 of this Chapter) has created new potential opportunities to obtain useful genetic material for GE of organisms. It is now feasible to synthesize tailored versions of any gene, gene cluster or promoter.

1.3 Vector construction for gene transfer into higher organisms: general aspects

The introduction of foreign DNA into bacterial or yeast cells is called *transformation*. In animal cells the term *transfection* is used for the same process, in order to avoid confusion. The reason for this is that transformation refers to phenotypic changes taking place when cells are underway from being normal to becoming malignant cancer cells. For plant cells, both designations may be used. In the GE context, transformation and transfection relates to the same phenomena: inherited changes that are due to the introduction of foreign, exogenous DNA.

The process of expression vector construction is based on the same methods and tools as cloning vector construction. In principle, eukaryotic expression vectors do not differ from their prokaryotic counterparts. A basic eukaryotic expression vector must contain:

i) A eukaryotic promoter that secures the transcription of the transgene;
ii) A multiclonic site (MCS), i.e. a DNA sequence composed of recognition motifs for a number of restriction endonucleases;
iii) Eukaryotic transcriptional and translational stop signals;
iv) A DNA sequence that enables polyadenylation of the mRNA;

v) A selectable eukaryotic marker gene. The target gene can undergo a series of additions (e.g. insertion of specific promoter-intron combinations), deletions (of unwanted introns or codons), or other modifications (DNA sequence changes for preferential codon usage) to optimize for expression in the desired host.

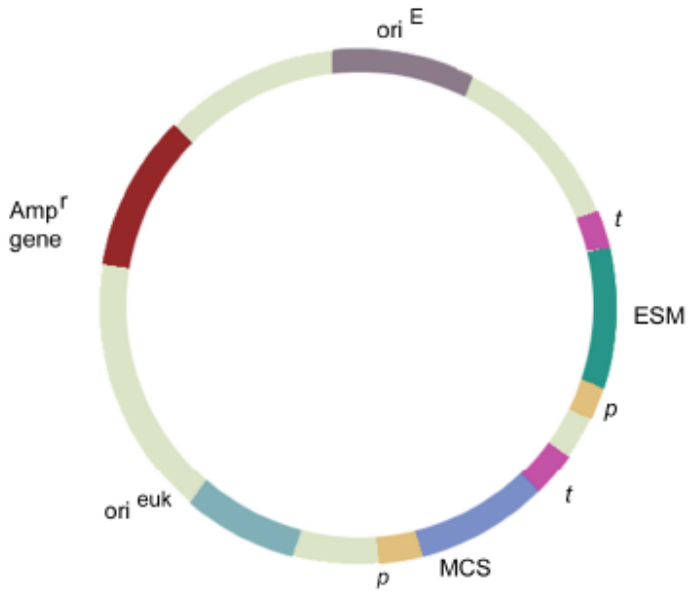

Figure 4.3. Outline of an eukaryotic expression vector. The major features are: promoter (p); the multiple cloning site (MCS) for a transgene, polyadenylation and termination signals (t); eukaryotic selectable marker gene (ESM); eukaryotic origin of replication (ori^{euk}); E. coli origin of replication (ori^{E}); an E. coli selectable marker gene (Amp^{r}).

Most eukaryotic vectors are shuttle vectors with two origin of replications and two selectable marker genes. One set functions in *E. coli*, the other in the chosen eukaryotic host cell. An outline of a generic eukaryotic expression vector is given in Fig. 4.3. It is important to emphasize that in real life there is no such thing as 'a generic expression vector'. All the elements inserted into a eukaryotic expression vector are carefully selected to be optimally functional in the target species and cell type of choice.

2. Insertion of genes into plants

Crop plants

There are three major reasons for developing transgenic plants. First, the addition of a gene(s) may improve the agricultural, horticultural, or ornamental value of a crop plant. Second, transgenic plants can act as living bioreactors for the inexpensive production of economically important proteins or metabolites. Third, plant genetic transformation (*transgenesis*) provides a powerful means for studying the action of genes during development and other biological processes.

Some of the traits that can be introduced into plants by a single gene construct or, possibly, a small cluster of gene constructs include: insecticidal activity, protection against viral infection, resistance to herbicides, protection against pathogenic fungi and bacteria, delay of senescence, tolerance of environmental stresses, altered flower pigmentation, improved nutritional quality of seed proteins, increased post-harvest shelf life, as well as self-incompatibility and male sterility and seed sterility. In addition, transgenic plants can be made to produce a variety of useful compounds, including therapeutic agents, polymers, and diagnostic tools such as antibody fragments. Alternatively, they can be engineered to synthesize viral antigenic determinants and, after ingestion, can be used as edible vaccines.

To date, over 140 different plant species have been genetically transformed, including many crop and forest species. Plant GE may have a big impact on plant breeding programmes because it promises to significantly reduce the 10 to 15 years that it takes to develop a new variety using traditional plant breeding techniques. Genetically modified (GM) plants are now prevalent in parts of the world and appear in processed food products worldwide.

Forest trees

The demand for wood is expected to grow by 20% in the next decade, while the world's forest cover declines at an annual rate of 9.4 million hectares, an area comparable to that of Portugal.[1] Breeding of trees is a slow process, partly due to their long generation time. Hence, it is conceivable that the utilization of transgenic trees or marker-assisted breeding may alleviate the gloomy prospects of the present.

Genomic sequencing projects and genome mappings have opened the road to transgenesis for several tree species, such as birch, pine, eucalyptus, spruce, oak, and acacia. The genus *Populus* (poplars) has been adopted as the model of choice due to advantages such as fast growth, amenability to tissue culture and genetic transformation, and a small genome (approximately 500 Mega base pairs).[2] Only China has reported the commercial release of transgenic poplar. In 2004 approximately 1.4 million insect-resistant trees were planted on 300–500 hectares. Insect resistance was achieved by transgenesis of *cry* genes from *Bacillus thuringiensis* (FAO, 2004). In the scientific community, GE of forest trees is considered an important avenue to domestication and increased yields. One of the arguments is that GE circumvents the long generation times that are typical for most forest trees. Most efforts so far have been devoted to improve lignin extraction during pulping. However, there have also been published promising results related to pathogen and pest resistance, bioremediation, acceleration and prevention of flowering, and herbicide resistance (for a review, see Boerjan, 2005). Most transgenic forest treelines obtained so far are derived from transformation of somatic embryonal tissues (somatic embryos) via co-cultivation with *Agrobacterium tumefaciens* (see below).

Fruit trees

Regeneration and transformation systems using mature plant material of woody fruit species have been achieved as a necessary requirement for trangenesis of cultivars. Once a useful transformant is isolated, unlimited production of the desired transgenic line can be achieved by *vegetative propagation*, the normal method of multiplying fruit trees. The only transgenic fruit tree being commercially produced at present is papaya (*Carica papaya*) resistant to PRSV (Papaya ringspot virus). In this case transformation was achieved by micoprojectile bombardment. More

[1](http://www.fao.org/forestry/site/28679/en/, accessed June 11, 2007).

[2]In addition to their value for wood products, members of the genus Populus provide a range of ecological services, including carbon sequestration, bioremediation, nutrient cycling, biofiltration, and providing diverse habitats, and this is the case for many other forest trees as well.

commonly, however, DNA has been transferred to fruit trees by disarmed and transgenic *Agrobacterium* strains. Recent overviews of transgenes and intended traits in fruit trees are now available (e.g. Petri & Burgos, 2005).

Vector considerations
There are a number of DNA delivery systems and expression vectors that work with a range of plant cells. Furthermore, most plant cells are *totipotent* – meaning that an entire plant can be regenerated from a single plant cell – so fertile plants that carry an introduced gene(s) in all cells (i.e. transgenic plants) can often be produced from genetically engineered tissue cultures. If the developed transgenic plant flowers and produces viable seed, the desired trait is passed on to successive generations.

Transformation with the Ti plasmid of Agrobacterium tumefaciens
The soil bacterium *A. tumefaciens* is a phytopathogen that, as a normal part of its natural life cycle, genetically transforms plant cells. This genetic transformation leads to the formation of crown gall tumours, which interfere with the normal growth of an infected plant. This agronomically important disease affects only *dicotyledonous plants (dicots),* including grapes, stone-fruit trees (e.g. peaches), and roses.

Crown gall formation is the consequence of the transfer, integration and expression of genes of a specific segment of bacterial plasmid DNA – called the T-DNA (transferred DNA) – into the plant cell genome. The T-DNA is actually part of the 'tumour-inducing' (Ti) plasmid that is carried by most strains of *A. tumefaciens*. Depending on the bacterial strain that is host to the Ti plasmid, the length of the T-DNA region can vary from approximately 12 to 24 kilobase pairs (kbp).

Ti Plasmid-Derived Vector Systems
The simplest way to exploit the ability of the Ti plasmid to genetically transform plants is to insert the desired recombinant DNA sequence into the T-DNA region and then use the Ti plasmid and *A. tumefaciens* to deliver and insert this gene(s) into the genome of a susceptible plant cell. Although the Ti plasmids are effective as natural gene transfer vectors, they have several serious limitations as routine cloning vectors.

First, it is not possible to regenerate transformed cells into mature crop plants without prior removal of some genes contained in the Ti plasmid.
Second, naturally-occurring Ti plasmids are large (approximately 200–800 kb). For recombinant DNA experiments, however, a much smaller version is preferred, so large segments of DNA that are not essential for the cloning vector purposes are removed.
Third, because the Ti plasmid does not replicate in the bacterium *Escherichia coli,* the convenience of perpetuating and manipulating Ti plasmids carrying inserted DNA sequences in this laboratory bacterium does not exist. Therefore, in Ti plasmid-based vectors, an origin of replication that can be used in *E. coli* is added.
To overcome these constraints, recombinant DNA technology has been used to create a number of Ti plasmid-based vectors. These vectors are similarly organized and contain the following components:

(i) A selectable marker gene, such as the antibiotic resistance gene neomycin phosphotransferase (*nptII*), that confers kanamycin resistance to transformed plant cells. Because *nptII*, as well as many of the other marker genes used in plant transformation, is prokaryotic in origin, it is necessary to put it under the control of plant (eukaryotic) transcriptional regulation signals, including both a promoter and a

termination/polyadenylation sequence, to ensure that it is efficiently expressed in transformed plant cells.

(ii) An origin of DNA replication that allows the plasmid to replicate in *E. coli*. In some vectors, an origin of replication that functions in *A. tumefaciens* has also been added.

(iii) The right border sequence of the T-DNA region. This region is absolutely required for T-DNA integration into plant cell DNA, although most cloning vectors include both a right and a left border sequence.

(iv) A polylinker (MCS, multiple cloning site) to facilitate insertion of the cloned gene into the region between T-DNA border sequences.

Based on these alterations, a number of different Ti-plasmid based constructs have been used to bring recombinant genes into plant cell cultures from which mature plants may be regenerated. Two examples of such constructs are given in Fig. 4.4. Further developments include Ti-plasmid constructs designed to give recombinant gene expression in mitochondria or chloroplasts (see Section 6 of this chapter). The Ti-plasmids are propagated in *E. coli* before being transferred to *A. tumefaciens* for transformation of plant cell cultures.

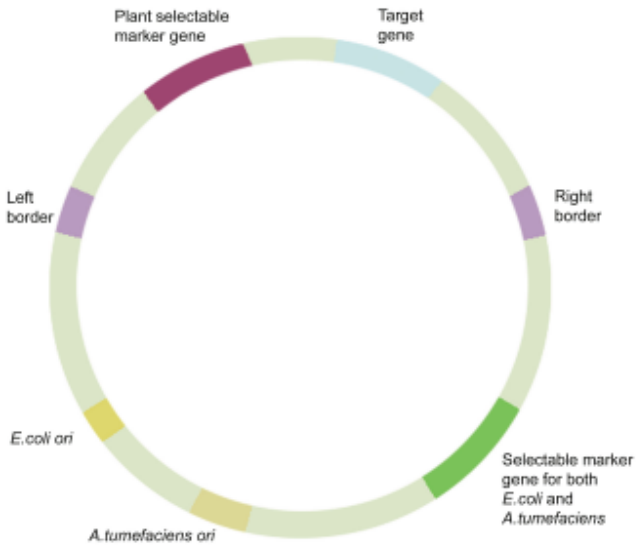

Figure 4.4. A cloning vector derived from a Ti plasmid. This binary vector has origins of DNA replication (ori) for both E. coli and A. tumefaciens. A bacterial selectable marker gene can be used in both hosts. Both the transgene and the plant selectable marker gene are inserted between the T-DNA left and right borders.

Gene delivery methods

Although *A. tumefaciens*-mediated gene transfer systems are effective in several species, *monocotyledonous plants (monocots)*, including the world's major cereal crops (rice, wheat and maize), are not readily transformed by *A. tumefaciens*. However, by refining and carefully controlling conditions, protocols have been devised for the transformation of maize (corn) and rice by *A. tumefaciens* carrying Ti plasmid vectors. Many of the early plant transformation experiments were conducted with limited-host-range strains of *Agrobacterium*. However, more recently, wide-host-range strains that infect most plants have been tested and found to be effective, so many of the plant species that previously appeared to be refractory to transformation

by *A. tumefaciens* can now be readily transformed. Thus, when setting out to transform a new plant species, it is necessary to determine which *Agrobacterium* strain and Ti plasmid are best suited to that particular plant.

When the difficulties in transforming some plant species first became apparent, a number of procedures that could act as alternatives to transformation by *A. tumefaciens* were developed. Several of these methods require the removal of the plant cell wall to form *protoplasts*. Plant protoplasts can be maintained in culture as independently growing cells, or, with a specific culture medium, new cell walls can be formed and whole plants can be regenerated. In addition, transformation methods have been developed that introduce cloned genes into a small number of cells of a plant tissue from which whole plants can be formed, thereby bypassing the need for regeneration from a protoplast. At present, most researchers favour the use of either Ti plasmid-based vectors or microprojectile bombardment to deliver DNA into plant cells.

Microprojectile bombardment (also called *biolistics*), is the most important alternative to Ti plasmid DNA delivery systems for plants. Gold or tungsten spherical particles (approximately 0.4 to 1.2 micrometers (μm) in diameter or about the size of some bacterial cells) are coated with DNA that has been precipitated (with $CaC1_2$, spermidine, or polyethylene glycol). The coated particles are then accelerated to high speed (300 to 600 metres/second) with a special apparatus called a *particle gun (or 'gene gun')*. The original version of the gene gun used a small amount of gunpowder to provide the propelling force. The device that is currently used employs high-pressure helium as the source of particle propulsion (Fig. 4.5). The projectiles can penetrate plant cell walls and membranes; however, the particle density used does not significantly damage the cells. The extent of particle penetration into the target plant cells may be controlled by varying the intensity of the explosive burst; altering the distance that the particles must travel before reaching the target cells, or using different-sized particles.

Once inside a cell, the DNA is detached from the particles and, in some cells, integrates into the plant DNA. Microprojectile bombardment can be used to introduce foreign DNA into plant cell suspensions, callus cultures, meristematic tissues, immature embryos, protocorms, coleoptiles, and pollen in a wide range of different plants, including monocots and conifers, plants that are less susceptible to *Agrobacterium*-mediated DNA transfer. Furthermore, this method has also been used to deliver genes into chloroplasts and mitochondria, thereby opening up the possibility of introducing exogenous (foreign) genes into these organelles.

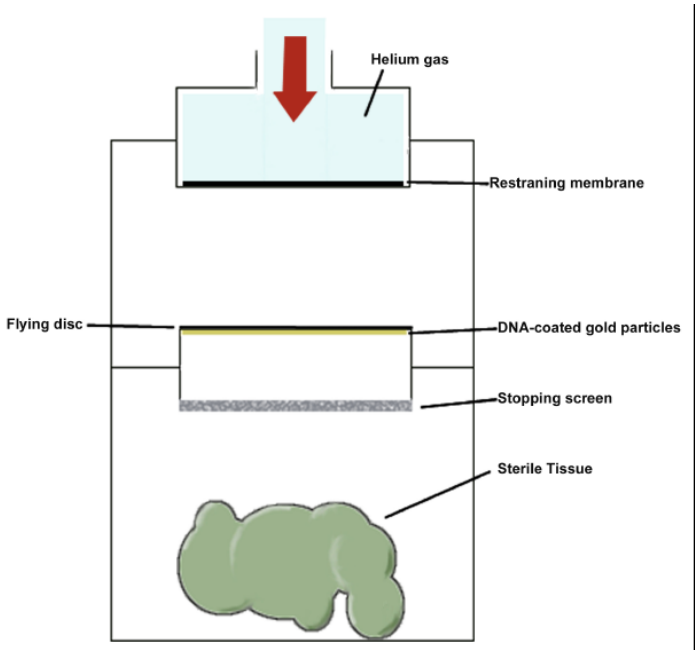

Figure 4.5. Schematic representation of a 'gene gun' (microprojectile bombardment apparatus). The plastic restraining membrane bursts when the helium pressure reaches a certain point. The released gas then accelerates the flying disk containing the DNA-coated gold particles on its lower side. The gold particles pass the stopping screen, while the flying disc is held back, and penetrate the cells of the sterile tissue.

Typically, plasmid DNA dissolved in a buffer is precipitated onto the surface of the microprojectiles. Using this procedure, it is possible to increase the transformation frequency by increasing the amount of plasmid DNA; however, too much plasmid DNA can be inhibitory. It is estimated that there are approximately 10,000 transformed cells formed per bombardment. With this technique, cells that appear to be transformed, based on the expression of a marker gene, often only transiently express the introduced DNA. Unless the DNA becomes incorporated into the genome of the plant, the foreign DNA may be degraded eventually.

The configuration of the vector that is used for biolistic delivery of foreign genes to plants influences both the integration and expression of those genes. For example, transformation is more efficient when linear rather than circular DNA is used. Moreover, large plasmids (>10 kb), in contrast to small ones, may become fragmented during microprojectile bombardment and therefore produce lower levels of foreign gene expression. However, large segments of DNA may be introduced into plants using *yeast artificial chromosomes (YACs)*. The YACs were engineered to contain plant selectable markers as well as yeast selectable markers. In a number of experiments the presence of distantly situated plant selectable marker genes in transformed plant cells indicates that the entire YAC, along with the entire inserted foreign DNA, was probably transferred. DNA hybridization experiments revealed that YACs up to 150 kbp in total size have a good chance of being stably integrated into the plant cell.

Use of reporter genes in transformed plant cells

It is essential to be able to detect the recombinant DNA that has been integrated into the plant genome so that those cells that have been transformed and are expressing the vector gene cassette can be identified. Furthermore, in studies of plant transcriptional regulatory signals and the functioning of these signals in specific plant tissues (such as leaves, roots and flowers), it is often important to be able to quantify the level of expression of a gene with a readily identified product.

Quantification and other applications require the use of *reporter genes* that either permit transformed cells to be selected or encode an activity that can be assayed. To these ends, a number of different genes have been tested as reporters for transformation, including genes that can be used as dominant selectable markers and genes whose proteins produce a detectable response to a specific assay.

Many of these reporter genes are from bacteria and have been equipped with plant-specific regulatory sequences for expression in plant cells. Dominant marker selection provides a direct means of obtaining only transformed cells in culture. For example, in the presence of the antibiotic kanamycin, only plant cells with a selectable marker gene *nptII* gene can grow. The inclusion of marker genes encoding antibiotic resistance in transgenic plants has raised concerns. The antibiotic resistance genes that are used as selectable markers might be transferred to pathogenic soil or gut microorganisms. Moreover, it is possible that the products of some marker genes might be either toxic or allergenic. The presence of some reporter genes and their products may limit the market potential of the commercial product. To allay these concerns, strategies for the production of transgenic plants without any marker genes have been developed (Darbani et al., 2007).

Gene expression considerations

When genetic transformation of crop plants became routine, research efforts were directed toward introducing a wide range of recombinant plant and bacterial genes into plants. The transformed plants were assayed for the production of the foreign protein and studied physiologically to assess how the presence of an additional, novel protein affected the whole plant. Many of these early experiments utilized promoters that were expressed constitutively (i.e. they were always 'on', and not regulated) in a range of plant cells. More recently, many additional plant promoters have been isolated and characterized, and used to express foreign proteins in specific cells at certain times during the growth and development of the plant. For example, instead of the strong constitutive 35S promoter from cauliflower mosaic virus (*35S CaMV promoter*), which is expressed in all plant tissues and throughout the life of the plant, researchers have used the promoter for the small subunit of the photosynthetic enzyme ribulosebisphosphate carboxylase, which is active only in photosynthetic tissues such as leaves. Similarly, plant promoters active only in specific tissues, such as roots or flowers, or only during periods of environmental stress (e.g. the pathogenesis-related promoters), or in the presence of chemical inducers, have been used to control the expression of some foreign genes.

The level of expression of a foreign protein under the control of the 35S CaMV promoter is often lower than desired. To address this problem, it is necessary to test different promoter/gene constructs in plants to see if more effective promoters can be found.[3] In addition to the promoter, several other elements may enhance foreign gene expression. These include enhancer sequences (found from one to several hundred nucleotides upstream of the promoter sequence), introns (that may stabilize messenger RNA), and transcription terminator sequences (see Chapter 3 for further details).

[3]In one series of experiments, recombinant DNA constructs that contained all or some of the following elements were tested: the 35S promoter, the nopaline synthase gene transcription terminator, from one to seven tandemly repeated enhancer elements, and a DNA sequence from tobacco mosaic virus called (omega) that increases gene expression at the translational level. The most active construct contained seven enhancer elements and directed a much higher level of foreign gene expression in both transgenic tobacco and rice plants than when the 35S promoter alone was used. These promoter constructs directed a wide range of foreign gene expression in different transgenic plant lines. This variation is probably due to the site within the plant genome where the T-DNA is inserted. Nevertheless, this work shows that it is possible to engineer promoters that are much stronger than the naturally occurring 35S promoter. With this approach, it should be possible to engineer promoters that are tissue specific, developmentally regulated, and robust.

3. Insertion of genes into animals

Applications for transgenic animals

As livestock animals and their products constitute a major factor in human nutrition, the purposeful genetic modification of livestock animals has special implications. Any additional safety risks introduced by the genome modification, whether real or only perceived, are highly unlikely to be accepted by either the regulatory authorities or consumers. Traditional GE approaches involve new recombination events between unrelated DNA sequences, something which has been considered as a potential risk and is currently limiting the acceptability of the technology.

The production of transgenic animals has focused mainly on producing models, for instance in the mouse, for basic and medical research. In terms of commercially important livestock species, work has revolved around specialized non-agricultural purposes such as pharmaceutical production and xenotransplantation. To a lesser extent, agricultural applications to improve animal production traits and food quality have also been pursued. The first reports of transgenic livestock came in the 1980s, and focused on introducing growth-promoting genes into pigs and sheep. The present century, however, has already seen transgenic swine (EnviroPigs) carrying a bacterial phytase gene driven by a salivary gland-specific promoter. Phytase breaks down phosphates in the pigs' feed, reducing phosphorus excretion in the manure by up to 75%, and thus reduces environmental pollution (Golvan et al., 2001).

In spite of the low efficiency of the microinjection methods, a number of transgenic livestock have already been established, e.g. pigs with growth hormone transgenes and sheep with keratin-IF-I transgenes for improved wool quality. 'Transgenic animal bioreactors' are based on the fact that animal cells are required to synthesize proteins with the *appropriate post-translational modifications* (see Chapter 3). Transgenic animals are being used for this purpose. Milk, egg white, blood, urine, seminal plasma, and silk worm cocoons are candidates for the sources of recombinant proteins at an industrial scale.

Transgenesis to engineer disease-resistant livestock is another goal pursued. Mastitis (mammary gland infections) costs the US dairy industry approximately USD 2 million annually, and has a similar impact in Europe. *Staphylococcus aureus* is a major mastitis pathogen, and it is highly sensitive to lysostaphin. Lysostaphin-transgenic cattle, expressing the antimicrobial peptide in their mammary epithelium, excrete the product in their milk. Transgenic cows resist *S. aureus* mammary gland infections, and their milk kills the bacteria in a dose dependent manner (Wall et al., 2005).

The process of evaluating transgenic pigs as potential donors for *xenotransplantation* to humans involves a number of complex steps. It is one of the most widely discussed applications of transgenesis and cloning, although it does not seem to be a viable choice in the near future (Vajta & Gjerris, 2006).

Transgenic chickens could be used to improve the genetic make-up of existing strains with respect to built-in (in vivo) resistance to viral, bacterial and coccidial diseases, better feed efficiency, lower fat and cholesterol levels in eggs, and better meat quality. Avian researchers have also suggested that the egg, with its high protein content, could be used as a source of pharmaceutical proteins. By analogy to the mammary glands of livestock, the expression of a transgene in the cells of the reproductive tract of a hen that normally secretes large amounts of ovalbumin could lead to the accumulation of a transgene-derived protein that becomes encased in the eggshell. The recombinant protein could either be fractionated from these sterile packages or

consumed as a nutraceutical with breakfast. The expected annual yield of recombinant protein from one hen is 0.25 kg. Currently, as 'proof-of-principle' experiments, transgenic chickens that synthesize monoclonal antibodies, growth hormones, insulin, human serum albumin, and alpha-interferon have been created. Production of germ line transgenic chickens has also been achieved by using a retrovirus-based vector (Koo et al., 2006).

Vector and gene delivery considerations
Microinjection of foreign DNA (*expression plasmids*, see Section 1.3) into pronuclei of zygotes has been the method of choice for the production of transgenic domestic animals. This method is simple, but very inefficient because
(i) a large number of embryos are lost and
(ii) gene transfer rates are very low.

The low transgenesis rates result in enormous production costs. A transgenic cow would come with a price tag of at least USD 300,000 (Wells et al., 1999).

Following microinjection *the transgene is randomly integrated into the host genome*, which can be associated with insertional mutagenesis, unpredictable expression levels of the transgene and unwanted pathological side effects. However, a systematic analysis of potential pathological side effects putatively associated with the random integration and expression of a specific transgene in transgenic domestic animals has not yet been reported (Deppenmeier et al., 2006).

The need for a better method of livestock transgenesis was a major driving force behind the development of *nuclear transfer technology* that led to the generation of Dolly the sheep. In the following years, methods to introduce transgenes into the germ line of various animal species were presented, but they were often too inefficient and costly for practical applications. *Nuclear transfer (cloning)* is a possible way to generate transgenic animals in different species. However, this approach is both difficult and burdened by extremely high failure rates. The vast majority of clones die at various stages of development or shortly after birth. This phenomenon has been termed 'cloned offspring syndrome', and seems to be due to faulty e*pigenetic programming* of the donor genome (Vajta & Gjerris, 2006).

Hence, the use of *lentivirus vectors* for introduction of transgenes into the germ line (see the following) was a major breakthrough, which now seems to make production of transgenic livestock for agricultural and medical purposes feasible (Maga, 2005).

Viral vectors and delivery vehicles
Viral vectors can be divided into two groups according to the basic life cycles of their parental viruses. They are either *non-integrating* or *integrating*. Only the latter can be used for transgenesis, because the genomes of the former would be lost during the cell divisions of early embryonic development.

The majority of available integrating viral vectors are based on representatives of the large family *Retroviridae*. *Lentiviruses*[4] belong to this family. Recently, a lot of attention and effort has been focused on the construction of non-integrating expression vectors, but the most promising vector systems at present seem to be based on various lentivirus-based constructs (Jackson et al., 2006;

[4]Lentiviruses have been isolated from sheep (visna/maedi virus), goats (caprine arthritis encephalitis virus), cattle (bovine immunodeficiency virus), horses (equine infectious anemia virus, EIAV), cats (feline immunodeficiency virus), monkeys (simian immunodeficiency virus), and humans (human immunodeficiency virus, HIV). The best studied example of a lentivirus is HIV.

Vajta & Gjerris, 2006.) All retroviruses contain single-stranded RNA genomes. They carry with them a viral enzyme, reverse transcriptase, which transcribes the RNA genome into a DNA copy that is made double stranded (ds) by a DNA polymerase. During infection of host cells, the dsDNA is integrated into the host genome as a provirus, and serves as a template for the production of progeny virus particles.

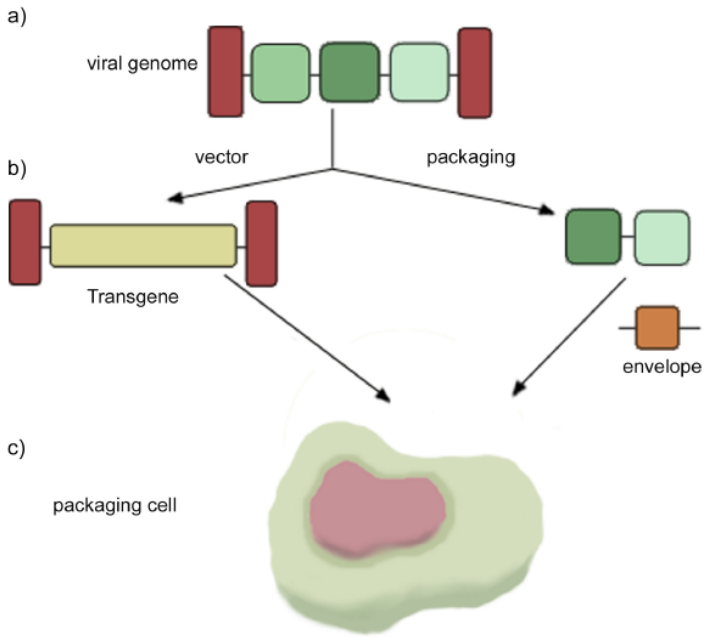

Figure 4.6. The basic principle of lentivirus vector construction. (a) Lentivirus genome depicting viral genes surrounded by LTRs (long terminal repeats) with their promoter and other cis-acting elements. (b) Division of the viral genome into a vector, containing the transgene, and a packaging unit. A gene expressing a suitable envelope (env) protein is supplied to broaden the spectrum of cells that accept vector infection. The packaging cells (c) express the viral proteins necessary for production of infectious virus particles in trans.

The basic concept of viral vector development is simple (Fig. 4.6) (for a review see Pfeifer, 2004): (i) identify viral genes relevant for pathogenesis, replication and production of infectious virus particles; (ii) delete all viral protein coding sequences; (iii) incorporate the transgene in the viral vector; and (iv) produce virus particles that carry the vector genome in packaging helper cells that provide essential viral proteins in *trans*. The vector virus particles are replication defective. During their use in a transgenic process the vector genome can only carry out a single round of infection. Hence, the integrated proviruses cannot produce progeny virus, but its genes can be efficiently transcribed by the host cell transcriptional system.

An important safety concern with lentivirus vectors is the possibility of insertional activation of cellular *oncogenes* by random integration of the vector into the host genome (see Chapter 8). The newest generation of such vectors are therefore self-inactivating (SIN). This is achieved by deletion of the lentivirus enhancer and promoter sequences, leaving the transgene promoter as the only transcriptionally functional element (Pfeifer, 2004).

Lentivirus transgenesis has been achieved for mice, rats, pigs, cattle, and chickens. In swine, infection of early zygotes with lentivirus-vectored transgenes has given high frequency of stably transgenic piglets. Zygote infection has not worked out well for cattle, while infection of bovine

oocytes before *in vitro* fertilization has been successfully carried out. Lentivirus vectors can also be used to *transfer transgenes into cells before their nuclei are transferred into enucleated bovine oocytes*. Bovine foetal fibroblasts have been used as nucleus donors (Hofmann et al. 2003; 2004). *RNA interference (RNAi)* has recently emerged as a novel method to knock down gene expression in mammalian cells. Lentiviral vectors carrying promoter-driven expression of short inhibitory (si) RNAs have recently been shown to induce efficient gene silencing in mice (Rubinson et al., 2003). Lentiviral RNAi vectors may prove valuable for gene expression knock down in farm animals as well. Such vectors might be used therapeutically to inhibit expression of disease-promoting genes (Pfeifer, 2004).

Cloning livestock by nuclear transfer

In a highly publicized case, a sheep named Dolly was cloned by transfer of a nucleus from a mammary (udder) cell of an adult organism. This was the first demonstration of pluripotency (totipotency) of a nucleus of a differentiated adult cell. Since the cloning of Dolly, somatic cell nuclei have been used to clone cattle, goats and pigs. In these cases, the nuclear transfer procedures are similar (Fig. 4.7). Briefly, embryonic, foetal or adult donor cells are isolated, cultured and genetically modified. Although not always feasible with adult cells, prolonged culture is preferred because experimenters have additional time to carry out successive genetic alterations, such as inactivating both alleles of a locus or creating multiple gene changes. After establishing a cell line with a specific genetic modification(s), individual donor cells are fused to an enucleated oocyte with short-duration electric pulses. For example, two 2.5 kilovolts per centimetre (kV/cm) pulses for 10 microseconds each are used to fuse adult cattle fibroblast cells with enucleated oocytes. The pulses simultaneously induce cell fusion and oocyte activation. Each fused cell is cultured to the blastocyst stage before being transferred into the uterus of a pseudopregnant female. At birth, genotype analysis is used to confirm the presence of the transgene.

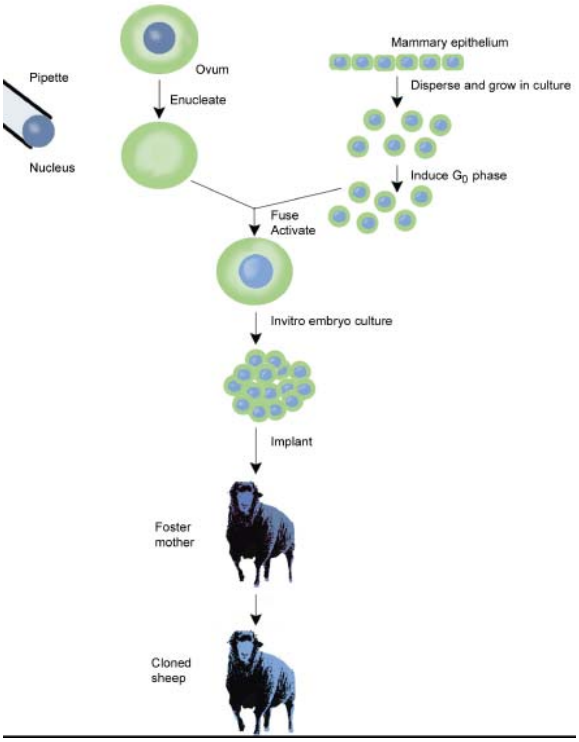

Figure 4.7. Cloning of sheep by nuclear transfer.

Transgenic fish

As natural fisheries become exhausted, production of this worldwide food resource will depend more heavily on aquaculture. In this context, enhanced growth rates, tolerance to environmental stress and resistance to diseases are some of the features that may be created by transgenesis. To date, transgenes have been introduced by microinjection or electroporation of DNA into the fertilized eggs of a number of fish species, including carp, catfish, trout, salmon, Arctic char, and tilapia. The pronuclei of fish are not readily seen under a microscope after fertilization; therefore, linearized transgene DNA is microinjected into the cytoplasm of either fertilized eggs or embryos that have reached the four-cell stage of development. Unlike mammalian embryogenesis, fish egg development is external; hence, there is no need for an implantation procedure. Development of transgenic fish occurs in temperature-regulated holding tanks. The survival of fish embryos after DNA microinjection is high (35% to 80%), and the production of transgenic fish ranges from 10% to 70%. The presence of a transgene is scored by PCR analysis of either nucleated erythrocytes or scale DNA. Founder fish are mated to establish true-breeding transgenic lines. Many of the initial studies with transgenic fish have focused on examining the effect of a growth hormone transgene on growth rate. In one study, a transgene consisting of the promoter region from the antifreeze protein gene of the ocean pout (*Zoarces americanus*), the growth hormone cDNA from salmon, and the termination-polyadenylation signals from the 3' end of the antifreeze protein gene from the ocean pout were injected into eggs of Atlantic salmon. In general, the transgenic salmon were larger and grew faster than the non-transgenic controls. This expression system was chosen to enhance the transcription of the growth hormone in cold waters. An 'all-fish' construct was assembled to avoid possible biological incompatibilities that might arise from using a growth hormone gene from non-fish sources. For even greater specificity an 'all-salmon' growth hormone construct was formulated and microinjected into sockeye salmon eggs. After approximately one year, the transgenic salmon were approximately eleven times heavier than the non-transgenic salmon. However, there was no difference in size between adults. Theoretically, the faster growth of young salmon would lower the cost of the feed and lessen the pollution of coastal waters in the vicinity of the holding pens. There is the further possibility that aquaculture with transgenic fish can be carried out within contained facilities. Regardless, the full impact of the accidental release of transgenic fish on natural populations must be considered if they are raised in ocean pens. Genetically engineered fish with enhanced phenotypic traits have yet to be implemented into commercial applications. This is partly because of the difficulties in reliably predicting the ecological risk of transgenic fish should they escape into the wild (Devlin et al., 2006).

4. Insertion of genes into microorganisms

Applications for transgenic microorganisms

A high number of bacteria and yeasts have been genetically engineered for production of industrially, nutritionally and medically important eukaryotic gene products under *contained* conditions. Yeasts, e.g. *Saccharomyces cerevisiae* and *Pichia pastoris*, are often the organisms of choice for such purposes. The reasons for this are mainly that bacteria do not carry out the post-translational modifications of transgenic proteins that are necessary for their authentic structure and proper functioning. Consequently, yeasts have been used to produce recombinant proteins from eukaryotic genes. A number of bacteria have, however, been made transgenic for the purposes of *environmental bioremediation* and as *probiotics*. The use of such genetically engineered bacteria implies direct or indirect release to the environment.

Sites contaminated by metals (e.g. Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Cr^{2+}) and *xenobiotics* (e.g. trichloroethylene, PCBs (polychlorinated biphenyls), dioxins, trinitrotoluene, PAHs (polycyclic

aromatic hydrocarbons), nitroglycerine) pose enormous health and environmental problems. At present, contaminated sites are treated by physical, chemical and thermal processes following excavation and transportation. The cost of removal of one m^3 of contaminant from a one acre contaminated site is estimated at USD 0.6–2.5 million. In contrast, the cost of bioremediation by transgenic plants or microorganisms is estimated at USD 2–5000. In addition, bioremediation causes minimum site disruption, stabilizes the soil against erosion, and concentrates heavy metals (Davison, 2005).

Naturally occurring microorganisms capable of degrading a variety of toxic compounds under laboratory conditions have been isolated. However, as many of the xenobiotic pollutions are novel to the ecosystems, microorganisms have not evolved appropriate metabolic pathways to degrade them. This is where transgenic microorganisms may fill a void in bioremediation strategies (Pieper & Reineke, 2000).

Probiotics (the name is derived from the Greek 'for life') have been used for *c.*100 years to treat a variety of mucosal surface infections, such as those of the gut and vagina, but the use of these traditional treatments diminished after the advent of antibiotics. However, these agents are now being reconsidered as alternatives to antibiotics because of the rise in antibiotic-resistant strains of bacteria. Probiotics have many potential therapeutic uses, but have not been universally accepted because of a lack of understanding of their action. Lactic acid bacteria (LAB) have been modified by traditional and GE methods to produce new varieties. Modern techniques of molecular biology have facilitated the identification of probiotic LAB strains, but only a few LAB have been modified by recombinant-DNA technology because of consumer resistance to their introduction to markets, especially in Europe (Ahmed, 2003; Celec et al., 2005).

Vector considerations
A eukaryotic gene will not function in a prokaryotic organism because there is no mechanism for removing introns from transcribed RNA. Furthermore, a eukaryotic gene needs prokaryotic transcriptional and translational control sequences to be properly expressed. Special strategies are therefore required for cloning and expressing eukaryotic coding regions in prokaryotic cells. The intron problem is overcome by the synthesis of cDNA copies of functioning mRNAs, and the necessary control sequences are added by the 'cut-and-paste' techniques described in Section 1.3. In addition to expression plasmids, other vectors that allow for cloning and expression of larger DNA fragments than plasmids can cope with are available for bacterial systems. *Bacteriophage λ vectors* for use with *E. coli* can accept inserts in the range of 15–20 kbp, while *cosmids* can cope with up to 45 kbp. Cosmids combine the properties of plasmids and λ vectors.

Gene delivery methods
Transformation is the process of introducing expression vectors into bacterial cells. Uptake of DNA is usually achieved either by *$CaCl_2$ precipitation* or by *electroporation.* The former method implies treatment of the cells with ice-cold $CaCl_2$ followed by exposure to high temperature ($42^{\circ}C$).

Electroporation is based on the fact that uptake of free DNA can be induced by exposing bacteria to a high-voltage electric field. The expression is a contraction of the descriptive phrase 'electric field-mediated membrane permeabilization'.

For some bacteria, *conjugation*, the natural system of transmitting plasmids from one bacterial strain to another, has been used to transport an expression plasmid from a donor cell to a recipient cell that is not easily transformed by the aforementioned techniques.

Gene expression considerations
There is no single strategy to obtain optimal expression of every cloned gene. The features that are being manipulated to modulate gene expression include the promoter and terminator sequences, the strength of the ribosome-binding sites, the number of transgene copies, and whether the transgene is to be plasmid-borne or integrated into the host cell genome. Furthermore, it is important to consider the final cellular location of the transgene product, the efficiency of translation and the intrinsic stability of the product in the host cell. Most cloned genes have distinctive properties that require considerable time and effort before the optimal expression level is found.

Insertion precision
For GE bacteria that are to be, or that may indirectly become, released into the environment it is essential, for efficiency as well as biosafety reasons, that the transgenic DNA be neither easily lost or transferred to other organisms, e.g. by HGT (horizontal gene transfer; see Chapter 13). The integration of the transgenic construct into the bacterial genome may prohibit both loss and unintended spread of DNA.

For integration of the transgenic construct DNA into a chromosomal site, the input DNA must share some sequence similarity with the chromosomal DNA, and there must be a physical exchange, homologous recombination (HR), between the two DNA molecules. It was initially thought that recombination required at least a sequence similarity of some 50 nucleotides for HR to occur. This has, however, been shown not to be mandatory, a fact that opens the way for the integration of additional construct copies, or part of copies, in untargeted locations in the recipient genome (Ikeda et al., 2004; see also Chapter 13 and references therein).

5. Location of the inserted genes

Random insertions
The transgene DNA may integrate into or adjacent to plant genes and perturb their expression by either decreasing or increasing their expression. The transgene could be expressed in an unanticipated manner through actions from promoters in adjacent plant genes or via interactions of plant gene open reading frames (ORFs) with promoter elements in the plant transgene. Transgene rearrangements during integration can create spurious open reading frames (ORFs) and spurious ORFs could allow the transgene to produce unintended gene products. Recombination due to repeated sequences in the transgene may result in intralocus instability or may lead to ectopic recombination. Furthermore, effects of gene silencing can interfere with the desired gene expression (Haslberger, 2006). These and other areas of scientific ignorance and knowledge gaps of importance to risk assessment and management are further discussed in Chapter 8.

Gene targeting
Many scientists now recognize the unavoidable and unpredictable consequences of the present methods for transgenesis, whether based on naturally occurring or synthesized DNA/RNA. Hence**,** strategies to perform gene targeting, i.e. to insert the gene construct into a predetermined location in the genome have been pursued. This has been achieved, at a very low efficiency, by *homologous recombination* (HR) strategies. The purpose is to perform precise, site-specific modifications of the genome to introduce, functionally delete or subtly alter target genes or their regulatory sequences. Homologous recombination is, however, an extremely rare event in mammalian cells. Furthermore, although transfected gene constructs may find their predetermined sites, other copies of the construct may integrate randomly into other locations of the genome.

Superior phenotypic characteristics in livestock have been linked to quantitative trait loci (QTL). Many QTL are associated with point mutations, single-nucleotide polymorphisms. Hence, for genetic improvement of livestock, *oligonucleotide-mediated gene modification (OGM)* may be a safer and more acceptable strategy than GE transgenesis or HR-based approaches (Laible et al., 2006).

The OGM techniques are based on single-stranded oligonucleotides (ssODNs). They contain mismatches with regard to the target gene in the recipient genome. Upon transfection into the animal cell, the mismatches are introduced into the genomic target sequence. This in turn will give a changed or 'improved' protein product from the targeted gene. Thus, this is an approach that avoids some of the potential biosafety concerns related to the insertional mutagenesis results that may arise from untargeted integration of transgenes. At present, this technology is far from efficient enough for livestock animal applications, but future development and refinement may change this situation.

6. Future prospects for gene transfer methodologies

Gene 'stacking'

Most organismal characteristics and traits are the result of the cooperation between a number of genes. Hence, in order to obtain useful changes, a cluster of transgenes has to be transferred to the recipient organism. Progress towards second and third generation genetically modified organisms (GMOs), with nutritional, environmental or other benefits that consumers may appreciate, has been slow, and will continue to be so until the bottleneck of having methods to manipulate multiple genes or traits has been removed. The theoretical potential for sophisticated metabolic engineering in plants is enormous, and could lead to the development of plants able to grow in inhospitable environments, and provide healthier foodstuffs and improved raw materials. Similar statements have been made for transgenic animals. However, most metabolic processes that are targets for manipulation depend on the interaction between numerous genes. Hence, effective metabolic engineering will only be achieved by controlling multiple genes in the same, or interconnected, biochemical pathways (Halpin, 2005). For instance, three carotenoid biosynthesis genes have been engineered into 'Golden rice' to make it produce provitamin A. Efficient provitamin A absorption may, however, require that the resorbable iron content is enhanced. This will necessitate the introduction of three additional transgenes.

Significant progress in multigene transgenesis has been made during the last few years. A variety of conventional and new techniques has been employed. Despite imperfections, plant biotechnologists consider that they provide a promising framework for future improvements. Two or more genes can be sequentially introduced into an organism by conventional iterative procedures. A plant containing one transgene can be crossed with individuals harbouring other transgenes, or it is re-transformed by new transgenes. For example, crossing plants expressing different Bt toxins (cry genes) can provide an efficient way to delay the emergence of Bt-resistant pests. Yet despite some success stories, the iterative strategies for obtaining multi-transgenic plants have several significant limitations. Principal among these is the fact that the trangenes will not be linked, and will be sited in different random loci in the recipient genome. Furthermore, the procedures will be very costly and slow. Finally, a high number of selectable marker genes will be necessary, and this will not be easily accepted by regulatory authorities and the public. Although several strategies have been developed to remove marker genes, these are not foolproof and this may hinder the acceptance of such multi-transgene organisms.

Alternative strategies for obtaining multi-transgenic plants are now being exploited. These include co-transformation with multiple independent transgenes and 'linked effect transgenes'. The latter refers to two or more 'effect genes', each with its own promoter and terminator, that are positioned contiguously on DNA that will transfer as a single entity into the recipient genome, e.g. on a single T-DNA for *Agrobacterium*-mediated transformation. All these procedures are, however, limited by the fact that it is not possible to ensure that the transgenes are expressed at similar levels, even when they are physically linked. Ways to overcome these difficulties are sought through constructing polycistronic transgenes, polyprotein expression systems and chimeric transgenes for multiple gene expression (Halpin et al., 2005).

The 'stacking' of transgenes in crops offers the potential to provide multi-toxin resistance to particular pests, nutritional value enhancement, resistance to biotic and abiotic stress, and bioremediation of xenobiotics. Plant raw materials, such as fibres, oils and starch, may be produced more cost-effectively and be environmentally benign for processing by industry. Entirely new industrial and therapeutic products may be produced in crops in a substantial manner. Edible plant vaccines may offer immunologically superior and cost-effective alternatives to traditional vaccines (Singh et al., 2006).

Chloroplast transgenesis
In nuclear transgenic plants, expression of multiple genes requires introduction of individual genes and time-consuming subsequent backcrosses to reconstitute multi-subunit proteins or pathways, a problem that is compounded by variable expression levels, as well as unpredictable insertion sites, expression levels and genome stability of the transgenic plants. In order to accomplish expression of multiple genes in a single transformation event, several genes can now be introduced into the chloroplast genome.

In plant and animal cells, the *monocistronic translation* of nuclear messenger RNAs (mRNAs) that contain only one translational unit poses problems in engineering multiple genes in plants. In contrast, most chloroplast genes of higher plants are co-transcribed. Multiple steps of chloroplast mRNA processing are involved in the formation of mature mRNAs. Expression of *polycistrons* via the chloroplast genome provides a unique opportunity to express entire pathways in a single transformation event. Additionally, chloroplast GE, according to its proponents, is an environmentally friendly approach resulting in containment of foreign genes and hyperexpression (Bogorad, 2000).

Chloroplast GE is rapidly becoming the transformation method of choice for the next wave of transgenic products in crop plants, particularly for plant-made pharmaceuticals (PMPs). Chloroplast GE has been designed in order to obtain high levels of gene expression needed for target protein production, which can be up to 45% of the total soluble proteins produced in the cell (De Cosa et al., 2001), while limiting the amount of vertical gene flow from the maternally inherited chloroplasts.

Artificial chromosomes: YACs, BACs and MACs
Artificial chromosomes are DNA molecules of predictable structure which are assembled in vitro from defined constituents that are similar to natural chromosomes. The first artificial chromosomes have been constructed in yeast (*Saccharomyces cerevisiae*). They include *centromeres*, *telomeres*, and *origins of replication* as essential components. These yeast artificial chromosomes (YACs) can be introduced into cell lines. They carry much larger amounts of DNA than usually can be employed in microinjection. Microinjection of a 450 kb genomic YAC harbouring the murine tyrosinase gene resulted in transgenic mice which showed position independent and copy number dependent expression of the transgene. Lactoglobulin and human

growth factor were expressed in the mammary gland of transgenic rats. Artificial chromosomes can also be constructed in bacteria (BACs), which can be genetically modified more easily. Transgenic mice were generated via pronuclear injection of BACs and germ line transmission and proper expression of the transgene was achieved. However, to date, transgenic livestock have not been reported upon transfer of a YAC construct. This may be attributed to the inherent problems of this technology, such as difficulties in isolating YAC DNA with sufficient purity and the inherent instability with a tendency for deleting regions from the insert.

Mammalian artificial chromosomes (MACs) have been engineered by employing endogenous chromosomal elements from YACs or extra chromosomal elements from viruses or BACs and P1 artificial chromosomes (PACs). MACs with a size of 1–5 Mb were formed by a *de novo* mechanism and segregated like normal chromosomes upon introduction into cell lines. A human artificial chromosome (HAC) containing the entire sequences of the human immunoglobulin heavy and light chain loci has been introduced into bovine fibroblasts, which were then used in nuclear transfer. Transchromosomal offspring were obtained that expressed human immunoglobulin in their blood.

Satellite-DNA based artificial chromosomes (SATAC) are neochromosomes that are formed by *de novo* amplification of pericentric heterochromatin yielding chromosomes from 10 to 360 megabases. These can serve as chromosomal vectors for exogenous DNA. Transgenic mice have been generated by microinjection of SATACs into pronuclei of zygotes. The additional chromosome showed germ line transmission over three generations. Microinjection of SATACs was also compatible with the development of bovine embryos. Transgenic embryos could be identified by staining for the presence of a reporter gene and FISH detection of the extra chromosome.

Synthetic biology (see the following) offers new opportunities to make useful forms of artificial chromosomes.

Nanobiotechnology (NBT)
The size domain of nanotechnology is a billionth of a metre. Nanobiotechnology is thus defined as the use of nanoscale or nanostructured objects in the size range of 1 nm (nanometer) to 100 nm. *Nanocarriers* are materials or devices of nanoscale made up of different biodegradable materials such as natural or synthetic polymers, lipids or phospholipids, and even organometallic compounds. They offer attractive solutions for DNA transformation of cells and organisms. There are, however, a number of unsolved health and environmental biosafety issues related to the use of nanocarriers as gene delivery vectors (Hoet et al., 2004).

Synthetic biology
Synthetic biology is interpreted as the engineering-driven building of increasingly complex biological entities for novel applications. Some scientists even predict that the first man-made cell, capable of replication and evolution, fed only by small molecule nutrients, is now possible within the next decade or so (Forster & Church, 2006). Two of the synthetic biology application areas most significant for engineering of transgenic organisms are represented by *artificial gene networks* and *de novo synthesis of large DNA sequences*. Genomic-scale DNA synthesis is already becoming increasingly possible today. Furthermore, DNA synthesizing of an entire intracellular pathway, composed of genes from various species, is becoming feasible. Such approaches will include optimal codon usage, adapted secondary mRNA structures, tailored regulatory elements (e.g. promoters, enhancers, introns), and MCS strategies that allow the modular replacement of specific genes by improved versions (Heinemann & Panke, 2006). At this point, it is important to emphasize the fundamental difference between engineering in biology and

in, for instance, chemistry or physics. Biological systems have the capacity to replicate and to evolve. This may interfere with the short- and long-term stability of engineered pathways, constructs and organisms, and will require constant monitoring of the integrity of the systems.

RNAi technology

In addition to the traditional strategies for vector construction and genetic modification strategies described, RNAi (interference) technology (see Chapter 3) is now becoming a new way to improve the contents and fight the diseases of crop plants (Sen & Blau, 2006). Furthermore, plant virus vectors for transfer and expression of transgenes in crop plants are coming into use (Chung et al., 2006).

Hybrid technologies

It seems quite safe to predict the future development of transgenic organisms based on fusions and hybrids between transgenesis, nanobiotechnology, RNAi technology, and synthetic biology. Such developments will include tailored single transgenes, multimodular DNAs or artificial chromosomes more efficiently delivered to cells and organisms by different types of nanocarriers. The nanocarriers may be loaded with protein ligands that target the DNA constructs to specific cell types and facilitate the transport from the cell surface to the nucleus, and stable integration into the recipient cell genome.

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Chapter 5

Basics on the Fifth Nucleotide in DNA, 5-Methyldeoxycytidine: A Regulatory Genetic Signal

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In mammalian as well as in plant DNA, and in the DNA of many other organisms, there occurs a fifth nucleotide, 5-methyldeoxycytidine (5-mC), in addition to the traditionally recognized four nucleotides A, C, G, and T. Although the presence of 5-mC in DNA has been known for a long time, only during the last 30 years has there been progress in elucidating its functional significance. This brief chapter will give an introduction to the field and address biological processes in which 5-mC has been shown to assume a major role.

1. On the early history of 5-mC

The fifth nucleotide, 5-methyldeoxycyidine (5-mC), was first described in DNA from the tubercle bacillus (Johnson & Coghill, 1925) and in calf thymus DNA (Hotchkiss, 1948). Subsequently, 5-mC had a biochemical future as 5-hydroxymethyl-C (5-hm-C) in the DNA of the T-even bacteriophages. The biological function of this C modification was never elucidated. Daisy Dussoix and Werner Arber (Arber & Dussoix, 1962; Dussoix & Arber, 1962) discovered the phenomena of restriction and modification in bacteria. It was recognized later that DNA modifications, such as 5-mC and/or N^6-methyladenosine (N^6-mA), had important biological consequences. A major endeavour followed in many laboratories that worked on the biochemistry of DNA modifications in bacteria and their phages (review by Arber & Linn, 1969). Around 1970, Hamilton Smith and his colleagues discovered the restriction endonucleases (Kelly & Smith, 1970) whose application to the analyses of DNA was pioneered by Daniel Nathan's laboratory (Danna & Nathans, 1971). It was soon appreciated that enzymes, whose activity was compromised by the presence of a 5-mC or an N^6-mA in the recognition sequence, could be of great value in assessing the methylation status of a DNA sequence (Waalwijk & Flavell, 1978; McClelland & Nelson, 1988).

In 1975, two papers (Holliday & Pugh, 1975; Riggs, 1975) alerted the scientific community to the importance of methylated DNA sequences in eukaryotic biology. At more or less the same time, my laboratory at the Institute of Genetics in Cologne independently analyzed DNA in the human adenovirus and in adenovirus-induced tumour cells for the presence of 5-mC residues (Günthert et al., 1976) and discovered that integrated adenovirus, perhaps any foreign, DNA had become *de novo* methylated (Sutter et al., 1978). DNA methyltransferases in human lymphocytes were studied early on by Drahovsky and colleagues (1976). Vanyushin's laboratory in Moscow analyzed the DNA of many organisms for the presence of 5-mC and N^6-mA (Vanyushin et al., 1968).

Church and Gilbert (1984) were the first to develop a genomic sequencing technique, based on the chemical modification of DNA by hydrazine, and thus provided a means to survey all possible C-residues for the occurrence of 5-mC in a sequence. The bisulfite sequencing technique introduced by Marianne Frommer and colleagues (Frommer et al., 1992; Clark et al., 1994) allowed for a positive display of methylated sequences. This method and some of its modifications have now become the 'gold standard' in analytical work on DNA methylation. The

method is precise and yields reproducible results but is laborious and expensive. At present, however, there is no better method available.

Constantinides, Jones and Gevers (1977) reported that the treatment of chicken embryo fibroblasts with 5-azacytidine, a derivative of cytidine which was known to inhibit DNA methyltransferases (reviewed by Jones, 1985), activated the developmental programme in these fibroblasts leading to the appearance of twitching myocardiocytes, adipocytes, chondrocytes, and others in the culture dish. Their interpretation, at the time, that alterations in DNA methylation patterns activated whole sets of genes involved in realizing a developmental program, has stood the test of time. There is now a huge body of literature on changes in DNA methylation during embryonal and foetal development (for an early contribution to this topic, see Razin et al., 1984). The observation of inverse correlations between the extent of DNA methylation and the activity of integrated adenovirus genes in adenovirus type 12-transformed hamster cells (Sutter & Doerfler, 1980 a; 1980 b) elicited a surge of similar investigations on a large number of eukaryotic genes. Today, it is generally accepted that specific promoter methylations in conjunction with histone modifications (acetylation and methylation, among others) play a crucial role in the long-term silencing of eukaryotic genes (Doerfler, 1983). There is no rule, however, without exceptions – Willis and Granoff (1980) have shown that the genes of the iridovirus frog virus 3 (FV3) are fully active, notwithstanding the complete 5€ -CG-3€ methylation of the virion DNA and of the intracellular forms of this interesting viral genome.

Since many foreign genomes in many biological systems and hosts frequently became *de novo* methylated, several authors have speculated on whether this phenomenon reflects the function of an ancient cellular defence mechanism (Doerfler, 1991; Yoder et al., 1997) against the uptake and expression of foreign genes, much as the bacterial cell has developed the restriction modification systems to counter the function of invading viral genomes. In eukaryotes, integrated foreign viral, in particular but not exclusively, retrotransposon genomes, which make up a huge proportion of the mammalian and other genomes, are frequently hypermethylated (Bestor, 1998). This finding is in keeping with the cellular defence hypothesis of *de novo* methylation mechanisms. In my laboratory at the Institute of Genetics in Cologne (Schubbert et al., 1997) and also by others (Forsman et al., 2003), these considerations have prompted investigations on the stability of food-ingested DNA in mammals as a possible source of foreign DNA taken up with high frequency by mammalian organisms.

In research on the function of 5-mC, many questions remain to be investigated: How have the patterns of DNA methylation, i.e. the distribution of 5-mC residues in any genome, evolved over time? How different are these patterns from cell type to cell type and under what conditions are they preserved, even interindividually maintained in a given species? In what way do these patterns codetermine the structure of chromatin by providing a first-line target for proteins binding preferentially to methylated sequences (Huang et al., 1984; Meehan et al., 1986) or by being repulsive to specific protein-DNA interactions?

Chromatin structure and specific patterns of DNA methylation, which differ distinctly from genome region to genome region, are somehow related. There is growing experimental evidence that the presence of 5-mC residues affects the presence of a large number of proteins in chromatin. However, we do not understand the actual complexity of these interactions or the role that histone modifications can play in conjunction with DNA methylation in the control of promoter activity. Imaginative speculations abound in the literature but there is little novel experimental evidence. I suspect we will have to unravel the exact structural and functional biochemistry of chromatin before real progress on these crucial questions will become possible. A recent review (Craig, 2005) phrased the chromatin enigma thus: ‘there are many different

architectural plans … leading to a seemingly never-ending variety of heterochromatic loci, with each built according to a general rule'.

With the realization and under the premise that promoter methylation could contribute to the long-term silencing of eukaryotic genes, researchers have approached the fascinating problem of genetic imprinting. Several groups provided evidence that genetically imprinted regions of the genome can exhibit different methylation patterns on the two chromosomal alleles (Sapienza, 1995; Chaillet et al., 1995). For one of the microdeletion syndromes involving human chromosome 15q11-13, the Prader-Labhart-Willi syndrome, a molecular test was devised on the basis of methylation differences between the maternally and the paternally inherited chromosome (Dittrich et al., 1992).

Problems of DNA methylation, of the stability and flexibility of the patterns of DNA methylation are also tightly linked to many unresolved questions on reproductive and/or therapeutic cloning. In an effort to correlate gene expression with survival and foetal overgrowth, imprinted gene expression has been investigated in mice cloned by nuclear transfer or in embryonic stem (ES) cell donor populations from which they were derived. The epigenetic state of the ES cell genome appears to be extremely unstable. Variation in imprinted gene expression has been observed in most cloned mice. Many of the animals survived to adulthood despite widespread gene dysregulation, indicating that mammalian development may be rather tolerant to epigenetic aberrations of the genome. These data imply that even apparently normal cloned animals may have subtle abnormalities in gene expression (Humpherys et al., 2001). In cloned animals, lethality occurs only beyond a threshold of faulty gene reprogramming of multiple loci (Rideout et al., 2001). However, malformations are frequent among cloned animals which appear also to have a limited lifetime.

Similarly, the idea of replacing defective genes with their wild type versions or of blocking neoplastic growth by introducing cogently chosen genes and stimulating the defences against tumours and metastases has captured the fascination of many scientists working towards realistic regimens in gene therapy. However, many unsolved problems have remained with viral gene transfer vectors: (i) Stable DNA transfer into mammalian cells was frequently inefficient; (ii) The site of foreign DNA insertion into the recipient genomes could not be controlled; (iii) The integrates at random sites were often turned off unpredictably due to cellular chromatin modifications and/or the *de novo* methylation of the foreign DNA.

Of course, there have been prominent voices cautioning against the premature application of insufficiently scrutinized concepts and techniques (cited in Stone, 1995). Adenovirus vectors proved highly toxic in topical applications to the bronchial system of cystic fibrosis patients (Crystal et al., 1994). In a tragic accident, the administration of a very high dose of a recombinant adenovirus, which carried the gene for ornithine-transcarbamylase, led to the death of 18 year old Jesse Gelsinger (Raper et al., 2003). Retroviral vectors as apparent experts in random integration were thought to assure continuous foreign gene transcription in the target cells. By using a retroviral vector system, ten infant boys suffering from X-linked severe combined immunodeficiency (X-SCID) had presumably been cured. However, the scientific community was alarmed soon thereafter by reports that two of these infants developed a rare T-cell leukemia-like condition (Hacein-Bey-Abina et al., 2003). Presumably, the integration of the foreign DNA construct had activated a protooncogene in the manipulated cells – perhaps a plausible explanation and in line with long-favoured models in tumour biology.

In this latter context, I submit to consider a different concept. The possibility exists that the insertion of foreign DNA into established mammalian genomes, with a preference at actively

transcribed loci, can alter the chromatin configuration even at sites remote from those immediately targeted by foreign DNA insertion (Doerfler, 1995; 2000). In cells transgenic for adenovirus or bacteriophage lambda DNA, extensive changes in cellular DNA methylation (Heller et al., 1995; Remus et al., 1999) and cellular gene transcription patterns (Müller et al., 2001) have been documented. Foreign DNA insertion at one site may, hence, affect the genetic activity of a combination of loci which might be disseminated over the entire genome. The chromosomal sites of the cellular genes thus afflicted might depend on the location of the initial integration event. Oncogenic transformation of the cell, according to this model, would ensue because of alterations in specific combinations of genes and loci and in extensive changes in the transcriptional programme of many different genes.

If valid, this concept could shed doubts on apparently useful procedures in molecular medicine – the generation of transgenic organisms, current gene therapy regimens, perhaps even on the interpretation of some knock-out experiments. The functional complexities of the human, or any other, genome cannot yet be fathomed by the knowledge of nucleotide sequences and the current textbook wisdom of molecular biology. At this stage of our 'advanced ignorance' in biology, much more basic research will be the order of this and, I suspect, many future days, in order to be able to heed the primary obligation in medicine – *primum nil nocere*.

2. Onward to new projects

Today, the concept of an important genetic function for 5-mC in DNA has been generally accepted. Moreover, many fields in molecular genetics have included studies on the fifth nucleotide in their repertoire of current research: regulation of gene expression, structure of chromatin, genetic imprinting, developmental biology, even in *Drosophila melanogaster* (Lyko et a., 2000), an organism whose DNA has been previously thought to be devoid of 5-mC, cloning of organisms, human medical genetics, cancer biology, defence strategies against foreign DNA, and others. Progress in research on many of these topics has been rapid, and the publication of a number of concise reports within the framework of Current Topics in Microbiology and Immunology is undoubtedly timely (Doerfler & Böhm, 2006 a; 2006 b). When screened for 'DNA methylation' in early June of 2007, PubMed[1] responded with a total of 12,357 entries dating back to 1965; a search for 'DNA methylation and gene expression' produced 5,322 citations.

A conventional review article on DNA methylation or on one of its main subtopics, therefore, would have to cope with serious limitations, omissions and oversimplifications. With more than 30 years of experience in active research in the field, I wish to briefly outline questions, problems and possible approaches for further research. Seasoned investigators in the field undoubtedly will have their own predilections. For the numerous newcomers to studies on DNA methylation, my listing might provide an introduction, or more likely might arouse opposition, which will be just as useful as an aid to initiate original research.

1. Chromatin structure

Patterns of DNA methylation in the genome and the topology of chromatin structure and composition are tightly linked. Studies on the biochemical modifications of histones – amino acid sequence-specific acetylations and methylations (Allfrey et al., 1964; and many references since) have revealed the tip of the iceberg. A much more profound understanding of the biochemistry of all the components of chromatin and their possible interactions with unmethylated or methylated DNA sequences will have to be elaborated. I would rate such studies as the number one priority

[1] PubMed is an online reference service of the National Library of Medicine and the National Institutes of Health.

and primary precondition for further progress in the understanding of the biological significance of DNA methylation.

2. Promoter studies

We still do not understand the details of how specific distributions of 5-mC residues in promoter or other upstream and/or downstream regulatory sequences affect promoter activity. It is likely, though still unproven, that there is a specific pattern for each promoter, perhaps encompassing only a few 5€ -CG-3€ dinucleotides, which leads to promoter inactivation. It would be feasible to modify one of the well-studied promoters in single, or in combinations, of 5€ -CG-3€ sequences and follow the consequences for promoter activity with an indicator gene. Moreover, for each methylated 5€ -CG-3€ sequence, the promotion or inhibition of the binding of specific proteins, transcription factors and others will have to be determined. It is still unpredictable whether there is a unifying system applying to classes of promoters or whether each promoter is unique in requiring specific combinations of 5€ -5m-CG-3€ residues for activity or the state of inactivity. Of course, in this context, the question can be answered as to whether the activity of a promoter can be ratcheted down by methylating an increasing number of 5€ -CG-3€ dinucleotides step by step in increments of one.

3. Correlations between DNA methylation and histone modification in eukaryotic promoters

In what functional and enzymatic ways are these two types of modifications interrelated? Can one be functional without the other; is one the precondition for the other one to occur? Ever since the search began for the class of molecules which encodes the genetic information, the 'battle has raged', as it were, between proteins and DNA to exert the decisive impact. A similar, though less fundamental, debate on the essential mechanisms operative in long-term gene inactivation is occupying the minds of researchers today. In most instances, the 5-mC signal is relevant mainly in long-term gene silencing. For frequent fluctuations between the different activity states of a promoter, the DNA methylation signal would be a poor candidate for a regulatory mechanism, because promoter methylation is not easily reversible.

4. On the mechanism of *de novo* methylation of integrated foreign or altered endogenous DNA

One of the more frequent encounters for molecular biologists with DNA methylation derives from the analysis of foreign DNA which has been chromosomally integrated into an established eukaryotic genome. Foreign DNA can become fixed in the host genome not only after infection with viruses, but also in the wake of implementing this integration strategy in the generation of transgenic organisms. In knock-in and knock-out experiments, in regimens of gene therapy and others, investigations on this apparently fundamental cellular defence mechanism against the activity of foreign genes – *de novo* methylation – has both theoretical and practical appeal. During the embryonic development of mammals, methylation patterns present at very early stages are erased and new patterns are re-established *de novo* in later stages. Hence, we lack essential information on a very important biochemical mechanism. There are only few systematic studies on the factors that influence the generation of *de novo* methylation patterns. Size and nucleotide sequence of the foreign DNA as well as the site of foreign DNA insertion could have an impact, but in what way remains uncertain. Other aspects of *de novo* methylation relate to the availability, specificity and topology of the DNA methyl-transferases in the chromatin structure.

5. Levels of DNA methylation in repetitive DNA sequences

Studies on repetitive DNA sequences and their functions are one of the very difficult areas in molecular biology, mainly for the want of new ideas to contribute to their study. Perhaps, the elucidation of the patterns of 5-mC distribution in these sequences could shed light on possibly novel approaches of how to proceed further. Repetitive DNA sequences, particularly

retrotransposon-derived DNA or endogenous retroviral sequences, are in general heavily methylated. Exact studies on the methylation and activity of specific segments in the repetitive DNA are available only to a limited extent. The difficulty for a systematic analysis certainly lies in the high copy number and the hard to prove or disprove possibility that individual members of a family of repetitive sequences might exhibit different patterns.

6. Foreign DNA insertions can lead to alterations of DNA methylation *in trans*

Studies on this phenomenon have occupied our laboratory for several years, and we are still investigating whether these alterations might be a general consequence of foreign DNA insertions or occur only under distinct conditions. We, therefore, propose to pursue the following strategies:

(i) Random insertion of a defined cellular DNA segment with a unique or a repetitive sequence at different chromosomal sites and follow-up of changes in DNA methylation in different locations of the cellular genome. In this context, methylation patterns in unique genes and in retrotransposons or other repetitive sequences will be determined.

(ii) In individual transgenic cell clones transgene location should be correlated with methylation and transcription patterns in the selected DNA segments. Could the chromosomal insertion site of the transgene be in contact with the regions with altered DNA methylation at the level of interphase chromosomes?

(iii) Studies on histone modifications in or close to the selected DNA segments in which alterations of DNA methylation have been observed.

(iv) Influence of the number of transgene molecules, i.e. the size of the transgenic DNA insert, at one site on the extent and patterns of changes in DNA methylation in the investigated *trans*-located sequences.

(v) I consider this topic of fundamental importance because its pursuance could shed light on unforeseen and unforeseeable problems arising during the generation of transgenic (gene manipulated) organisms, the cloning of organisms and in gene therapeutic strategies, possibly also in knock-in and knock-out experiments that are so frequently the basis of medically relevant conclusions. While the technical advantages and potential economic spin-off in the mentioned fields have been heralded in an exaggerated way, basic research dealing with the consequences of foreign DNA insertion has been deplorably under-represented.

7. Stability of transgene and extent of transgene methylation

Hypermethylated transgenes appear to be more stably integrated than hypomethylated ones (Hochstein et al., 2007). A refined approach to this problem could be to fix genomically differently pre-methylated transgenes and follow their stability in individual cell clones.

8. Enzymes involved in *de novo* methylation of integrated foreign DNA

It is still uncertain which DNA methyltransferases or which combinations of these enzymes are involved in the *de novo* methylation of integrated foreign DNA. Enzyme concentration by itself might not be the rate-limiting step. Rather, chromatin structure and the topical availability of DNA methyltranferases could be the important factors that need to be investigated.

9. The role of specific small RNAs in triggering DNA methylation

There is a lack of studies on this problem in mammalian systems.

10. Complex biological problems connected to DNA methylation

A great deal of very interesting research on DNA methylation derives from the work on epigenetic phenomena, on genetic imprinting, and more generally, from the fields of embryonal

development, medical genetics and tumour biology. From the presently available evidence, DNA methylation or changes in the original genomic patterns of DNA methylation are most likely implicated in any one of these phenomena.

Concluding remarks

The structural and functional importance of the 'correct' patterns of DNA methylation in all parts of a mammalian genome is, unfortunately, not well understood. The stability, inheritability, and developmental flexibility of these patterns all point to a major role that these patterns play in determining structure and function of the genome. Up to the present time, studies on the repetitive sequences, which comprise > 90% of the DNA sequences in the human or other genomes, have been neglected. We only have a vague idea about the patterns of DNA methylation in these abundant sequences, except that the repeat sequences are often hypermethylated, and that their patterns are particularly sensitive to alterations upon the insertion of foreign DNA into an established genome. Upon foreign DNA insertion into an established genome, during the early stages of development, or when the regular pathways of embryonal and/or foetal development are bypassed, e.g. in therapeutic or reproductive cloning, patterns of DNA methylation in vast realms of the genome can be substantially altered. There is very little information about the mechanisms and conditions of these alterations, and investigations into these areas could be highly informative. By the same token, a thorough understanding of these problems will be paramount and a precondition to fully grasp the plasticity of mammalian genomes. Moreover, it is hard to imagine that, without this vital information at hand, we will be able to apply successfully our knowledge in molecular genetics to the solution of medical problems. A vast amount of basic research still lies ahead. I suspect that, in the futile hope of making 'quick discoveries' and, consequently, in neglecting to shoulder our basic homework now, we will only delay the breakthroughs in biomedical research that all of us hope for.

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Chapter 6

Understanding the uncertainties arising from technological interventions in complex biological systems: The case of GMOs

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Technological control of and intervention in complex biological systems inevitably create risks and concerns about unexpected or unidentified outcomes. The lack of empirical data (evidence) and scientific consensus, as well as the various types of uncertainty embedded in dynamic biological processes limit the knowledge sources regulatory agencies can draw on to effectively assess the health and environmental impacts of novel technologies. Thus, contested scientific knowledge, and intrinsic uncertainty surrounding biological processes create an arena where the lack of conclusive evidence can serve differing interests. For instance, industry can advocate the beneficial impacts of their novel products whereas other interest groups, claim that application of the same products involves unacceptable risk to health or the environment. The divergent groups may all present rational agendas given their contrasting risk-benefit perspectives, objectives and values within the dynamic discourse of knowledge formation. The commercial introduction of genetically modified organisms (GMOs) has revealed a broad range of views among scientists and stakeholders on risk perspectives and if and how GMOs should be regulated. The 'science-based' risk assessment of GMOs has resulted in different policy outcomes dependent on how the regulatory agencies involved have assessed various types of, or lack of, data to reach conclusions in the face of uncertainty. In this chapter we describe and contextualize the broader scientific uncertainties present in the process of risk assessment of GE and GMOs. The discussion is structured as follows:

1. Lack of scientific understanding of the biological processes involved and affected

Uncertainty is the driving force of science and hence there will always be tension and a time-lag between the science-based regulatory agencies' immediate need for robust knowledge and the relative, iterative process of knowledge production itself. In many cases, especially with new technology, the regulatory decision making is done in the absence of 'certainty', and hence it is vulnerable to various types of subjective assumptions about the risks and benefits involved. The types of uncertainties surrounding GE and GMOs can be divided into three broad classes:

(i) Reducible uncertainty, due to lack of knowledge and the novelty of the activity, which can be addressed with more research and focused collection of empirical data.
(ii) Irreducible uncertainty due to inherent randomness, variability and complexity in the biological system under consideration.
(iii) Uncertainty arising from ignorance given that the prevailing operating paradigms and models do not adequately represent the biological system evaluated.

A holistic approach to the potential risk issues of GE and GMOs involves appreciation of these various types of uncertainty and encourages its explicit consideration and communication. This can be challenging and controversial because a holistic approach often questions the basic assumptions behind the science, i.e. problem framing, hypothesis formulation, model choice, and the use of and reliance on specific methods and assumptions for data production and interpretation (Section 1.1), the extent to which reliable, reproducible data can be obtained at all (Section 1.2), and whether the prevailing paradigms in which data are produced are sufficiently representing the system investigated so that no unforeseen effects will materialize (Section 1.3).

1.1 Uncertainty in data quality and production

Data quality. Access to peer-reviewed quality data is essential for a 'science-based' risk assessment. In order to gain regulatory approval, commercial developers of GMOs often submit their own test results to document the expected behaviour of the GMO and its products in the exposed system, and hence, its safety. Some experimental data on the safety of GMOs are also available in the peer-reviewed literature (Vain, 2007). Yet, knowledge gaps are routinely identified during regulatory risk assessment of GMOs. These gaps are often due to missing data (lack of relevant studies) or because the previously published studies have too narrow a scope or have focused on aspects of the biological system with only limited relevance to the biosafety of the GMO itself. To address the lack of direct empirical data and studies, a number of substitute approaches and assumption-based reasoning are routinely included in regulatory risk assessment. Often, the concepts of *familiarity* (with the unmodified parent organism) and *substantial equivalence* (to the unmodified parent organism) are used to frame the safety investigations of the GMOs in the context of previous experience and current analytical methods (König et al., 2004). These concepts are developed and maintained within expert cultures and evaluations of the GMOs. Thus, inference, drawing from organismal history and comparative experiences and observations of the parent organism (of both the GMO and the GMO trait itself), form the starting point of all current risk assessments of GMOs.

Regulatory risk assessment is based on literature reports of evidence (data) and not on data produced independently by the regulatory agency itself. The data received by the regulatory agency is thus produced and contextualized within the objectives that initiated the study (e.g. to support the safe commercialization of the GMO). Due to the many potential sources of motivational bias in directly submitted (often with confidentiality claims) and peer-reviewed data, it is essential that the multitude of data sources used, and the inferences and assumptions made in the risk analysis are openly evaluated and clearly communicated (Marvier, 2002; Lövei & Arpaia, 2005; Meyer et al., 2005). Accordingly, the outcome of any risk assessment can be no more conclusive than the quality of the underlying data. This reliance on data quality and external providers of data seems often to be forgotten in the scientific debate on risk issues of GMOs; providing the ground for subjective expert opinions and value-influenced interpretations to provide the main 'data' basis for the arguments forwarded.

Data production: hypothesis formulation. Hypotheses define the problem framing that underlies all peer-reviewed research that in turn yields the data subsequently supporting the biosafety assessment of GMOs (Jewett, 2005). Understanding the processes behind hypothesis formulation

is thus critical for conceiving how scientific data are produced and peer reviewed. The subsequent downstream choice of models and methods used for testing the hypothesis also depends on the hypothesis itself. Because an unlimited number of hypotheses can be constructed for any given problem, an equally unlimited number of model and method choices are at hand. This is clearly the Achilles heel of science, as hypothesis development is limited to the researcher's preconceived ideas of the system and the paradigms within which the biological system are understood (Strohman, 1997). Moreover, the researcher's ethical values, research environment, funding sources, employment status and financial prospects, time constraints, and material/resource accessibility will influence if not determine the biosafety-relevant hypothesis generated (Lewontin, 1991). Thus, subjective choices and motivational bias in conceptualization of the hypothesis behind the research question (risk identification) may far exceed the uncertainties in the specific experimental design and data collection itself.[1] There are no internationally agreed upon detailed standards for the methods used for biosafety-relevant data collection[2]; partly due to the case-by-case nature of risk assessments and the large geographical differences in ecosystems. Thus, the quality of biosafety data must be understood and interpreted in the research and motivational context within which they are produced. Likewise, the absence of biosafety data may indicate ignorance, or a lack of or bias with respect to research focus, motivation, capacity, time, or financial or political research support.

Data production: choice and limitations of models. In most scientific studies, models of a biological system are designed to test hypotheses about a phenomenon, or a specific cause-effect relationship (e.g. an intended or unintended effect of a GMO). The assumption is that the model represents the natural system with respect to the relevant parameters measured. By definition, a model does not claim to represent the 'truth' and therefore cannot be argued to be false. In contrast, hypotheses are directly linked to the natural system and are falsifiable. There is at present little scientific consensus on the choices of models and methods to investigate the effects of GE and GMOs; this concerns both the proposed benefits and the undesired effects. This scientific uncertainty arises from incomplete understanding of the interactions among natural variables and the limitations inherent in simplified models in predicting the behaviour of multivariable natural systems. For instance, the potential for pollen flow from genetically modified (GM) crops to other crops, weeds and wild relatives is a biosafety-relevant question for regulators and scientists that can be addressed by a range of hypotheses and model choices of a highly complex natural system. Pollen flow raises issues such as:

- Economic and legal concerns with regard to how GM crops can be cultivated in co-existence with conventional and organic farming, including issues related to labelling, liability, and socio-economic aspects such as effects on traditional farming practices, product identity, seed quality control, and changes in farming infrastructure.

- Environmental concerns with regard to potential adverse effects from flow of transgenes (introgression) into cultivated species, weeds and wild species.

[1]For example, a company researcher holding a utilitarian view that GMOs are a simple extension of traditional breeding efforts would develop biosafety-relevant hypotheses that are likely to be quite different from a researcher with a previous background as an environmentalist viewing GMOs as novel entities with little in common with traditional breeding. Both researchers will develop biosafety-relevant hypotheses, but it is clear that these would differ substantially in the problem framing, resource requirements, models and methodologies, data interpretation and contextualisation, and hence, possibly in the outcomes.

[2]The Codex Alimentarius (2003) represents a collection of internationally adopted food standards, including principles for risk analysis and guidelines for safety assessment of foods derived from modern biotechnology. Environmental, ethical, moral and socio-economic aspects are not addressed in the Codex standards.

- Health concerns with regard to the potentially changed allergenic properties of pollen caused by the genetic modification, or health impacts caused by pollen flow from GM plants producing pharmaceutically-active compounds into crop plants entering the food chain.

These concerns can be seen through risk windows of many sizes, addressed by a number of hypotheses on the effect (or lack thereof) of GMOs on agriculture, and investigated with a broad range of methods and scales. Recently, farm-scale field trials on the biological effects of GM plants (compared to non-GM varieties) have been performed (Squire et al., 2003); an approach that certainly broadens the scope, system reliability and robustness of the data produced.

Data production: choice and limitations of methods. In the conducting of research, scientists make assumptions and inferences based on the paradigms within which they are trained and the research environment they are socialized into (Kuhn, 1962). The choice of models and methods to test a specific hypothesis is a variable of the research environment, resources, the competencies and instruments at hand, and most importantly, time constraints. Thus, researchers operating in different research environments will invariably choose different models and methods to address the same risk-relevant question. An example is the issue of addressing potential allergenicity of GMO products. This issue is exceedingly complex, and the mechanistic aspects of allergy development are not fully understood, even within the basic medical sciences. Thus, there is no single biological model or experimental standard available to evaluate the potential allergenicity of new products from GMOs.

Scientists have thus been drawing on the familiarity of the unmodified host organism(s) and have constructed a number of models, assumptions and comparative approaches to justify the claim of absence of allergenicity in GM products.[3] Not surprisingly, the assumptions behind selecting the most appropriate model and method choices have been questioned (Spök et al., 2005). There are few alternatives to testing in live organisms. Yet, selecting live test organisms, other than humans, inevitably raises questions about the relevance of the animal model chosen because there is no single animal model that can reliably solve allergenicity questions in humans. The choice of model system and methodological approaches will likely remain a contentious issue in the pre-marketing investigations of the safety of GE and GMOs.

It is important to be fully aware of the limitations of the methods and models used when considering and concluding from the outcome of biosafety-relevant studies (Andow, 2003). Often, various interest groups (sometimes also the 'objective' scientists behind the study itself) are eager to conclude more broadly from the studies than what the applied methods, models and produced data allow. The assumptions underlying the study, the choice of hypotheses, the interpretation of published data, as well as the significance of the absence of data, can lead to unsupported claims about the intended or unintended effects of GMOs. For instance, one frequently hears that 'there is no data to suggest that unintentional effects occur'. Such an argument raises two questions:

1. Have relevant studies been done *at all* to produce data that address the question?

[3]These include (i) computer-assisted bioinformatics-based comparisons of the new proteins (produced by the GMO) to known protein allergens, (ii) examinations of the stability of the protein in experimentally simulated gastrointestinal tract systems, and (iii) experimental and theoretical consideration of the overall concentration, composition and stability of the protein (e.g. heat stability). It is clear that these methodologies require numerous subjective decisions regarding the exact experimental conditions applied. Some examples of assumptions that depend on the model choices include assumptions that the allergenic site can be identified in proteins based on 2-D amino acid composition and not 3-D structure, and that the protein digestive capacity of the gastrointestinal tract of humans can be adequately constructed by mixing specific concentrations of enzymes and chemicals in test tubes.

2. If studies are available, what were the motivations, objectives and hypotheses behind the production of the data, and are the tested hypotheses, models and methods sufficiently robust to support such a statement?

An example of this type of argument, not infrequently also found written in biosafety risk assessment documents, is 'there is no data to suggest that plant transgenes have transferred horizontally into bacteria'. It is often unclear if such an argument is made because the authors have examined the range of peer-reviewed studies that have used suitable methods to produce risk-relevant data, or simply that no relevant studies have been done and considered in the assessment.[4]

In conclusion, beyond the explicit awareness and communication of the rationale behind the risk conceptualization, hypothesis formation and choice of models and methods, scientists must clearly communicate the limitations of their methods and experimental approaches. Likewise, regulators must explicitly consider the problem framing behind the hypothesis construction, the context behind the model choices, and the methodological limitations embedded in the data when drawing on experimental studies in risk assessments.

1.2 Indeterminacy due to inherent randomness in biological systems

Biological systems are highly complex and may not be easily quantified or explained by quantitative methods. Random variation in baseline data in conjunction with complex, multi-scale network interactions between molecules, cells, organisms, physical environments, and environmental variables (temperature, season, geography, etc.) can lead to meaningless quantification efforts; and hence indeterminacy (Funtowicz & Ravetz, 1990; Wynne, 1992). Whereas precise numbers (such as the rate of gene flow, or degradation kinetics of a protein) can be obtained within various experimental model systems, their quantitative mean and range as a variable in changing geographical and environmental contexts rarely have the same level of precision.

Regulatory decision makers often face exact numbers presented in experimental data, but in reality, robust range estimates are unachievable.[5] The regulators or scientific advisory board must therefore make judgments as to whether to base the assessment on the empirically-determined numbers at hand (given the limitations of the models and methods by which they were obtained), or make their own subjective predictions of the number ranges in real life.

[4]Re-examining the available literature on monitoring gene transfer from plants to bacteria, two groups of scientists independently concluded that previous studies that have examined this risk scenario have used methods that are unable to resolve the issue (Heinemann & Traavik, 2004; Nielsen & Townsend, 2004). It was found that the currently applied sampling methods for monitoring of gene transfer from GM plants to soil or human intestinal gut microorganisms are too insensitive and effectively have only examined a few grams of sample material from the gut or soil. These severe limitations in the data were not previously exposed in regulatory risk assessment documents.

[5]Consider, for instance, the example of gene flow from GM bacteria to wild-type bacteria. Laboratory models readily provide the opportunity to quantify gene transfer frequencies between defined bacterial populations grown under simplified laboratory conditions. However, are these numbers (or even the absence of detectable transfer) relevant to the broad range of natural conditions or bacterial species these GM bacteria encounter? We argue, not at all. For example, published studies suggest gene transfer processes occurring in complex environments such as soil can vary more than a billion-fold, even within a gram of soil (Nielsen et al., 1997). This is due to the locally highly variable microhabitat that soil represents (soil types, plant roots, rock surfaces, animal manure, water logging, etc. (Nannipieri & Smalla, 2006)). Thus, laboratory-obtained numbers are most often irrelevant, and neither encompass the high spatial-temporal variation in gene transfer rates in nature, nor incorporate the effects of selection or genetic drift with equally constrained quantitative approaches (McHughen, 2006). Thus, most vertical and horizontal gene transfer frequencies remain practically indeterminable in all complex environments since the full set of environmental conditions cannot be fully conceived or examined.

A closer look at the quantitative aspects of biosafety-relevant studies reveals that indeterminacy is an intrinsic component in many, if not most, of these and hence they are of little direct quantitative value. Subjective assessments and supportive claims must therefore be constructed to support their informative value in risk assessments. For instance, given that pollen flow is shown to occur between GM and non-GM plants, frequency estimates of this process are only relevant to risk assessment if they are robust to variations in environments and conditions such that the process can be reliably quantified (McHughen, 2006). In most cases, this will not be the case as the measured frequencies represent a snapshot taken in a given farm-field context.

While we appreciate the value of numbers, they may be more useful to identify relevant processes for subjective assessment within a qualitative risk perspective. Nevertheless, risk assessment documents frequently make use of specific numbers drawn from empirical studies. Perhaps this is done unconsciously for the purpose of constructing an argument (providing exact numbers that erroneously give the impression of high accuracy) to support their final risk conclusions rather than cautiously communicating the context (and the associated uncertainty) in which they where produced.

In conclusion, complex natural systems have cause and effect relationships in multiple dimensions, therefore often making them untenable to current experimental methodologies that seek to produce exact numbers that can support quantitatively oriented risk assessments. Nonetheless, precise numbers quantifying risk-relevant scenarios remain the preferred support and basis for regulatory decision making, perhaps since this conveys an impression of numerical certainty in the assessment (Meyer et al., 2005).

1.3 Ignorance arising from conceptual limitations in the operating paradigms of the biological system

Risk from GE and GMOs arises because there is uncertainty about casual chains in the intervened complex biological system. Yet, on the surface, successful applications of GM techniques appear to demonstrate an increased knowledge of the biological systems that have been genetically modified. However, intervening at more powerful levels does not imply that the intervention is more controlled. In fact, the intervention may increase the level of ignorance by widening the gap between the levels where human intervention is possible and the levels where accumulated knowledge, experience and consensus confer predictability on the processes involved and affected. For example, whereas the random introduction of novel DNA fragments into the genome of most organisms is now a routine technique in molecular biology laboratories, the corresponding knowledge and predictive power of the unintended cellular, organismal and environmental effects are only partially understood. Due to the lack of a coherent understanding of how genomes function, it is today impossible to predict precisely how the introduced genes will function in the new host organism and how the modification will affect the organisms' own gene functions and regulations (see Chapters 3 and 8). It is, with little scientific support, often assumed by GMO developers that the new transgene-encoded product will act independently of the many thousand proteins and metabolites active in the same cellular environment.[6]

[6]Yet, there are many examples of ignorance of unintended effects of transgene insertions (Cellini et al., 2004; Prescott et al., 2005; Filipecki & Malepszy, 2006), and without doubt most of those observed have never reached the peer-reviewed literature. This is because the reports on unintended negative effects (ignorance) available in the peer-reviewed literature represent only those experimental studies for which the authors (including the journal editor) have had a motivation to publish. Since most developers of GMOs are companies with no incentives or duties to publish negative research findings (i.e. that would create investor uncertainty on the safety and predictability of the core technology), it is clear that the published studies represent only a minor fraction of the observed unintended effects to date. Moreover, in GE-based plant breeding most undesired events are excluded from further breeding seasons (similar to traditional plant breeding programmes), resulting in exclusion of most events with undesired or unintended

An overriding philosophical concern with the scientific approaches applied to the reduction of ignorance in GE and GMOs is that current methodology directs and shapes the research questions raised in regard to details within the system itself (reductionism), often producing little coherent understanding of the larger system (holism).[7] The absence of a holistic research focus can be explained by relative lack of comparatively precise methods and inability to test a defined, detailed and single cause-effect based hypothesis. Moreover, the results produced from more holistically oriented approaches are necessarily with lower mechanistically based explanatory power, often less reproducible and not patentable due to inherent variation in the processes within and between organisms. Thus, due to a lowered immediate commercial potential they become less valued and attractive to pursue within the current single hypothesis- and patent-driven scientific approaches. In science philosopher Thomas Kuhn's view (1962), scientists work well within defined paradigms focusing on specific mechanistic (and therefore patentable) aspects of the system. Thus, it can be questioned to what extent discipline-oriented researchers and research institutions are effectively trained, organized and motivated to take on broad cross- and multi-disciplinary approaches that may be required to advance the broader understanding of the implications of technological interventions.

The ecotoxicological risk perspective (paradigm) has been influential in shaping risk concepts in biosafety. This unwittingly contributes to further ignorance since chemicals follow a different environmental route and degradation pathway than transgenes (Karlsson, 2006). Chemicals have a release-dependent concentration decline with a given breakdown time in the environment. In contrast, (trans)genes follow the path of the host genome, possibly eventually also the path of sexually compatible and some incompatible species (through vertical and horizontal gene transfer). Hence, the initial release concentration of the (trans)gene may have little predictive power of the persistence time, degradation routes, or amplification and spread of the transgene in the environment over time. Thus, ecotoxicological risk models (based on the premise that exposure dose predicts response) have no or little utility in predicting the environmental behaviour of released transgenes, where exposure dose does not predict response. This is explained by the conceptually different contexts and behaviour of the evaluated entities, i.e. non-replicating chemicals versus replicating genes and organisms.

2. Lack of scientific consensus on the effects caused and observed

There are divergent opinions among scientists about the occurrence and relevance of potential adverse effects arising from GE and GMOs, the definition of potential 'adverse effects', and what action to take (if required at all) to prevent potential harm (Myhr & Traavik, 1999; 2003). Various scientific experts draw or make inferences from their specific scientific disciplines to support their views and framing of the risk issues debated.[8] Because experiences and traditions, paradigms, problem framing, models, and methodologies differ sharply among scientific disciplines, there may be little common ground for single scientific disciplines to independently

characteristics. Several years of subsequent selection-based breeding of the novel GM plant events lead to an increase in familiarity with the event (plant cultivar) and hence, to a reduction in the level of overall ignorance.

[7]For example, there are massive efforts to elucidate and engineer single metabolic and signal transduction pathways within cells, but the corresponding wider perspective on how these pathways act in concert, within organisms, and respond to variations in the organism's environment, is less understood.

[8]For instance, agricultural biotechnologists often make inferences about the safety of GM plants based on the long tradition of safe use and predicted behaviour of and familiarity with conventional crop plants. Implicit in this is the assumption that the insertion of species-foreign genes does not substantially alter the genetics and physiology of the modified plants beyond the inserted trait. Some ecologists, on the other hand, refer to experiences catalogued from the introduction of exotic species to make inferences on the anticipated knowledge gaps about the novel GM plants that may only materialize as a negative effect after years of cultivation and widespread distribution. Implicit in this is the assumption that GM plants may have substantially different genetics that can produce unpredictable properties.

solve broadly framed biosafety concerns. Thus, while acknowledging the variation in the different disciplines' problem framing and risk conceptualization, the broad demand for 'more research' on biosafety issues is not necessarily sufficient to build consensus among scientists and stakeholders on risk issues and to reduce uncertainty.

In fact, more research may lead to increased uncertainty due to the discovery and exposure of novel processes and factors not previously considered that might also cast doubt on the adequacy of the scientific methods used in previous studies (Sarewitz, 2004). Yet, keeping in mind the subjective context of scientific practice and data production, few would disagree that continued research on biosafety issues would contribute to improve the safe use of GMOs. The lack of scientific consensus is a normal and often *the* driving part of science, and is not a particular risk feature of GE and GMOs. Sarewitz (2004) denotes this observation as an 'excess of objectivity', referring to the observation that available scientific knowledge can legitimately be interpreted in different ways to yield competing views of the problem and therefore differences in society's response. Meyer et al. (2005) argue that the current lack of data and the subjective constituents, particularly integral values, within data production in biosafety hinder scientific consensus building on the effects caused and observed. Moreover, a non-uniform response is seen among experts to new studies reporting deviations from safety assumptions further exemplifying the values, stakes and subjective interpretations underlying the discourse on the safety of GE and GMOs.

A main challenge in regulatory risk assessment is how to interpret and weigh conflicting studies of which some may indicate an undesired effect arising from the activity, whereas others, perhaps the majority, indicate no observable negative effects. Thus, in other words, should biosafety assessment be exclusively based on mainstream science and the leading scientists' views on what type of studies to pursue and their interpretation of data? Further, how should contrasting data and minority views be communicated in the conclusions of a risk assessment?[9] There is no clear policy on how to deal with contrasting studies during regulatory risk assessment, leaving their inclusion or exclusion, and interpretation open to subjective assessments made by the members of the regulatory body. Often, the presence of conflicting safety studies in the regulatory risk assessment phase may never reach the risk communication phase, due to the perceived need of providing the public with an unambiguous risk conclusion that is not intended to communicate that there is uncertainty.

2.1 Disagreements between experts on data interpretation and 'sound science'

How can experts disagree on study design and the interpretation of data if knowledge production itself is the outcome of unbiased rational thought and approaches? Postmodernist philosophers question whether scientists can ever be neutral and objective. The subjective components of science in hypothesis construction, experimental design, data interpretation, contextualization, and communication are rarely as heavily exposed as in the discourse on the biosafety of GMOs. The idealized view of an objective approach in science has long been dismissed by the philosophers of science and by those scientists taking a broader interest in their own field of research. For instance, more scientific journals now have a strict policy requiring scientists to declare conflicts of interest in their published studies, making transparent the motivational factors that can bias the study or its interpretation (Lexchin et al., 2003, Fontanarosa et al., 2005).

[9]Historically, early indications of the harmful effects of BSE, dioxins, and a number of pesticides (EEA, 2001) were reported, but these studies could not compete with mainstream scientific views and the leading opinion makers of the time, and were thus not considered in the regulatory decisions. Yet, there is ample support in the scientific literature that some contested scientists in the minority and dismissed scientific studies have been proven correct. A number of studies claiming undesired effects have also been correctly dismissed, and some studies may yet await acknowledgement or dismissal.

Although it is undisputable that ethical values and bias in data production and interpretation form a core part of scientific knowledge production, the effect thereof is rarely explicitly considered in biological risk assessment or in the public or scientific discourse on how to most efficiently address safety concerns in GE and GMOs. Since it is strongly argued by GMO developers that risk assessment should be 'science-based', a broader consideration of the subjective components of data production is rare. The understanding and identification of the impact of values in biological risk assessment is often confused because the impact occurs at several levels:

> (i) Values shape *knowledge production* by affecting problem framing, hypothesis construction, model choice, experimental design, data interpretation, contextualization, and communication of studies motivated by curiosity-driven data production prior to the applied biosafety context, or studies motivated by the issue or mission-oriented production of safety data supporting the GMO.
>
> (ii) Values shape biological *risk assessment* by affecting risk conceptualization, problem framing, data interpretation, evidence weighting, considerations of expert opinions, how poor data quality, conflicting studies or the absence of relevant studies are dealt with, to what extent precautionary-oriented approaches should be taken, and which stakeholders and experts should be a part of the assessment. All these factors will eventually lead to a biased risk communication that is supportive of the risk management plan.
>
> (iii) Values shape governmental *risk policy* regarding the laws, liability regime, labelling requirements, and regulatory systems developed for GE and GMOs, the political process determining the composition of, and the design of, the type of decision-making bodies that will conduct the final GMO risk-benefit analysis (of which the biological risk assessment is one of several components), the prioritizing of GE and GMO investment and incentives, and the allocation of resources to biosafety research and broader resource input to curb or shape public opinion. The impact of values in risk assessment and management policies is exemplified by institutional and legislative changes instigated by changes in the political leadership.

Those singly advocating a 'science-based' regulatory system, with the objective of admitting and considering only certain types of data in the risk assessment process, are either deliberately ignorant of the strong influence in the science and regulatory process of the aforementioned exemplified values or have an agenda that benefits from not exposing their own values.[10] The 'science-based' approach can be advocated within a supportive governmental system and a society that share a particular set of values, and hence, they do not necessarily need to be acknowledged as part of the data production and risk assessment process. However, the inherent subjectivity and value component must be explicitly considered and acknowledged when the underlying values supporting the 'science-based' approaches to biosafety are not shared among stakeholders in the global GMO marketplace.

Disagreement between scientists on biosafety issues can be naively explained by pointing to the different 'quality' of the scientists involved. The quality discrepancies may be attributed to the fact that scientists have different overall skills, access to the disputed data, practical knowledge of the methodology, and reach beyond their area of competence, as well as they may apply wrong models, or fail to adequately incorporate related contrasting studies in their contextualization, etc. The construction of the concept of 'sound science' can be seen in this perspective, in which the

[10]They may implicitly advance specific (utilitarian) values that can include limited product regulation and requirements for safety studies, allocation of burden of proof to those voicing safety concerns, decisions to proceed in the face of uncertainty, support for rapid market access of new products, no labelling or liability provisions, broad patent opportunities, corporate control over genetic resources, etc.

concept is used to discredit scientists with opposing views and to claim support for a specific interpretation of the data underlying the safety assessments of GE and GMOs. Thus, the implicit claim of unsound science in some controversial biosafety-relevant studies may often be the result of confusion created by special interests, rather than uniform consensus among independent scientists, representing a broad set of values, on errors in the methodology of a specific study. For example, the study by Quist and Chapela (2001), reporting unexpected introgression of transgenes into corn landraces in Mexico was highly controversial after being published in the leading scientific journal *Nature*.[11] One can speculate as to how many of the peer-reviewed and published, or confidential business information-confined, biosafety studies conducted today follow a quality standard that would stand up to similarly intense and close scrutiny.[12]
The current discourse on the safety of GMOs is taking place within the natural sciences using concepts such as 'science-based', 'sound-science', 'familiarity', and 'substantial equivalence' and is often portrayed as getting the 'right' interpretation of controversial studies. As argued here and elsewhere (Meyer et al., 2005), closer examination of the discourse reveals that subjective assessments, value disagreements, bias, and conflicts of interest define the agendas for the discourse. Thus, disagreement on factual issues can be seen as a strategic discourse adopted to advance and bolster public and regulatory support for the specific objectives of the actors, and discredit those with opposing values and views (Thompson, 2002). Different value sets and risk perceptions direct those scientists who see little uncertainty in GMOs to promote a regulatory-limited, expert-driven, rational, and based-on-available-data-only approach to biosafety. In contrast, those scientists who perceive higher uncertainty and the value-laden context of risk assessment demand more research to fill knowledge gaps, precaution, and individual consumer autonomy and broader stakeholder involvement in the risk analysis.

3. Summary

Biosafety data do not arise from an objective process of data and knowledge accumulation, but represent the scientist's choice of methods and the interpretational context, as determined by the biological, ethical, political, and economic objectives, in which the data is produced. It is important to acknowledge the subjective context underlying all data production, processing, interpretation, and presentation as defined by values, preferences, assumptions, audience, and policies. A transparent handling of these integral components of science and regulatory practice would drastically enhance the quality of data available to regulatory risk assessment and the social robustness of risk analysis while refocusing the ongoing scientific discourse on the safety of GMOs. The future public credibility and trustworthiness of scientists active in the field of

[11]Distinguished scientists, many with strong motivational bias (economic interest in GM plant production) attempted to discredit the study (Christou, 2002). Such unusual peer pressure was made that the Nature editor subsequently wrote that the study should not have been published. Yet, subsequent independent studies conducted by the Government of Mexico confirmed the main observations in the Nature-published study (Alvarez-Morales, 2002), and there is today little scientific controversy over the conclusion that corn transgenes were, at some stage, present in the native corn population of Mexico (Cleveland et al., 2005; Ortiz-Garcia et al., 2005). What remains controversial is the extent to which the transgenes become distributed within the genome of single corn plants. However, this latter aspect is of minor importance to the main observation: that transgenes were present where by law they should not have been. Because the application of all experimental methods requires subjective considerations, any group of influential scientists can discredit the methodology behind most published peer-reviewed studies in any science journal and portray it as 'unsound science'. This exemplifies the science philosopher Bruno Latour's (1987) description of science as an activity where competing knowledge claims are advanced through various networks of scientists, where the stronger network leads the knowledge claim, and competing views struggle for acknowledgement. There have, to our knowledge, been few attempts from those highly vocal in discrediting the Quist and Chapela study to make the Mexican Government publish their three independent studies confirming the presence of transgenes in Mexican corn. If science was an objective unbiased struggle to advance knowledge, should not this be expected?

[12]See also Ioannidis (2005) for an informative discussion on the probability that a research claim is true, taking into account the number of studies conducted, study power, effect size, financial interest and prejudice, bias in model design, data analysis and presentation, and competition in the research field.

biosafety depend on how they identify and acknowledge their objectives and subjective influence on problem framing and choice of methodologies.

Virtually all the broader uncertainties in the science behind GMO safety assessments examined here are not unique to gene technology, but are present in any modern technology assessment. Although this chapter focuses critically on uncertainties, it should not be interpreted as advocating a specific position in disfavour of technological developments in GE and GMOs. Technological advances are always made in the face of uncertainty. Uncertainty is thus not a barrier to scientific progress, but is the main driver of new discoveries, creativity, and inventions. Dogmatic claims assuming 'certainty', rather than uncertainty, stall science (Pollack, 2003). It is the duty of all scientists to identify and challenge the paradigms, values and assumptions shaping their scientific approaches in a reflective and transparent way to ensure that their knowledge claims continually strive for the highest quality.

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Chapter 7

GE Applications and GMO Release: The Ethical Challenges

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1. Introduction

The term 'genetic engineering (GE)' is very broad, covering an assortment of ways to analyse and manipulate genomes of living organisms. The public supports different applications of GE to different degrees. Genetically modified (GM) medicines and genetic tests, for instance, are considered to carry invaluable benefits, and hence they tend to be accepted. The utilization of GM animals, GM fish and GM crops, however, is strongly opposed. Different levels of support for different GE applications may be explained by the public conception of potential benefits and risks involved. Such risks are related to the potential for unintended adverse health and environmental effects as well as to social and economic aspects.

Different applications of GE and GMO usage represent various types of risk. For instance, with GE medical applications such as a GM vaccine, a GM drug or somatic cell gene therapy, the beneficiary coincidently carries the potential risks. For germ cell line gene therapy, however, diseases may be cured by genetic 'surgery', and the 'improved' genome will be passed on as a new genotype in the next generation. Accordingly, the risk of harm may be transferred to future generations. Issues that present putative risks across generation gaps, raise questions concerning moral obligations. They involve the challenge of balancing the ethical consideration of human needs today against the opportunities for future generations to fulfil their needs. The situation becomes even more complex when society and the environment may experience the risk. For instance, we do not know with certainty if GM crops will promote general welfare by providing more nutritious food or help to ensure food safety. Neither can we be sure that GM crops do not cause unintended effects on non-target organisms or threaten biodiversity. Inevitably, solutions to such dilemmas should be based on ethical reflections such as: How to act when the long-term consequences are unknown? How sure is 'sure enough'? Who are the affected parties? Good answers to these questions demand safety requirements for health and the environment, taking a long-term perspective, consideration for present and future members of society, and a presumption of democratic decision making. To meet these challenges, we will in this chapter argue that:

- A number of ethical issues, as well as choice of perspectives and value commitments, affect risk assessment and management of GE applications and GMOs.

- A more holistic approach to GE applications and GMO risk issues is needed to account for the present lack of scientific understanding and for the complexity of ecosystems.

2. The role of ethics

Decisions to apply new technology and innovations must be based on evaluations of the assumed benefits versus the potential risks of adverse effects to ecosystem, human and animal health. In addition, a decision must include an evaluation of the values that are important to enhance or to protect, which are directly linked to a community or governmental choice of level of protection. In general, the most fundamental distinction in ethics may arguably be drawn between the outcome of a decision (consequence ethics) and the means for taking decisions (deontological ethics) (Box 7.1).

Box 7.1 Consequence ethics and deontological ethics

Consequence ethics are mainly concerned with the outcome of actions, and what is right depends on the benefits achieved or the good outcome. A classical approach is utilitarian, meaning that the morally 'right' action is the one that optimizes the goal for the whole moral community (Bentham 1789; Mill 1871). Utilitarianism is usually an ethical foundation for risk-cost-benefit analyses. Risk-cost-benefit approaches are often used in the evaluation of technology development, introduction and implementation. Accordingly, an activity may be considered ethically acceptable if its benefits outweigh its costs. In deontological ethics, on the other hand, the moral rightness of an action is independent of its actual consequences (Kant 1781). Deontological ethics prescribe that moral rules need to be applied when making decisions. Such rules may prohibit an action irrespective of the best intentions and/or outcome. Such moral rules may include respect for human autonomy and dignity. Some environmental ethicists have argued that rights and duties should be extended to animals and to the environment, and not relate to humans only (Regan 1980).

GE applications and the release of GMOs involve a lot of challenges to the quality of decision making. The differences in perception between governments, among the scientists and within the public are related to the underlying ethical issues, as well as to choice of perspectives and value commitments that affect frameworks of risk assessment and management of GE applications and GMOs. Most often cost-benefit analyses are chosen as the fundament for risk regulatory frameworks. However, a strict application of risk-cost/benefit analyses does not cope appropriately with the current lack of scientific understanding and the complexity of the human and environmental systems that are to become the recipients of the GE applications and the GMOs. Therefore, application of cost-benefit analyses may, for instance, lead to unintended ecological effects such as long-term adverse effects on health, decreased biodiversity and harm to dynamic ecosystem processes being ignored. Such analyses also fail to take into account the deeper ethical bases that shape the scientific and public opinions. Hence, applications of cost-benefit analyses that only rely on quantitative valuations without qualitative considerations may appear to be a too narrow approach to GE application and GMO release decisions, by being 'blind' to natural and cultural values that are difficult to measure (Wynne, 2001). In addition, it is difficult to quantify environmental costs. They are qualitatively different from straightforward costs carried directly by producers and consumers and are often linked to value questions. Environmental costs are difficult to measure, and adverse effects may develop over long time frames. The benefits of reducing environmental costs and

risks are most often of non-monetary value. The environment may hence be neglected in standard practice and the incentives for reducing environmental risk and cost may be absent.

Different approaches grounded in deontological ethics have as a common feature a demand for equality and justice of something that is considered as important (as rights, income, and access to resources) (Dobson, 1998). Deonotological ethics imply that moral rules need to be considered when making decisions. Consequently, issues of risk and benefit distribution must include balanced ethical considerations concerning the needs of the present versus future generations, as well as for animals and the environment. Furthermore, for the purpose of avoiding serious, unintended ecological effects it may be necessary to develop new ethical models as alternatives to the anthropocentrically grounded approaches that are mostly used at the present. There are distinct philosophical differences between giving priorities to protection of human interests, i.e. anthropocentrism, versus preservation of ecosystems, i.e. ecocentrism (Box 7.2).

Box 7.2 Antropocentric versus ecocentric approaches

In an anthropocentric context, the environment is protected to promote human welfare, i.e. for recreation purposes, or as a source for gaining new knowledge. Since ecosystems contain huge amounts of unknown information, and biodiversity centres represent valuable genetic pools for future possibilities for humans, i.e. agricultural and medicinal development, protection might be in humankind's best interest (Daily et al. 2000; Pimentel et al. 2000). Hence, human interests provide a powerful set of motives for protecting the environment against activities that may have severe consequences (i.e. reduced biodiversity) for present and future generations. Ecocentrics emphasize the need for a change from the anthropocentric domination and exploitation of the environment towards a greater respect for the integrity of the animals and the environment (Dobson 1998, Westra 1998). Biocentrics argue that as humans, we must provide rights to species and habitats and hence it is our duty to respect their integrity (Regan 1980). Respect for ecosystem integrity is considered important, and preservation and protection of biological, ecological and genetic processes are necessary, irrespective of the instrumental value to humans.

In an ecocentric context, release of a GMO or a GE vaccine into the environment may be morally justified when it protects the diversity of the species in the community, and does not cause adverse effects to ecosystem processes. Involvement of ecocentric ideologies will legitimize a holistic approach to risk-associated studies. Such an approach may also focus on changes in both biotic and abiotic factors (both physical and chemical factors that are non-living), for instance the effects on soil, water and air. This ideology differs from anthropocentric GMO governance with respect to value commitments and factual beliefs. Hence, ethical issues do affect the significance of frames and approaches in environmental risk regulation. Involvement of ecocentric and biocentric ideologies will, for instance, entail awareness of the complexity of ecosystems and hence legitimize interdisciplinary scientific initiatives and a holistic approach to risk-associated approaches.

3. Risk assessment and risk management

The Cartagena Protocol on Biosafety was adopted in 2000, and 141 countries have ratified it so far. Many countries have adopted national regulations for GMO use and release as well. The international and national regulations do, simply by their existence, acknowledge the risks of GE applications. By extension, authorities have realized the need to employ precaution in order to protect human and animal health and the environment. However, it is necessary to reflect on the fact that the risk assessment and management strategies prescribed through regulations are developed within particular frameworks. They include (as mentioned) values and preferences in relation to the natural environment and the promotion of human health.

Risk assessment includes hazard identification, risk characterization and risk estimation, while risk management comprises value judgements with regard to acceptability, trade-off criteria and adaptation of strategies for coping with the risk aspects identified during the assessment. Risk assessment has been considered a strictly 'scientific' process, while social and political factors are involved at the risk management and communication stage. However, in reality, it is obvious that risk assessment also involves value judgements. They relate to conception and acceptance of consequences that should be avoided, and also to the processes of risk characterization and investigation. Such judgments are most often made before initiation of the risk assessment, and serve as 'lenses' through which adverse effects and lack of knowledge are viewed, perceived and defined. For instance, if the decision makers demand that complete and supportive information or credible scientific evidence is needed before cause-effect relationships are claimed, lack of knowledge may be downplayed or overlooked in situations with high complexity. Waiting for scientific evidence of harm implies postponement of precautionary measures and preventive actions until a product or an activity is proven harmful, or until plausible cause-effect relations are established. On the other hand, in situations characterized by lack of knowledge and complexity, it may not be possible to get conclusive scientific evidence of adverse effects. A reductionistic approach awaiting conclusive scientific evidence may then fail to protect humans and animal welfare. Hence, the quality of a risk assessment will depend on the value aspects considered important to protect, and the harm that needs to be avoided by the scientists and the decision makers involved.

The present GMO risk assessment procedures are dependent on information produced and owned by the very same companies whose products are being assessed. This means that there is a conflict of interest linked to risk assessment. A further obstacle for independent risk assessment is the difficulty in obtaining access to this information (Myhr & Traavik, 2002), since it is often claimed to be confidential business information. Access to information, i.e. the risk assessment performed by the companies that develop GE applications and the GMOs, and accumulation of knowledge via independent peer review is needed in order to ensure transparency and confidence (Nielsen, 2006). In addition, this is essential for identifying lack of knowledge and for directing further research activities in areas of uncertainty and ignorance.

3.2 Scientific uncertainty and complexity

Before releasing any new living organism or genetically modified DNA construct into a new location or ecosystem, important questions concerning environmental and health effects need to be answered. A number of hypothetical effects, both beneficial

and harmful, have different degrees of scientific support, mostly due to lack of relevant research. At present, very little research to approve or reject such hypothetical claims has been carried out. Without hard data that specifically address the issues, it is impossible to assess health and environmental impacts, and more critically, the exposure levels to be recommended. The present lack of scientific understanding is of ethical significance in the context of research that should be initiated and also of how this research should be carried out (see Chapters 4, 6 and 8–15).

3.2.1 The need for early warning research

The report *Late lessons from early warnings: the precautionary principle 1896-2000*, published by the European Environment Agency (EEA, 2001), describes 14 cases where lack of precaution has had human, ecological and economic costs. The most relevant of the cases in our context may be the horizontal transfer (HGT, see Chapter 13) of antibiotic resistance genes, the endocrine disrupting effects of chemical pollutants and the bovine spongiform encephalopathy (BSE) story. In all 14 cases, 'dissident' scientists predicted and had preliminary results indicating the problems that later became evident. Such scientists were marginalized and discredited by mainstream science as well as by the economic stakeholders involved. Recently, we have experienced GE-relevant cases directly, through the histories of Drs Arpad Pusztai and Ignacio Chapela.

The necessity of learning from past failures, and to heed early scientific evidence of risks, is emphasized in the EEA report. The selected cases are analysed historically with focus on the decisions taken (or not taken) at a given time, and correlated to the knowledge at that specific time. The report describes how lack of scientific proof of harm was misinterpreted as evidence of safety both in science and in policy, and that the failure to respond caused human, ecological and economic costs. For instance, throughout the DES (synthetic oestrogen diethylstilbestrol) case there were official assertions of safety, i.e. that there was no risk of transmission to the foetus (Ibarreta & Swan, 2001). DES had been prescribed since 1947 to pregnant women in order to prevent spontaneous abortions. The pharmaceutical industry, the medical scientists and the regulators did not acknowledge the 'early warnings' indicating that DES could cause harm. As early as in 1938, it was reported that DES could increase cancer in laboratory animals. Several subsequent studies proved that DES could cause cancer in the cervix and vagina of rodent species. However, the acceptance that DES could cause teratogenic effects and was a transplacental carcinogen first came in 1971, ten years after the limb reduction effects of thalidomide were revealed. Before that it was generally assumed that the placenta protected the foetal environment from external exposure. The DES case illustrates how narrow risk-assessment frameworks are, and how the choice of null hypotheses may hamper both initiation and acceptance of early warning based research.

The 14 cases in the EEA report have exemplified the risk of bias towards safety conclusions when hypotheses that dominate mainstream science are treated with blind reliance. The DES case had its tragic toll because it was generally accepted that the placenta protected the foetus against hormone-related harms. Hence, no risk-associated studies to confirm or reject this assumption-based hypothesis were initiated.

The DES, and the other cases in the EEA report, highlight the problem of 'omitted research', an expression used for important research lacking intellectual, economic or political incentives for being carried out.

We have experienced the dramatic consequences of ignoring early warnings quite recently. Following the BSE (mad cow disease) scandal in UK, a *Science* commentary asked: '*What happens when the premise underlying a scientific risk assessment is wrong and, as a result, the risk is vastly understated? In the case of so-called mad cow disease, or bovine spongiform encephalopathy (BSE), people die, an industry suffers, and a country panics*' (Gavaghan, 2000). A very highly respected BSE researcher commented: '*From my perspective, unwelcome scientific advice about an epidemic spread of BSE worldwide, and especially about the undeniable possibility of transmission of the BSE agent to humans, was dismissed*' (Manuelidis, 2000). In other words, when harm cannot be proven by science, in part because the kind of scientific research in question has not yet been carried out, the developer and/or proponent of a product maintains the legal presumption that it causes no harm by its action, and the 'public and the environment' carry the burden of proof.

In relation to GMOs, claims are made that early warnings represent 'snap-shots' and 'worst-case scenarios', not reality, and therefore they should not be published (Shelton & Sears, 2001). This issue has recently been exemplified by the controversies arising following the *Nature* report that Mexican maize was contaminated with transgenic DNA from GM maize (Quist & Chapela, 2001). The report caused an extensive debate concerning methods used for detection of GM contamination and with regard to the significance of the preliminary findings (Kaplinsky et al., 2002). A temporary climax was reached by an editorial note in *Nature* (Editors' comment, 2002) claiming 'the evidence available was insufficient to justify publication of the original paper'. In this case, there has been extensive interference in the process by actors (media, the public, non-governmental organizations, and industry) not normally active in the scientific process. The focus has been on the researchers and their context, and very little has been done to confirm or refute the claimed biologically and ecologically adverse impacts. This case illustrates the extent of scientific disagreements, and ethical dilemmas that surface when there are close ties between public and academic science and private enterprise.

Just like early safety proclamations, early warnings may later be proven wrong. It is, however, important to publish them in order to inform other scientists and regulators. This in turn will become the basis for follow-up research designed to confirm or reject them. If such 'early warnings' are not reported, evidence required for the application of the Precautionary Principle may not be known, and governments may end up making decisions in the absence of proper scientific understanding.

3.2.2 Reductionism, scientific uncertainty and complexity

The 'central dogma' (see Chapters 2–4) was the basis for molecular biology and GE. Approaches based on reductionism were both productive and unavoidable in the early developmental stages of GE. Lately, however, a growing acceptance of an unanticipated complexity and unpredictability in the relationships between DNA-RNA-protein has emerged. New techniques, such as genomics, proteomics and metabolomics (see Chapters 4 and 8) have been developed to cope with complex interactions, the cooperation and coordination of multiple genes and the dynamics of

total genomes. This is not to deny that reductionistic approaches may present very fruitful ways to study phenomena, since they will involve few variables under controlled and contained conditions. However, some results of reductionistic assumptions, such as the belief that large-scale behaviour of GMOs can be extrapolated from effects studied in small-scale models, do not hold validity and do not represent reality. To extrapolate from one context to another, i.e. from small to large-scale release, leaves questions concerning the environmental fate of GM plants unanswered (Wolfenbarger & Phifer, 2000).

Interactions with the environment are organized on a higher level than the DNA level. For instance, the same gene may not have the same expression level in different organisms (Bergelson et al., 1998). A transgene may result in other proteins in the recipient than in the donor plant (Prescott et al., 2005). These and other examples show that extrapolation of data from small-scale to large-scale, or from one context to another, does not necessarily represent reality. Growth conditions are geographically and climatically different and may make it difficult to identify the cause-effect relationships of impact. Such extrapolations may therefore, in fact, increase the uncertainty.

Furthermore, unpredictable effects of GMO use and release may arise due to interactions between the introduced transgenes(s) and the recipient genome, or unanticipated interactions between the GMO and the ecological system. Hence, one needs to be aware that there will always be an inevitable gap between limited experimental conditions and reality, i.e. the consequences of an activity can never be fully predicted. This is because uncertainties regarding the behaviour of complex systems may not be directly linked to lack of knowledge, which can be reduced by performing more research. Consequently, resolving uncertainty and complexity requires a) more comprehensive studies of ecological effects by GMO utilization (see Chapters 4 and 8–15) and b) epistemic discourses that involve different scientific disciplines. This will ensure diverse considerations and enhance critical evaluation of methods, processes and results that may be of relevance to risk assessment (see also Chapter 6).

4. GMOs in the Third World

In a Third World context, GM crops in particular have attained a lot of focus. For instance, it is argued that GM crops may enhance global food security, and must therefore be used in poverty alleviation strategies. However, there is a need to consider the implications of the fact that most GM crops are developed and distributed by Western, resource-rich companies with little connection to regional and local realities in the South. For instance, small-scale resource poor farming does not have the same ability to apply management strategies that come with the new technology, as does large-scale farming. Features that distinguish small-scale low input farming from industrial farming (high input) necessitate adoption of procedures for introduction and management of GMOs that are specially designed for such systems. Hence, there is a need to understand the political, socio-cultural and ecological basis for the release of GMOs, not only for large-scale agriculture but also for small-scale, resource-poor farming (Cleveland & Soleri, 2005). Also, internationally recognized strategies for poverty reduction, conservation of biodiversity and sustainability need to be acknowledged when introducing GM crops in poverty alleviation strategies. In

addition, since environmental security is an essential part of successful poverty alleviation, food security strategies have to be environmentally sustainable. In the context of sustainable development, local acceptance and applicability of new farming practices entail that the knowledge and worldviews of local farmers need to have a central role. These needs initialize the development of competence and capacity as well as inclusion and application of traditional knowledge, relating to biodiversity conservation and use as well as to socio-cultural aspects. Broad involvement may also help to integrate different viewpoints and enable wider considerations of risk. This may also enrich the process of scientific investigation by providing knowledge of local conditions and resources. However, many countries in the Third World have yet to implement national regulatory frameworks for regulation of GE applications and GMOs, and many of these countries also lack scientific and administrative capacities to ensure a sustainable introduction of GE applications and release of GMOs. Hence, the need for biosafety capacity building in the Third World is urgent.

5. Implications of a gene ecology approach

Traditional science is challenged with respect to its ability to address complex ecological risk issues, and consequently also the role science plays in policy making. In response, some scientists and sociologists have presented alternatives to traditional scientific activity. Weinberg (1972) introduced the term 'trans-scientific' to describe questions 'which can be asked by science and yet which cannot be answered by science'. Weinberg challenged the authority of science in policy-relevant decision-making processes, and suggested that political and/or additional processes should be essential. Funtowicz and Ravetz (1990; 1993) have introduced the concept of 'post-normal science'. This contrasts traditional and applied science when it comes to responding to uncertainty and inadequacy in quality or 'fitness of purpose' in policy-related research. Post-normal science entails a broad and integrated view for approaching problems in science, by taking into account both the factual and value dimension of the scientific method. This insight rests on two axes, decision stakes and system uncertainty, and the interrelationship between them.

With regard to biotechnology and GE it has recently been argued that there is a need for more comprehensive approaches, such as epigenetics and systems biology, to take into account the inherent complexity. We support this point of view, realizing that the present lack of scientific understanding and the complexity of the recipient ecosystems necessitate implementation of the precautionary principle and precautionary-motivated risk-associated research (see Chapter 17). Such precautionary research is motivated by post-normal science and is a part of what we have defined as the gene ecology approach (Box 7.3).

Box 7.3 Gene Ecology

Gene Ecology is a new interdisciplinary field that is unique in its combination of genetics and biochemistry with bioethics, the philosophy of science, and social studies of science and technology. It builds on innovative work in the areas of genomics, proteomics, food science, ecology, evolution, intellectual property, indigenous rights, participatory technology assessment, and globalization. This systemic approach reverses the trend toward the more reductionistic qualities of the component sciences.

Gene ecology is a central discipline for the comprehensive evaluation of gene-based technologies.

Gene ecology research starts with a list of 'ifs', 'perhapses' and 'maybes' and the objective of the research is to:

- Adopt precautionary motivated research
- Replace uncertain presumptions of risk with science-based comprehension
- Establish experimental models, experimental designs and methods that reflect the ecological interactions and complexity of ecosystems
- Conduct ethical analyses that are closely linked to the understanding of how GE may affect the well being of humans, animals and the natural environment
- Establish a more integrated basis for assessment of the ethical implications of science and regulations related to GE applications.

6. Social robustness

The present concerns of the public with regard to use of GE can be seen as requests for a dialogue with scientists and regulators. This can only be achieved if the public concerns are taken seriously and approached with respect. If this is the case, the debate may attempt to differentiate between specific GE applications and the various arguments for and against a specific GE application. The key determinants with regard to risk perception are distribution of risks and benefits, voluntarism and consent, and degree of familiarity, visibility and control. Perception and acceptance of risk are intertwined, and are influenced by individual as well as cultural and social values (Renn, 1998). Hence, a normative baseline for judging relevance and acceptability of potential adverse effects varies in time and space, and depends on both scientific understanding and other factors, such as social values within a religious, cultural or national context. The public consideration of GE risks represents a broad view that is not exclusively based on scientific risk assessment.

It has been generally believed that gathering more knowledge about technology will reduce the public scepticism. Contrary to this, several reports have highlighted that regardless of the level of knowledge, the public still holds sceptical attitudes towards GE (Gaskell et al., 2000). For instance, the Eurobarometer surveys reveal that high levels of public knowledge do not reduce the demand for more control of GE applications (Eurobarometer, 2006). According to Nielsen (1999), the sceptical group of the public may be separated into two distinct fractions, 'the traditional' and 'the modern', while the proponent groups share characteristics with 'techno-optimists and entrepreneurs'. The proponents of the technology put emphasis on practical benefits, view science and progress as 'a good thing', and estimate risks to be minor and manageable. The 'traditional group' represents 'the blue argument' and voices concern about the rightness of technological intervention and progress on the basis of moral and religious values. The 'modern' sceptics, on the other hand, argue on the basis of a more environmentalist critique and consider present knowledge too limited to allow some GE applications.

GE proponents have assumed that resistance and scepticism to GE applications are based on ignorance and emotions and may hence be labelled 'irrational'. Indeed, it is

possible that over time the present lack of knowledge will be reduced and scientific uncertainty will be either resolved or recognized as 'non-reducible'. Objections related to inherent values, on the other hand, will remain as aspects of GE. Inherent values vary between individuals and socio-cultural contexts. Such 'value-based' arguments are considered the opposite of scientific facts. This view leads to prolonged separation of values and facts, and reinforces stereotypical dichotomies between scientific and public perception of science (Levidow & Marris, 2001).

Differences in perspectives may be considered complementary rather than contradictory. Consequently, value-based arguments should not be underestimated in decision making, and inherent values need to be included independent of their scientific validity. The future of GE may depend on whether the developers and regulators are prepared to increase transparency and involvement of more than just 'scientific facts'. In this case, more awareness concerning scientific uncertainty as well as ethical, cultural and social issues must be raised. It is crucial to recognize that the scientific, economic and social contexts are intertwined with regard to the quality of risk assessment and management. New institutions for participatory processes are needed to strengthen dialogues between stakeholders, with respect to selection of working hypotheses, burden of proof formulations and evaluation of evidence (public participatory methods are further described in Chapter 34).

Conclusion

Ethically responsible decision making must be based on the best available knowledge, but also on the conception of missing knowledge. This requires awareness of the relevant scientific uncertainties and knowledge gaps involved. While it is widely acknowledged that good risk assessment demands uncertainty and ignorance estimations, the common instruments to make uncertainties and scientific ignorance visible are still limited.

Although research on such topics has made significant progress during the last decade, valuable and useful instruments to represent ethical principles need to be established. Furthermore, the reliability of decision making is not only related to the quality of data supporting technical solutions, but also to whether the data are relevant for risk specific goals and conclusions. Ethical aspects relate directly to the scientific description of the risk assessments and management of GE, taking into account the adverse effects and unexpected effects that need to be avoided, as well as the benefits we need to achieve. This may initiate creative thinking about designs of risk-associated research. Truly creative thinking must include proper monitoring of the promised benefits and potential health and environmental risks as well as social, ethical and cultural issues that the communities find important to protect. Adequate evaluation methods can include stakeholder participatory methods: deliberative processes for uncertainty and ignorance assessments, for accommodation of scientific disagreements, and for integration of stakeholder interests and perspectives.

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Chapter 8

Genetically Engineered Cells and Organisms: Substantially equivalent or different?

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The dynamic and interconnected regulation of the genome is now slowly being revealed. The genome does not function in a constant, stable and linear fashion, but is instructed by and fine-tunes its activities according to networks of signals received from the external ecosystem and the internal environment of the organism. The genomic signal pathways may be modified by ecosystem variation as well as by physiological changes in the organism. Thus, the chromatin structure, the genome, the epigenome, the transcriptome, the proteome, the metabolome, and the interactome are interlinked and intertwined in various ways with information transfer in multiple directions.

Integration of foreign DNA into an established genome may have unanticipated side-effects, e.g. in terms of chromatin changes, genome instability, unexpected protein products from the transgene(s), and influence on overall organismal gene expression patterns, in quantitative as well as qualitative terms, of the recipient organism. In this chapter we discuss and exemplify, from a precautionary point of view, the changes that may occur in modified genomes and the consequences they may have. We structure the discussion as follows:

1. Lack of precision in recombinant DNA techniques
2. Changes in the genome
3. Changes in the transcriptome
4. Changes in the proteome
5. Changes in the metabalome
6. Changes in the epigenome
7. Changes in the interactome
8. Concluding remarks

1. Lack of precision in recombinant DNA techniques

Genetic engineering (GE) techniques are presented by many as a tool for the safe and predictable production of GMOs. The intended change in gene expression in GMOs is, however, often not simply a matter of transcription and translation of the inserted recombinant DNA sequences, as symbolized by the Central Dogma model (see Chapters 3, 5, 9, and 13). While achieving a stable, single-copy recombinant DNA insertion is the aim of the genetic engineer, it is not the norm.
Available methods for transfer of gene constructs into cells are inefficient and imprecise (see Chapter 4). Insertional mutagenesis is a default consequence of recombinant DNA

insertions. The resulting phenotypic consequences of the insertion events are largely determined by the characteristics of the gene transfer vector and the location of and number of copies inserted per cell.

While many emphasize the precision of recombinant DNA techniques, none of the currently available methods permit predetermination of *where* in the recipient cell-DNA our gene construct will be inserted, or the number of copies that will be inserted into GMOs of commercial relevance. The specific locations of the inserts may nevertheless substantially influence the functions of the inserted DNA as well as its effects on the cell's own genes. For instance, within the same transformed/transfected mammalian cell culture we will find cells with quite different characteristics.

These, in principle indefinite number of variants arise due to varying insertion sites and number of full or partial DNA copies. In addition to full vector copies, a number of rearranged or truncated versions, some of them quite small, may be inserted into some cells. These aberrant versions can still influence the integrity and functions of the recipient genome, and they may go undetected by conventional testing.[1] Impacts arising from uncharacterized insertions cannot be predicted from characterized insertions. Furthermore, if the characterized inserts are identical between, for example, two recombinant maize lines (events), but the insertion sites are different, one *cannot* extrapolate any biosafety conclusions from one line (event) to the other. The context of the insert would obviously be different, as would be the genes that may be affected directly or indirectly and therefore also the resulting plant phenotype.

The integration of foreign DNA (transgene) in a new host genome may influence any of the gene expression control processes described in Chapters 1 and 3. New gene products may also arise and the transgene product may also vary in its properties. For instance, read-through transcription, initiated somewhere in the insert and ending outside it, or initiated in adjacent regions and ending in the insert, may be sources for novel RNAs and recombinant proteins.[2]

The consequences of insertion may, as earlier stated, vary considerably according to the exact insertional locations and/or construct organization. This is valid for the expression of the inserted transgene as well as for changes in the recipient organism's own genes and their expression levels. The insertion may have effects by introducing a change in chromatin structure, the topography as well as the proteins binding to the DNA (Recillas-Targa, 2006), or by inducing changes in DNA methylation patterns and other epigenetic characteristics (see Chapter 5). Furthermore, cis-acting regulatory DNA motifs may be present in the insert, or may arise from the 'new' sequences created by integration that

[1]It is a common phenomenon for transgene constructs to integrate in multiple places in the genome, and for very small parts of the construct to integrate independently of full-sized versions (for recent comprehensive reviews, see Filipecki & Malepszy, 2006; Latham et al., 2006).

[2]Abortive transcription from read-through might, for example, produce novel short and double-stranded (ds)RNA molecules. A risk factor emerging from the production of novel dsRNA is the potential to induce gene silencing either locally, or on other genes. The same dsRNA can have different effects at different concentrations, in some cases showing non-specific effects at concentrations lower than those needed to induce silencing (Zhao et al., 2001). It should also be appreciated that any new RNA transcript may undergo, as described in Chapter 3, a large series of modifications that result in 'a family' of different RNA molecules, all derived from the same original source. The family members do not necessarily give rise to the same proteins or even proteins with similar functions.

can alter the expression level of genes adjacent or even distant to the insert.

GE cell cultures may be used to produce recombinant products *under contained laboratory conditions*. This implies that the product that the gene is coding for (e.g. insulin) is extensively purified before it is taken out of the laboratory, while the GE cells and DNA are destroyed inside the laboratory. Such applications of GE may, in principle, be made safe. However, when recombinant cells are developed and placed in the open environment, changes in the gene expression levels and small metabolite contents will vary according to changing ecosystem conditions.

Under the influence of given sets of ecosystem variables, the recombinant organisms may over time expose phenotypic traits that have environmental or consumer health implications. 'Consumers' may include a number of wildlife species in addition to humans and domestic animals. From biosafety/risk assessment/regulatory points of view it is hence imperative to reveal whether, compared to its unmodified counterpart, a GMO has experienced changes in the interacting regulatory parts, its *'interactome'*: the genome, epigenome, transcriptome, proteome, and metabolome working as overlapping layers of information involved in cellular function (Box 8.1). Only when minimal changes are observed will it be justified to claim 'substantial equivalence'.

Box 8.1 The '-omes' and the '-omics'

Genome: 1) The entire collection of genetic material in an organism, virus or organelle. 2) The haploid set of chromosomes (DNA) of a eukaryotic organism.

Genomics: The study and development of genetic and physical maps, large-scale DNA sequencing, gene discovery, and computer-based systems for managing and analysing genomic data.

Proteome: The full complement of proteins that are found in a particular cell or tissue under a particular set of circumstances. May include information on their relative or absolute abundance.

Proteomics: The study of the structure and expression of proteins, and of the interactions between proteins.

Interactome: The complete collection of all physical protein-protein interactions that can take place within the cell.

Interactomics: The study and construction of comprehensive sets of protein-protein interactions.

Transcriptome: The full complement of expressed gene transcripts, including alternative splice variants that are found in a particular cell or tissue under a particular set of circumstances. This may include information on the relative or absolute abundance of transcripts.

Transcriptomics: The study of the full complement of expressed gene transcripts. Several techniques have been developed for parallel analysis of the expression of thousands of genes, most notably cDNA microarrays and oligonucleotide arrays.

Metabolome: The assembly of substrates, metabolites, and other small molecules that is present in a population of cells.

Metabolomics: Study of the structure and distribution of all metabolites (small molecules), particularly organic compounds.
Functional genomics: A whole spectrum of approaches, under development, to ascertain the biochemical, cellular and/or physiological properties of each and every gene product and its regulation. These include near-saturation mutagenesis (i.e. screening hundreds of thousands of mutants to identify genes that affect traits as diverse as embryogenesis, immunology and behaviour), high-through put reverse genetics (methods to systematically and specifically inactivate individual genes), and elaboration of genetic tools.
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2. Changes in the genome

The whole purpose of a transgenesis process is of course to change the genome of the recipient organism. There are a number of possible, unpredictable consequences of DNA insertions in GMOs. They may be sorted into the following categories:

1. Genome destabilization
2. Chromatin changes with consequences for transgene as well as genome gene expression
3. *De novo* methylation of the transgene or spread of the transgene methylation pattern to endogenous genes, i.e. epigenomic effects
4. Introduction of new regulatory elements, e.g. promoters, enhancers and dehancers, known or hidden splice sites, start codons, terminators, etc. These may cause:
    a. Unpredictable, environment-dependent level of transgene expression, and
    b. Unpredictable, environment-dependent influence on expression pattern of recipient genome in terms of:
        i. Signal transduction-dependent *promoter* effects
        ii. Signal transduction-dependent *enhancer/silencer* effects
        iii. Signal transduction-dependent effects of transferred DNA methylation patterns
5. Activation of endogenous mobile elements (‘jumping genes’). Once activated, they may engage in:
6. Reinsertion at new chromosomal loci
7. Horizontal gene transfer to other individuals or species
8. Unanticipated and unpredictable changes in gene products, e.g. by posttranslational modifications
9. Silencing or over-expression of genes.

Some prominent uncertainties are related to the fact that the recipient organism receives a new promoter/enhancer. These elements govern the gene expression levels of their attached transgenes, but after insertion, they may also change the gene expression and methylation patterns in the recipient chromosome(s) over long distances up- and downstream from the insertion site. Promoters/enhancers function in response to signals received from the internal or external environment of the organism. For a GMO this may result in unpredictability with regard to:

- The chromatin organization and contents of the recipient genome
- The expression level of the inserted transgene(s)
- Altered expression of a large number of the organism's own genes
- Altered influence of geographical, chemical (i.e. *xenobiotics*) and ecological variables of the environment
- Transfer of vector sequences within the chromosomes of the organism, and vertical and/or horizontal gene transfer to other organisms.

Few published studies have been devoted to the clarification of such putative changes in GMOs.

2.1 Observations from studies of GM plants[3]

Agrobacterium-mediated gene transfer to plants can result in insertion site mutations of the T-DNA, leading to truncations, interspersions, or other complex rearrangements of the recombinant DNA. Superfluous T-DNA integration frequently accompanies *Agrobacterium*-mediated transformation, where whole and partial copies of the transgenes become integrated.

For example, a molecular analysis of *Agrobacterium*-transformed *Arabidopsis thaliana* plants revealed that 80% of the transformants had a single insertion event; of these, only 22% contained a single copy of the transgene (the desired number for stable integration and expression in transgenic lines), and the remainder of these single-insertion events contained incomplete T-DNAs, tandem T-DNAs, or T-DNA fragments. These results indicate that even relatively simple T-DNA insertions undergo large- or small-scale rearrangements during the transformation process.

Plants transformed via particle bombardment methods are often more likely than *Agrobacterium*-mediated transformed plants to demonstrate complex integration patterns. The majority of integrated DNA is either arranged as multiple copies of the intact transgene, or as multiple copies with interspersed plant genomic DNA. Further, short recombinant DNA fragments may frequently integrate along with intact or rearranged multimers.

In a study of transgenes integrated into two lines of transgenic oat, 50 of the 82 transgene fragments identified (61%) were 200 bp or shorter. One study even reported the presence of bacterial DNA at a particle bombardment insertion site. As with *Agrobacterium*-mediated transformation, simple single copy insertion events tend to be the exception, and complex and errant integration the rule.

Given the complex transgene integration locus patterns accompanying transformation, developing a transgenic plant line requires careful selection of stable and high expressing transformation events for product development. However, the initial transformation process is not the only step where the transgenes might undergo significant rearrangement. Tissue culture is a common means to produce sufficient transgenic germplasm for further product development. During this process, undesirable tissue

[3] For further information and references, see the recent review by Latham et al., 2006.

culture-induced genetic rearrangements, termed *somaclonal variation*, can occur in both conventional and transformed lines.

Further along the development of the transgenic plant line is selective crossbreeding with elite crop germplasm for high agronomic performance. This process involves a number of introgressive hybridizations (introgression and subsequent backcrossing) to produce plants homozygous for the recombinant trait in the elite crop line. During this process, the complex nature of the recombinant DNA integration loci can lead to deviations in the expected Mendelian patterns of inheritance. [4] For instance, these irregular patterns have been observed during inheritance in lettuce (McCabe & Mohapatra, 1999), rice, maize, and barley. Subsequent selection procedures of the GM material may also introduce further genomic reorganizations (Hernandez et al., 2003).

2.2 Why do DNA rearrangements occur?

In plants, exogenous DNA transfer (e.g. with *A. tumefaciens* pathogenesis) elicits a wound response that activates nucleases and DNA repair enzymes. The transferred DNA is thus either degraded or used as a substrate for DNA repair, resulting in its potential rearrangement and incorporation in the genomic DNA (Takano et al., 1997). Furthermore, specific transforming plasmid structure and construct properties can enhance recombination events all along the transformation process. Indeed, some genetic elements can act as hotspots and undergo recombination at high frequency. This is, for example, the case for the 3' end of the CaMV 35S promoter, which contains an imperfect palindrome of 19 bp.

Illegitimate recombination can also occur in the borders of the Ti plasmid of *Agrobacterium tumefaciens*, especially in the right border that contains an imperfect palindromic sequence of 11 bp. The 3' end of the *nos* terminator is also theoretically highly prone to recombination (Kohli et al., 1999). Hot spots for recombination may lead to tandem transgene repeats with interspersed plant DNA sequences in a single genetic locus. Presence of several inserts may also result from multimerization in the plasmid before transformation or from multiple insertions.

A number of transgenic and genomic rearrangements have been reported for already commercialized transgenic crop plant varieties. The nature of these rearrangements and what they may mean in a risk assessment context is further discussed in Chapter 9.

3. Changes in the transcriptome

The intention of a transgenic process is to have the transgene expressed. Hence, the intended change is to add one transcript to the transcriptome of the GMO. However, as

[4]Given the likelihood of transgene reassortment during one or more of these steps in the production of a transgenic line, arriving at a stable and well-performing transgenic line requires the careful selection from many transformation events brought through development. Technical dossiers on commercial crop lines invariably suggest the stability of the inserted construct. Yet how robust are these analyses? Documentation of transgene locus structure (organization and copy number) and stability through inheritance in the scientific literature (as well as in applications for commercial approval) almost always rely on Southern blot analysis to demonstrate transgene copy number and integrity of the single-copy inserts. However, recent studies have determined that Southern blot analysis often lacks sufficient resolution to accurately determine copy number or transgene organization, and may have difficulties in detecting small rearrangments or solitary fragments (Hoebeeck et al., 2007).

discussed in Chapter 3, and earlier in this chapter, the inherent lack of insertion precision may lead to the expression of additional, unintended transcripts as well.
Although only a small number of published studies have been designed to reveal transcriptome aberrations in GMOs, there are published studies that exemplify the following:

1. Qualitative transcriptome changes, due to inefficient terminator motifs in a transgenic plant variety
2. Quantitative transcriptome changes, due to the influence of the transgene regulatory sequences on endogenous genes located close or distant to the insertion site.

3.1 Example of new transcripts originating from a plant transgene
New evidence suggests that the nos terminator sequence used in a number of transgenic plant varieties is a recombination hotspot, prone to read-through, and may contain a cryptic cis-acting splice sequence that could generate novel RNA molecules and proteins at any place it is inserted into the genome (Rang et al., 2005).

The Roundup Ready (RR) soybean varieties derive from a soybean line into which a gene coding for glyphosate-resistant enol-pyruvylshikimate-3-phosphate-synthase (EPSPS) was introduced. The insert and the flanking regions in RR soybean have recently been characterized. It was shown that a further 250-bp fragment of the *epsps* gene is localized downstream of the introduced *nos* terminator of transcription, derived from the nopaline synthase gene of *Agrobacterium tumefaciens*. At least 150 bp of this DNA region is transcribed in the RR soybean variety.

Transcription of the additional fragment depends on whether read-through events ignore the *nos* terminator signal located upstream. The data indicate that the read-through product is further processed, resulting in four different RNA variants from which the transcribed region of the *nos* terminator is completely deleted. Deletion results in the generation of open reading frames which might code for (as yet unknown) EPSPS fusion proteins. The *nos* terminator is used as a regulatory element in several other transgenic plants intended for food production. This implies that read-through products and transcription of RNA variants might be a common feature in such plants.

3.2 Examples of the activity of the 35S CaMV plant promoter in mammalian cells
In most of the transgenic crop plants commercialized, the transcription of the transgene is governed by the 35S promoter taken from the Cauliflower Mosaic Virus (CaMV). CaMV is a DNA-containing para-retrovirus that replicates by means of reverse transcription. It was earlier assumed that the 35S promoter exclusively functions in plants, and that it would therefore not represent a food/feed safety issue if the transgene under the control of such promoter would transfer horizontally. The following quote is representative of this assumption: ‘There have also been (scientifically unfounded) concerns that the strong plant virus promoter used to express transgenic DNA might be active in mammalian cells’ (Gasson & Burke, 2001).

There have now been published studies indicating that the 35S CaMV promoter has potential for transcriptional activation in mammalian systems, in addition to studies in

different yeast species. First, 35S promoter activity was demonstrated in human fibroblast cell cultures, thereafter in hamster cells, and very recently 35S promoter activity was established in human enterocyte-like cells (Myhre et al., 2006). Such cells line the surface of human intestines, and are hence highly relevant to whether uptake of transgenic DNA from the gastro-intestinal tract may have effects on the host if unintentionally taken up. However, no published studies have investigated 35S CaMV activity *in vivo*, and this is hence an obvious area of omitted research. This example illustrates how safety assumptions/claims made in the absence of experimental investigation on the issue can be misleading.

3.3 Example of upregulation of an endogenous gene under the influence of a transgene promoter

X-Scid is a disease linked to a defective gene on the X chromosome that leads to a total breakdown of the immune system due to lack of T cells. Victims are known in the media as 'bubble boys', having to live their short lives within totally contained plastic cubicles, since every kind of innocent infection will kill them.

A gene therapy protocol was developed in order to cure, or at least alleviate the symptoms of X-Scid victims. Bone marrow cells were taken from the patient and grown in culture. The cells were transfected with a vector that contains a healthy copy of the defective gene. The vector was a deletion mutant of MLV (murine leukaemia virus), with the transgene under control of a strong promoter. After having the bone marrow cells controlled for expression of the transgene, and observing a lack of any unwanted phenotypic characteristics, the cells were returned to the patient. The rationale was that the transferred healthy gene, following integration into the genomes of the bone marrow cells, should produce the proteins that make production of T cells possible, and hence provide the patient with a functional immune system.

In an initial series of 11 treated patients, the strategy seemed to work according to plan, until a tragic setback was recognized: one of the treated patients developed a highly aggressive type of cancer. It turned out that in treated cells from this patient, the gene transfer vector had integrated into a genomic location next to the *Lmo2* gene. This gene encodes a protein product that is known to be cancer causing when over-expressed. In the present case, the strong promoter of the gene therapy vector had forced the *Lmo2* gene to over-express. In a commentary article in *New Scientist* these events were dubbed 'Gene therapy's worst nightmare'. Yet what was observed was an illustration of the known insertion site unpredictability of current recombinant DNA techniques.

3.4 Does 'transvection' occur during transgenesis in mammalian cells?

A relevant question to ask is whether known, unknown or hidden DNA motifs in the gene vector, including its plasmid backbone sequences, may act as transcriptional enhancers and hence influence transcription of endogenous genes, whether integrated in the host genome or present on an un-integrated vector. Transcriptional enhancers are relatively short (30–500bp), *cis*-acting DNA sequences usually comprised of several binding sites for TF (transcription factor; see Chapter 3) activator proteins. The hallmark of enhancers is their ability to communicate with promoters, often activating genes over a large

distance. Some enhancers are able to activate promoters in *trans*, i.e. when the enhancer is on a different genomic entity than the promoter.

Recent studies (D'Aiuto et al., 2006) have demonstrated that a CMV (human cytomegalovirus) enhancer can increase the activity of its cognate promoter in *trans*, in the absence of factors that physically bring the enhancer into close proximity of the promoter. A process like this is called *transvection*. Interestingly, the authors also provided evidence that the CMV enhancer may activate other promoters in the modified host genome. Because such transactivation effects may result in unwanted or unexpected transcriptional activation of endogenous genes, these findings are important for conception of the range of transcriptional effects expected in various genetic engineering and gene therapy approaches.

4. Changes in the proteome

Inherent to a recombinant organism is one or more intended proteomic changes, namely the expression of the transgenic protein(s) that will confer the desired new trait or property.

As earlier indicated in the present chapter, integration of foreign DNA may lead to additional quantitative and qualitative differences in the expressed proteins in a modified cell. Chapter 3 outlined some of the cellular processes that may lead to unexpected protein products from any given gene sequence. All these processes also apply to transgenes as well. Unfortunately, there are few published studies that have systematically compared the proteomes of GMOs to their unmodified counterparts. There are, however, two examples that illustrate the profound and unpredictable differences in the biological functions of a recombinant protein when it is being post-translationally modified, i.e. glycosylated, in its new host organism.

4.1 An α-amylase inhibitor-1 gene transferred from common bean to pea

It was recently shown that expression of a recombinant plant protein (α-amylase inhibitor-1, αAI) from the common bean in a non-native host plant, i.e. transgenic pea, led to the synthesis of a structurally modified, probably aberrantly glycosylated form, of this inhibitor (Prescott et al., 2005). Employing models of inflammation, it was demonstrated that consumption of the modified αAI and not the native form predisposed the mice to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the modified αAI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these usually non-immunogenic proteins. This investigation demonstrated that recombinant expression of non-native proteins in plants may lead to the synthesis of structural variants with altered immunogenicity. The frequency at which alterations in structure and immunogenicity of recombinant proteins in new hosts occur is most often not known.

4.2 Production of recombinant protein in milk

The European Medicine Agency's (EMEA) decision in February 2006 to approve a recombinant product containing anthithrombin-α, had been eagerly awaited because it would be the first drug produced in a transgenic farm animal to reach the market. The

active ingredient, *human anthithrombin-α*, is produced by and purified from the milk of transgenic goats. GTC Biotherapeutics has been developing Atryn since 1993, principally for treating patients suffering from hereditary anthithrombin deficiency, a rare condition affecting one person in every 3–5000, that puts them at increased risk of deep vein thrombosis.

The decision of EMEA was, however, based on a lack of appropriate data to allay concerns about Atryn's immunogenicity. As pointed out by an anonymous editorial commentator in *Nature Biotechnology* (2006, 24: 368), the EMEA decision '*rather skirts around some of the underlying issues that transgenic protein producers have to face*'. These issues are discussed in Chapters 3 and 4, in addition to the present chapter of this book.

Of particular concern are different and unpredictable posttranslational modifications compared to native proteins. In the case of Atryn, this really seems to matter. Compared with a conventional anthithrombin-α product, Atryn's serum half-life was reduced seven- to ten-fold, necessitating infusion of the protein rather than a one-off injection.
One of EMEA's main concerns with Atryn was, however, its potential immunogenicity. The underlying problem is that it is extremely difficult to produce 'nature-identical' proteins in milk from transgenic animals. For instance, in cows, sheep and goats, glycosylated proteins typically contain N-glycolylneuraminic acid (NGNA), a modification which is virtually absent in native human proteins. Furthermore, the high concentration of protein produced in milk, around a gram per litre, overrides the glycosylation capacity of the mammary gland. Only rabbits and chickens have human-like glycosylation patterns. The *Nature Biotechnology* commentator concluded: '*Thus, if immunogenicity of milk-produced proteins turns out to be a generic problem, then a whole class of transgenic production methods may turn out to have a limited future. Chicken milk, anyone*?'

5. Changes in the metabolome

Unintended effects of transgenesis are closely related to changes in the metabolite levels. One of the major challenges is how to analyze the overall metabolite composition of GMOs in comparison to their unmodified counterparts. Metabolomics offer one possible solution.

The quality of crop plants is a direct function of the metabolite content. The metabolome determines the flavour, aroma and texture of crops, their storage properties, nutritional values and performance in the field. Genetic (metabolic) engineering has the potential to improve plant properties. However, problems may arise from such approaches because the organismal metabolism forms a large interconnected network. '*Just as the flap of a butterfly wing might cause a hurricane, changes in the flux of one branch might lead to unexpected changes in other parts of the network*' (Memelink, 2005).

A number of unexpected changes following genetic engineering have been seen in experimental studies with, for instance, *Arabidopsis* sp. and tomatoes (e.g. Romer et al., 2000; Hemm et al., 2003). Field trials with transgenic wheat lines have demonstrated how

profoundly the environment affects the metabolome of transgenic as well as unmodified varieties, but have also demonstrated important differences between a transgenic wheat line and its parental, unmodified counterpart (Baker et al., 2006).

Potatoes produce a number of toxic secondary metabolites, which are divided into two groups: the sesquiterpenes and the glycoalkaloids (PGAs). Whereas PGAs are largely produced and present in toxic quantities in both the foliage and 'green' potatoes, it is well documented that the levels of PGAs and sesquiterpenes are affected by biotic and abiotic stress. The development of GM potato varieties has made it prudent to ascertain whether there may be changes in the amounts or types of these secondary metabolites, either as a direct effect of the transgene or due to its interactions with environmental variables. One such study has been published by Matthews et al. (2005). Transgenic potato lines were exposed, along with non-transgenic lines, to a range of biotic and abiotic stresses and a range of environmental conditions in the field and store. Following stress, a comparison was made of levels of potato glycoalkaloid and sesquiterpene levels between the two groups. Significant differences were observed in the levels of both glycoalkaloid and sesquiterpene levels between transgenic and control material and between infected and noninfected material. The study did, however, also illustrate the profound impact that environmental parameters may have on the metabolome of transgenic as well as unmodified potatoes.

6. Changes in the epigenome

Epigenetic changes[5] can be induced in cells during the transgenesis process, and to become inherited in the consecutive generations (Filipecki & Malepszy, 2006). It is, however, difficult to ascertain whether epigenetic imprinting is due to the transgenesis or cell regeneration techniques. It is known from a number of organisms that an inserted DNA fragment may both transfer its own methylation pattern to the surrounding DNA and have its own pattern changed by the surrounding recipient DNA.
The transgenesis process may induce mutagenic-stress related mechanisms described as 'programmed loss of cellular control'. According to Filipecki and Malepszy (2006), this may lead to (i) genetic changes such as polyploidy, aneuploidy, chromosome rearrangements, somatic recombination, gene amplifications, point mutations, and excisions and insertions of retrotransposons, and (ii) epigenetic changes, including DNA methylation and histone modifications.

Regulation of gene expression by induced changes in DNA methylation is a very potent regulatory mechanism. DNA methylation is based on the existence of 'the 5th base' (see Chapter 5). Transgenesis may induce methylation changes in both directions:

- DNA *hypomethylation* leading to
    - Gene activation

[5]Epigenetics (see also Chapter 5) was introduced by Conrad Waddington in 1942 as the study of the processes by which genotype gives rise to phenotype. In 1987, Robin Holliday redefined epigenetics as: 'Nuclear inheritance which is not based on differences in DNA sequence'. Epigenetics encompasses heritable changes in DNA or its associated proteins except mutations in gene sequence. Many investigators in the field of epigenetics focus on histone modifications and DNA methylation, two molecular mechanisms that are often linked and interdependent.

    - Chromosome instability
- DNA *hypermethylation* leading to
    - Gene silencing
    - Chromatin remodelling
    - RNA-associated silencing.

In recombinant plants, DNA methylation changes may occur in both directions, but *hypomethylation* has been more frequently reported. Already in 1996 it was clearly demonstrated that different epigenetic expression states might arise in transgenic plants regenerated from the same material (Matzke & Matzke, 1996), and that these states are stably inherited to the following generations.

As pointed out earlier, the influence of the environment on the initiation and persistence of epigenomic programmes cannot be overestimated, but this is an area of omitted research. In spite of a considerable number of peer-reviewed articles concerning epigenetic consequences of transgenesis in model organisms such as *Arabidopsis*, the epigenomes of marketed, transgenic crop plants are virtually unknown.

7. Changes in the interactome

The concepts and technologies of classical molecular biology have dominated genetic engineering approaches during the last 50 years. This has favoured methods that have approached complex processes by separation and isolation of single pathways and molecules. Nonetheless, biologists have continually been aware that a fundamental characteristic of all biological organization is that functional units never exist in isolation. Biological complexity is based on synergistic cooperation achieved by interactions between the components of the cell (Uhrig, 2006). Proteins are essential for almost all biological processes. They operate entirely on the basis of interactions with other molecules, i.e. other proteins, nucleic acids, lipids, or low molecular metabolites and other compounds.

Only rarely is the protein monomer the functionally active form, as most often assumed when using transgenes. Comprehensive knowledge of protein interactions is therefore an important source of information to functionally annotate proteins and to understand and model processes on a genome-wide level (see also Chapter 3). That the transgenic protein product provides the intended function and trait (e.g. insecticidal effects or herbicide tolerance in plants) does not preclude that it contains additional active domains that become evident in its new genomic, biological and environmental host context. Such ‘novel’ domains may be inherent in the amino acid chain, or arise as a result of alternative folding due to host-specific post-translational modifications (see Chapter 3). The recombinant protein may therefore engage in complex formations with endogenous proteins and other cellular components when present in novel environments. This may, in turn, lead to activation or inhibition of cellular processes, or even create new intracellular processes. To what extent this occurs is unknown, since the studies needed for clarification are rarely conducted.

8. Concluding remarks

As stated by Haslberger (2006), there is a general need for a holistic and integrated basis for assessment of the properties and effects of GMOs. This conclusion was also drawn by a recent World Health Organization (WHO) report (2005). Lack of knowledge concerning the putative and unpredictable changes in the contents of GMOs discussed in this chapter have won increasing acceptance during recent years. A fact that has been reflected in a number of expert committee reports from international organizations such as WHO, the Food and Agriculture Organization (FAO), and the Organization for Economic Cooperation and Development (OECD). Many of the risk issues identified here that lack answers (see also Chapter 9) were identified before the first transgenic plants were commercially grown in 1996. The application of the modern '-omics' techniques can contribute to reveal many risk-relevant differences in composition between recombinant organisms and their isogenic, parental counterparts under relevant environmental conditions.

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Chapter 9

Genetic Engineering and Omitted Health Research: Still No Answers to Ageing Questions

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Introduction

Some of the most crucial scientific questions concerning the health effects of genetic engineering (GE) and genetically modified organisms (GMOs) were raised up to twenty years ago.[1] Most of them have still not been answered at all, or have found unsatisfactory answers. We believe, as Mayer and Stirling[2] said, 'in the end it is often the case that those who choose the questions determine the answers'. Will another twenty years pass before societies realize the urgent need for public funding of genuinely independent risk- and hazard-related research? The time for such investment is now, so that a new scientific culture with working hypotheses rooted in the Precautionary Principle (PP)[3] can discover other, possibly even more important questions of safety.

In this chapter we will mainly confine ourselves to putative health hazards related to GM plants used as food or feed, with some brief notes on GM vaccines as well as the novel RNAi- and nanobio-technologies. Our focus is not because we do not recognize the paramount, indirect threats to public health posed by social, cultural, ethical, and economic issues, as well as the complexities posed by the relevant legal and regulatory environments, but for reasons of space. In the specific context of food or feed safety assessment, 'hazard' may be defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The hypothetical hazards of whole GM foods, i.e. those hazards that have been realized so far, fall into a few broad categories.

First, there are those either related to the random and inaccurate integration of transgenes into recipient plant genomes, with uncertainty with regard to direct or indirect effects of the polypeptide product of the transgene, or uncertainty with regard to DNA types and circumstances promoting uptake and organ establishment of foreign DNA from mammalian gastro-intestinal tracts.[4] Second, there are those that might come from the purposeful production of potential hazards, such as allergens or powerful pharmaceutical products.

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[1]See for instance: Freese, W. and Schubert, D. (2004). Safety testing and regulation of genetically engineered foods. Biotechnology and Genetic Engineering Reviews 21: 299-324, or Pusztai, A. (2002). Can science give us the tools for recognizing possible public health risks for GM food? Nutrition and Health 16: 73-84.
[2]Mayer, S. and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024.
[3]Myhr, A.I. and Traavik, T. (2002). The precautionary principle: scientific uncertainty and omitted research in the context of GMO use and release. Journal of Agricultural and Environmental Ethics 15: 73-86.
[4]For a recent, authoritative review see: The Royal Society of Canada (2001). Elements of Precaution: Recommendations for the regulation of food biotechnology in Canada. An expert panel report on the future of food biotechnology prepared by the Royal Society of Canada for Health Canada, Canadian Food Inspection Agency and Environment Canada (ISBN 0-920064-71-x), www.rsc.ca/foodbiotechnology/index/EN.html

A number of scientific concerns have been raised in connection with public and animal health. In the following sections we will discuss, in some detail, a few of these. Some of them have been thoroughly discussed in excellent, recent reviews.[5]

Our contribution is based on 'gene ecology', *a new, cross-disciplinary scientific field aimed at providing holistic knowledge based on the Precautionary Principle.*[6] Some of the concerns we raise will also be relevant for environmental risk assessments of GMOs, due to the fact that the processes discussed can take place in large ecosystems as well as in the ecosystems at the scale of the human being.

Do we know whether any GM food/feed is safe for consumption?

For a composite material such as food/feed, reductionist approaches testing single components *in vitro* are highly unsatisfactory and cannot clarify important safety issues. In spite of the obvious need, very few studies designed to investigate putative effects of GM nucleic acids or food/feed on potential animal or human consumers have been published in peer-reviewed journals.[7] A consensus has emerged that the effects observed in some published studies[8] must be experimentally followed up. To date, this has not been done.

Most of the animal feeding studies conducted so far have been designed exclusively to reveal husbandry production differences between GMOs and their unmodified counterparts. Studies designed to reveal physiological or pathological effects are extremely few, and they demonstrate a quite worrisome trend[9]: Studies performed by the GM plant producers find no problems, while studies from independent research groups often reveal effects that should have merited immediate follow-up, confirmation and extension. Such follow-up studies have not been performed. There are two main factors accounting for this situation: The lack of funds for independent research, and the reluctance of producers to deliver GM materials for analysis.[10]

Can we rely on the transgenic DNA sequences given by GM food/feed producers?

If the transgenic DNA sequences given in the notifications differ from the inserted sequences found in the GM plants, the risk assessments made prior to approval of the GM plants for marketing do not necessarily cover the potential risks associated with the GM plants.
The most thoroughly studied transgenic events are:

- Bt-transgenic maize Mon810
- Bt- and glufosinate-transgenic maize Bt176
- Glyphosate-transgenic maize GA21
- Glufosinate-transgenic maize T25 (Liberty Link)
- Glyphosate-transgenic soybean GTS 40-3-2.

[5]See Footnote 1, and e.g. Pusztai, A., Bardocz S. and Ewen S.W.B. (2003). Genetically modified foods: potential human health effects. Pp. 347-371, in Food Safety: Contaminants and Toxins, edited by JPF D'Mello. CAB International.

[6] For further information see the homepages of GENOK-Norwegian Institute of Gene Ecology, www.genok.org and INBI-Centre for Integrated Research in Biosafety, www.inbi.canterbury.ac.nz

[7]Domingo, J.L. (2000). Health Risks of GM Foods: Many opinions but few data. Science 288: 1748-1749.

[8]E.g. Fares and El-Sayed (1998). Fine structural changes in the ileum of mice fed on endotoxin-treated potatoes and transgenic potatoes. Natural Toxins 6(6): 219-233; Ewen and Pusztai (1999). Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. The Lancet, Vol. 354, 16 October 1999.

[9]Pryme, I.F. and Lembcke, R. (2003). In vivo studies on possible health consequences of genetically modified food and feed – with particular regard to ingredients consisting of genetically modified plant materials. Nutr Health 17(1): 1-8.

[10]For documentation and further reading see Footnotes 1 and 2 and references therein.

Even amongst the most thoroughly studied and some of the oldest commercial GM plants, recent independent work has revealed that rearrangements occur in transgene inserts and the nature of the rearrangements varies. Deletions (Mon810, GA21, Bt176), recombination (T25, GTS 40-3-2, Bt176), tandem or inverted repeats (T25, GA21, Bt176), as well as rearranged transgenic fragments scattered through the genome (Mon810) have been reported.[11]

The transgenic modification techniques are prone to introduce such rearrangements because exogenous DNA transfer in plants elicits a 'wound' response, which activates nucleases and DNA repair enzymes. This may result in either degradation of the incoming DNA, or insertion of rearranged copies into the plant DNA.[12] In addition, the nature of the DNA constructs used to make transgenic plants may influence the rearrangement tendencies for a given transgenic event. Some genetic elements in the constructs may act as 'hotspots' and elicit recombination at high frequencies.[13]

While it was earlier assumed that integration of transgenic constructs took place at random locations in the recipient plant genome, it has now become apparent that integration sites are often concentrated in or near elements such as retrotransposons (T25, Mon810, GA21) and repeated sequences (Bt11 maize),[14] and this poses additional risks. Firstly, by introducing a new promoter or new enhancer motifs, transgenic insertions into, or close to, such elements may lead to altered spatial and temporal expression patterns of plant genes located close to and even far from, the insert. Secondly, a strong retrotransposon LTR promoter may upregulate the transgene expression level. Thirdly, defective retrotransposons may start 'jumping' under the influence of transacting factors recruited by the insert.[15] All these events may have unpredictable effects on the long-term genetic stability of the GMOs, as well as on their nutritional value, allergenicity and toxicant contents. These putative processes represent areas of omitted research with regard to health effects of GMOs.

[11]Hernandez et al. (2003). A specific real-time quantitative PCR detection system for event MON810 in maize YieldGuard based on the 3'-transgene integration sequence. Transgenic Research 12: 179-189; Holck et al. (2002). 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified MON810 MaisGard maize. Eur Food Res Technol 214: 449-453; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity?; Windels et al. (2001). Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 213: 107-112; Rönning et al. (2003). Event specific real-time quantitative PCR for genetically modified Bt11 maize (Zea Mays). Eur Food Res Technol 216: 347-354.

[12]Takano et al. (1997). The structures of integration sites in transgenic rice. The Plant Journal 11(3): 353-361; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity? In addition to cellular mechanisms controlling the transgene integration, subsequent selection procedures of the GE material may introduce further genomic reorganisations (Hernandez et al. (2003). A specific real-time quantitative PCR detection system for event MON810 in maize YieldGuard based on the 3'-transgene integration sequence. Transgenic Research 12: 179-189).

[13]This is the case for the 35S CaMV promoter that is present in most GEPs marketed so far, and also for the Ti plasmid of Agrobacterium tumefasciens and the nos terminator (Kohli et al. (1999). Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. The Plant Journal 17(6): 591-601; Collonnier et al. (2003). Characterization of commercial GMO-inserts: A source of useful material to study genome fluidity? Hot spots may lead to tandem transgene repeats with interspersed plant DNA sequences in a single genetic locus. Presence of several inserts may also result from multimerisation in the plasmid before transformation or from multiple insertions.

[14]Rönning et al. (2003). Event specific real-time quantitative PCR for genetically modified Bt11 maize (Zea Mays). Eur Food Res Technol 216: 347-354.

[15]Jank and Haslberger (2000). Recombinant DNA insertions into plant retrotransposons. Trends in Biotechnology 18: 326.

Are transgenic DNA and proteins taken up from the mammalian GIT (gastro-intestinal tract)?

If DNA and proteins from GMOs persist in, and are taken up from the mammalian GIT, this could theoretically (as will be explained further) ultimately lead to development of chronic disease conditions. The fate and consequences of DNA persistence and uptake is, however, not extensively studied, and therefore represents yet another area of uncertainty connected to GM plants.

It has generally been claimed that DNA and proteins are effectively degraded in mammalian GITs. This has been based on assumptions that have never been systematically examined.[16] A restricted number of recent publications have shown that foreign DNA and also proteins may escape degradation, persist in the GIT and even be taken up from the intestines and transported by the blood to internal organs in biologically meaningful versions.[17] These findings should not have come as such a surprise, since scientific articles from the 1990s[18] strongly indicated that this was an area of omitted research, as stated by a number of reports.[19]

Briefly summarized, there is evidence that relatively long fragments of DNA survive for extended periods after ingestion. DNA may be detected in the faeces, the intestinal wall, peripheral white blood cells, liver, spleen, and kidney, and the foreign DNA may be found integrated in the recipient genome. When pregnant animals are fed foreign DNA, fragments may be traced to small cell clusters in foetuses and newborns. The state of GIT filling, and the feed composition may influence DNA persistence and uptake. Complexing of DNA with proteins or other macromolecules may protect against degradation.

So far, only two published reports have investigated the fate of foreign/transgenic DNA in humans.[20] The consequences of DNA persistence and uptake thus represent yet another area of

[16]Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215.

[17]Schubbert et al. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Mol Gen Genet. 242(5): 495-504; Schubbert et al. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. Proc Natl Acad Sci USA 94(3): 961-6; Schubbert et al. (1998) On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet. 259(6): 569-76; Hohlweg and Doerfler (2001). On the fate of plants or other foreign genes upon the uptake in food or after intramuscular injection in mice. Mol Genet Genomics 265: 225-233; Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215; Einspanier et al. (2001). The fate of forage plant DNA in farm animals; a collaborative case-study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212: 129-134; Klotz et al. (2002). Degradation and possible carry over of feed DNA monitored in pigs and poultry. Eur Food Res Technol 214: 271-275; Forsman et al. (2003). Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract. Mol Gen Genomics 270: 362-368; Chen et al. (2004). Transfection of mEpo gene to intestinal epithelium in vivo mediated by oral delivery of chitosan-DNA nanoparticles. World Journal of Gastroenterology 10(1): 112-116; Phipps et al. (2003). Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci. 86(12): 4070-8.

[18]Wolff et al. (1990). Direct gene transfer into mouse muscle in vivo. Science 247: 1465; Jones et al. (1997). Oral delivery of poly(lactide-co-glycolide) encapsulated vaccines. Behring Inst Mitt. Feb (98): 220-8.

[19]E.g. a number of articles cited in Traavik, T. (1999). An orphan in science. Research Report for DN No. 1999-6, www.naturforvaltning.no/archive/attachments/01/05/Vacci006.pdf

[20]Forsman et al. (2003). Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract. Mol Gen Genomics 270: 362-368; Netherwood et al. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat Biotechnol 22(2): 204-209. In the former study, volunteers were fed rabbit meat. Rabbit retrotransposon sequences (RERV-H) were detected in the blood stream and in peripheral white blood cells for a considerable length of time after ingestion. In the latter study volunteers were fed epsps-transgenic (glyphosate-tolerant) soy as burgers and soy-milk. The transgenic DNA was detected in the small intestinal contents and bacteria. The volunteers were ileostomists, i.e. individuals in which the terminal ileum is resected and digesta are diverted from the body via a syoma to a colostomy bag.

omitted research. Extrapolating from a number of experiments in mammalian cell cultures and in experimental animals, it is conceivable that in some instances insertion of foreign DNA may lead to alterations in the methylation and transcription patterns of the recipient cell genome, resulting in unpredictable levels of gene expression levels and products. Furthermore, even small inserts may result in a 'destabilization' process, the end-point of which may be malignant cancer cells.[21] The BSE/new variant Creutzfeld-Jacob's Disease epidemics caused by prion proteins painfully illustrated the phenomenon of protein persistence, uptake and biological effects. Two recent publications indicate that this phenomenon may be more general that realized.[22] A hallmark of prion diseases and a number of other debilitating, degenerative diseases, e.g. Alzheimer's and Huntington's diseases, is deposition of 'amyloid fibrils'. Recent studies indicate that any protein can adopt a confirmation known as 'amyloid'[23] upon exposure to appropriate environmental conditions. Whether such conditions are more likely when proteins are expressed in different species and at very different concentrations, as is often the case for GM food/feed that are already in the marketplace, is unknown.

The consequences of protein persistence and uptake will vary with the given situation. Generally speaking, there is a possibility that toxic, immunogenic/allergenic or carcinogenic molecules may gain entry to the organism via cells in the gastrointestinal walls. The persistence of the Bt toxin Cry1Ab in faeces means a potential for spread on fields through manure. The ecological effects, e.g. on insect larvae and earthworms,[24] are presently a matter of sheer speculation.

Have the protein contents of GM food been altered in unpredictable ways?

Transgenes or upregulated plant genes may give rise to toxicants, anti-nutrients, allergens, and, putatively, also carcinogenic or co-carcinogenic substances. The concentration of a given transgenic protein may vary according to the location(s) in the recipient host cell genome of inserted GM construct DNA, and to environmental factors influencing the activity of the transgenic regulatory elements, e.g. the 35S CaMV promoter. The biological effects of a given transgenic protein, e.g. the Cry1Ab Bt toxin or the α-amylase inhibitor from beans when expressed in peas,[25] may be unpredictably influenced by post-translational modifications, alternative splicing,[26] alternative start codons for transcription, chimeric reading frames resulting

[21]E.g. Misteli, T. (2004). Spatial positioning: a new dimension in genome function. Cell 119: 153-156; Deininger, P.L. et al. (2003). Mobile elements and mammalian genome evolution. Curr Opin Genet Develop 13: 651-658; Costello, J.F. and Plass, C. (2001). Methylation matters. J Med Genet 38: 285-303; Gatza, M.L. et al. (2005). Impact of transforming viruses on cellular mutagenesis, genome stability, and cellular transformation. Environmental and Molecular Mutagenesis 45(2-3): 304-325.

[22]The first (Palka-Santani et al. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Gen Genomics 270: 201-215), based on feeding of gluthathione-S-transferase to mice, demonstrated undegraded protein in stomach/small intestinal contents, and trace amounts in kidney extracts, 30 minutes or more after feeding. Very significantly, incubation with stomach contents of control mice resulted in faster degradation than in feeding experiments. The second study concerned cattle fed cry1ab-transgenic maize Bt176 (Einspanier et al. (2001). The fate of forage plant DNA in farm animals; a collaborative case study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212: 129-134). Cry1Ab protein was detected in all parts of the GIT, and it was still detectable in the faeces.

[23]Demonstrated in a series of recent articles, e.g. Bucciantini et al. (2004). Prefibrillar amyloid protein aggregates share common features of cytotoxicity. J. Biol Chem 279: 31374-31382; Kayed et al. (2003). Common structure of soluble amyloid oligomers implies common mechanisms of pathogenesis. Science 300: 486-489.

[24]Zwahlen et al. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12: 1077-1086.

[25]Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T.J.V. and Hogan, S.P. (2005). Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. J Agric Food Chem 53: 9023-9030.

[26]Rang, A., Linke, B. and Jansen, B. (2005). Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. Eur Food Res Technol 220: 438-443.

from integration into the reading frame of a plant gene, and complex formation with endogenous plant proteins.

The influence of foreign DNA insertion on endogenous plant gene expression patterns may vary with local environmental factors, the actual insertion site(s), the number and stability of the inserts, transgenic promoter effects, methylation patterns of the insert(s), and post-transformational mutations in the transgenic protein coding as well as in regulatory sequences. Even a single nucleotide change may affect the properties of a protein, or it may create a new transcription factor binding motif. Detailed studies of these phenomena under authentic conditions are lacking, and hence we are confronted with yet another area of omitted research.

Could GM food/feed cause allergies?

One of the major health concerns related to GM plants is that the transgenic product itself, e.g. a Bt toxin, changed expression of endogenous plant genes, or chemical reactions that occur during the cooking of novel foods, may result in exposure to *allergenic* compounds. The risk assessment of allergens often follows an *allergenicity decision tree.*[27] These 'trees' are based on *in vitro* tests comparing a limited number of structures, usually only one, of the transgenic protein with known allergens. Hence, these comparisons are made in the hope that the protein isolated for the test matches all proteins produced from the same gene in the GM plant. In fact, this is unlikely because allergenicity tests are usually carried out with bacteria-, not *in planta*-produced versions of the transgenic protein. Glycosylation invariably takes place in plants, but not in bacteria, so this form of post-translational modification of both the transgenic protein and endogenous proteins would not be tested. Allergenic characteristics of proteins, and also their resistance to degradation in the organism, can be affected by glycosylation. Other protein modifications may also take place, adding to the unpredictability of transgenic products.[28]

Another important question related to allergenicity is whether post marketing surveillance can provide useful information about allergens in GM foods. For a number of reasons, this is not likely to happen.[29] Treatment of allergy is symptomatic, whatever the cause may be. The allergic case is often isolated, and the potential allergen is rarely identified. The number of allergy-related medical visits is not tabulated. Even repeated visits due to well-known allergens are not counted as part of any established surveillance system. Thus, during the October 2000 Starlink episode, it proved very difficult to evaluate Starlink (containing Bt toxin Cry9C) as a human allergen.[30] An additional reason for this was that the ELISA tests, used by FDA, that found no anti-Cry9C antibodies in suspected human cases, were dubious because bacterial, recombinant antigens were used instead of the Cry9C maize versions that the individuals had been exposed to.

Case: Bt toxins in Bt-transgenic GM plants

It is very important to be aware of the fact that the Bt toxins expressed in GM plants have never been carefully analysed, and accordingly, their characteristics and properties are not known. What is clear from the starting point, however, is that they are vastly different from the bacterial *Bacillus thuringiensis* protoxins, used in organic and traditional farming and forestry for

[27]Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 111: 1114-1121.
[28]Schubert, D. (2002). A different perspective on GM food. Nat Biotechnol 20: 969; Submissions on A549 High Lysine Corn LY038 http://www.inbi.canterbury.ac.nz/ly038.shtml
[29]Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect 111: 1114-1121.
[30]Bucchini, L. and Goldman, L.R. (2002). Starlink corn: a risk analysis. Environ Health Perspect 110: 5-13.

decennia.[31] The difference is evident already at the gene level, since the versions found in GMOs are engineered to produce active Bt toxins. By extrapolation, these have a number of potentially unwanted biological characteristics, ranging from solubilization of the protein under natural conditions and effects on insect and mammalian cells, to persistence and non-target effects in the environment.[32] In addition, the post-translational modifications that may influence conformations, cellular targets and biological effects of GM plant-expressed Bt toxins are unknown, and hence we once more identify an area of omitted research.

During the last few years a number of observations that may be perceived as 'early warnings' of potential health and environmental risks have appeared in the literature.[33] Most of them have, however, not been followed up by extended studies.

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[31]Stotzky, G. (2002). Release, persistence, and biological activity in soil of insecticidal proteins from Bacillus thuringiensis. Pp. 187-222 in: Deborah K. Letourneau and Beth E. Burrows: Genetically Engineered Organisms. Assessing Environmental and Human Health Effects. CRC Press LLC (ISBN 0-8493-0439-3).

[32]Andow, D.A. (2002). Resisting resistance to Bt-corn. Pp. 99-124 in: Deborah K. Letourneau and Beth E. Burrows: Genetically Engineered Organisms. Assessing Environmental and Human Health Effects. CRC Press LLC (ISBN 0-8493-0439-3).

[33]Human and monkey cells exposed to Bt-toxins from the extra- or intra-cellular environment are killed or functionally disabled (Taybali and Seligy (2000). Human cell exposure assays of Bacillus thuringiensis commercial insecticides: Production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environ Health Perspect online, 18 August 2000; Tsuda et al. (2003). Cytotoxic activity of Bacillus thuringiensis Cry proteins on mammalian cells transferred with cadherine-like Cry receptor gene of Bombyx mori (silkworm). Biochem J 369: 697-703; Namba et al. (2003). The cytotoxicity of Bacillus thuringiensis subsp. coreanensis A 1519 strain against the human leukemic T cell. Biochimica et Biophysica Acta 1622: 29-35). Influenza A infections in mice were changed from silent to lethal encounters by co-exposing the animals to Bt-toxin (Hernandez et al. (2000). Super-infection by Bacillus thuringiensis H34 or 3a3b can lead to death in mice infected with the influenza A virus. FEMS Immunology and Med Microbiol 209: 177-181). Farm workers exposed to Bt spores developed IgG and IgE antibodies to Bt-toxin (Cry1Ab) (Taylor et al. (2001). Will genetically modified foods be allergenic? Journal of Allergy and Clinical Immunology, May 2001, 765-771). The Bt-toxin Cry1Ac was found to have very strong direct and indirect immunological effects in rodents (Vazquez et al. (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Brazilian Journal of Medical and Biological Research 33: 147-155; Moreno-Fierros et al. (2000). Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from Bacillus thuringiensis induces compartmentalized serum, intestinal, vaginal and pulmonary immune response in Balb/c mice. Microbes and Infection 2: 885-890; Moreno-Fierros et al. (2002). Slight influence of the oestrous cycle stage on the mucosal and systemic specific antibody response induced after vaginal and intraperitoneal immunization with protoxin CryA1c from Bacillus thuringiensis in mice. ELSEVIER Life Sciences 71: 2667-2680). Earthworms exposed to Bt toxin Cry1Ab experience weight loss (Zwahlen et al. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12: 1077-1086). Cattle fed the Bt176 maize variety demonstrated undegraded Cry1Ab through the whole alimentary tract, and the intact toxin was shed in faeces (Einspanier et al. (2004). Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. Eur Food Res Technol 218: 269-273). Cry1Ab is much more resistant to degradation under field soil conditions than earlier assumed (Zwahlen et al. (2003). Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Mol Ecol 12: 765-775). Potentially IgE-binding epitopes have been identified in two Bt-toxins (Kleter and Peijnenburg (2002). Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential IgE-binding linear epitopes of allergens. BMC Structural Biology 2:8), and it should be added that many IgE-binding epitopes are conformationally not linearly determined. Finally, it is a matter of concern that Bt-toxins have lectin characteristics (Akao et al. (2001). Specificity of lectin activity of Bacillus thuringiensis parasporal inclusion proteins. J Basic Microbiol. 41(1): 3-6). Lectins are notorious for finding receptors on mammalian cells. This may lead to internalization and intracellular effects of the toxins. Occupational exposure to novel proteins, and potential allergic sensitization, has had little study, but could be of public health significance. An amazing number of foods have been proven to evoke allergic reactions by inhalation (Bernstein et al. (2003). Clinical and laboratory investigation of allergy to genetically modified foods. Genetically Modified Foods, Mini-Monograph, Volume 111, No. 8, June 2003). In this connection the findings of serum IgG/IgE antibodies to B. thuringiensis spore extracts (Bernstein et al. (1999). Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environmental Health Perspectives 107(7): 575-582), in exposed farm workers should be given further attention. Inhalant exposure to Bt-toxin containing GMP materials may take place through pollen in rural settlements and also through dust in workplaces where foods are handled or processed.

Case: Transgenic, glyphosate-tolerant (Roundup Ready) GM plants

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS) necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition. The transgene, *cp4 epsps*, used in genetically modified crops was isolated from an *Agrobacterium* strain. The whole idea is the combined use of the GM plant and the herbicide. Recent studies indicate that in some cases such GM plants are associated with greater usage of glyphosate than the conventional counterparts.[34] A very restricted number of experimental studies have been devoted to health or environmental effects of the GM plants or the herbicide itself. Some of these may be considered 'early warnings' of potential health and environmental risks, and they should be rapidly followed up to confirm and extend the findings.[35] Consequently, this is yet another area of omitted research.

Is the 35S CaMV promoter inactive in mammalian cells?

Cauliflower mosaic virus (CaMV) is a DNA containing para-retrovirus replicating by means of reverse transcription. One of the viral promoters, called 35S, is a general, strong plant promoter. It has been used to secure expression of the transgenes in most of the GMOs commercialized so far. Industry proponents have claimed unconditionally that the 35S is an exclusive plant promoter, and hence cannot, even theoretically, represent a food/feed safety issue.[36]

[34]Benbrook, C. Impacts of genetically engineered crops on pesticide use in the United States: The first eight years. Biotech InfoNet Paper No. 6, November 2003. www.biotech-info.net/technicalpaper6.html

[35]Mice fed GE soybean demonstrated significant morphological changes in their liver cells (Malatesta et al. (2002). Ultrastructural morphometrical and immunocytochemical analysis of hepatocyte nuclei from mice fed on genetically modified soy bean. Cell Structure and Function 27: 173-180). The data suggested that epsps-transgenic soybean intake was influencing liver cell nuclear features in both young and adult mice, but the mechanisms responsible for the alterations could not be identified by the experimental design of these studies. Treatment with glyphosate (Roundup) is an integrated part of the epsps-transgenic GMP application. A number of recent publications indicate unwanted effects of glyphosate on aquatic (Solomon & Thompson (2003). Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J Toxicol Environ Health B Crit Rev. 6(3): 289-324) and terrestric (Ono et al. (2002). Inhibition of Paracoccidioides brasiliensis by pesticides: is this a partial explanation for the difficulty in isolating this fungus from the soil? Med Mycol 40(5): 493-9; Blackburn and Boutin (2003). Subtle effects of herbicide use in the context of genetically modified crops: A case study with glyphosate (Roundup). Ecotoxicol 12: 271-285) organisms and ecosystems. Recent studies in animals and cell cultures point directly to health effects in humans as well as rodents and fish. Female rats fed glyphosate during pregnancy demonstrated increased foetal mortality and malformations of the skeleton (Dallegrave et al. (2003). The teracogenic potential of the herbicide glyphosate Roundup in Wistar rats. Toxicology letters 142: 45-52). Nile Tilapia (Oreochromis niloticus) fed sublethal concentrations of Roundup exhibited a number of histopathological changes in various organs (Jiraungkoorskul et al. (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia. Environ Toxicol 18(4): 260-7). A study of Roundup effects on the first cell divisions of sea urchins (Marc et al. (2002). Pesticide Roundup provokes cell division dysfunction at the level of CDK1/Cyklin B activation. Chem Res Toxicol 15: 326-331) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrod et al. (2003). The effect of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicoloy 185: 67-78) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard, S. et al. (2005). Differential effects of glyphosate and Roundup on human placental cells, Environ. Health Perspect. 113: 716-720). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

[36]E.g. Gasson, M. and Burke, D. (2001). Scientific perspectives on regulating the safety of genetically modified foods. Nat Rev Genet 2: 217-222.

In addition to studies in yeast[37] and in *Schizosaccharomyces pombe*,[38] there are published studies indicating that the 35S CaMV promoter *might* have potential for transcriptional activation in mammalian systems.[39] The final proof has become available during the last couple of years. First, 35S promoter activity was demonstrated in human fibroblast cell cultures,[40] thereafter in hamster cells,[41] and very recently a research group led by Terje Traavik (co-author of this chapter) has demonstrated substantial 35S promoter activity in human enterocyte-like cell cultures.[42] Such cells line the surface of human intestines. However, no published studies have investigated 35S CaMV activity *in vivo*, and this is therefore yet another area of omitted research.

Could the use of antibiotic resistance marker genes (e.g. nptII) present health hazards?

The antibiotic kanamycin is used extensively in crop genetic engineering as a selectable marker, *inter alia* in GM oilseed rape event lines such as MS1Bn x RF1Bn and Topas 19/2.
A selectable marker is a gene inserted into a cell or organism to allow the modified form to be selectively amplified while unmodified organisms are eliminated. In crop genetic engineering, the selectable marker is used in the laboratory to identify cells or embryos that carry the genetic modifications that the engineer wishes to commercialize. The selection gene is used once briefly in the laboratory, but thereafter the genetically modified crop has the unused marker gene in each and every one of its cells.

[37]Hirt, H. et al. (1990). Evolutionary conservation of transcriptional machinery between yeast and plants as shown by the efficient expression from the CaMV 35S promoter and 35S terminator. Curr Genet 17: 473-9.

[38]Gmunder and Kohli (1989). Cauliflower mosaic virus promoters direct efficient expression of a bacterial G418 resistance gene in Schizosaccharomyces pombe. Mol Gen Genet 220(1): 95-101; Probjecky et al. (1990). Expression of the beta-glucuronidase gene under the control of the CaMV 35s promoter in Schizosaccharomyces pombe. Mol Gen Genet 220(2): 314-6.

[39]The promoter initiates transcription in rabbit reticulocyte lysate (Ryabova and Hohn (2000). Ribosome shunting in the cauliflower mosaic virus 35S RNA leader is a special case of reinitiation of translation functioning in plant and animal systems. Genes & Development 14: 817-829) and in Xenopus oocytes (Ballas et al. (1989). Efficient functioning of plant promoters and Poly(A) sites in Xenopus oocytes. Nucleic Acids Research 17(19): 7891-7903). In the latter studies it was found that circular, supercoiled 35S CaMV driven expression plasmids were more active than linear forms. The CaMV genome carries structural and functional resemblance to mammalian Retroviridae and to Hepadnaviridae, which contains the human hepatitis B virus (HBV). A 19 bp palindromic sequence, including the TATA box of the 35S CaMV promoter, may act as a recombination hotspot in plants (Kohli et al. (1999). Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. Plant Journal 17(6): 591-601), and it is unknown whether this is also the case in mammalian cells. In a recent review article (Ho et al. (2000). Hazardous CaMV? Nat Biotechnol 18(4): 363) it was hypothesized that the 35S CaMV promoter might represent health hazards to human and animal consumers of transgenic plant materials. Against this it was argued that humans and mammals are continuously being exposed to CaMV particles through infected plant materials. This is true enough, but it is then forgotten that there are documented examples of animal species being resistant to intact viruses, but highly susceptible to infection by DNA from the same virus (Refs: Rekvig et al. (1992). Antibodies to eukaryotic, including autologous, native DNA are produced during BK virus infection, but not after immunization with non-infectious BK DNA. Scand J Immunol 36(3): 487-95; Zhao et al. (1996). Infectivity of chimeric human T-cell leukaemia virus type I molecular clones assessed by naked DNA inoculation. Procedures of National Academy of Sciences USA 93: 6653-6658; reviews: Traavik, T. (1999). An orphan in science. Research Report for DN No. 1999-6; Ho et al. (2000). Hazardous CaMV promoter? Nat Biotechnol 18(4): 363).

[40]Vlasak, J., Smahel, M., Pavlik, A., Pavingerova, D., and Briza, J. (2003). Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells, J Biotechnol 103: 197-202.

[41]Tepfer, M., Gaubert, S., Leroux-Coyau, M., Prince, S., and Houdebine, LM. (2004). Transient expression in mammalian cells of transgenes transcribed from the Cauliflower mosaic virus 35S promoter. Environ Biosafety Res 3: 91-97.

[42]Myhre, M.R., Fenton, K.A., Eggert, J., Nielsen, K.M. and Traavik, T. (2006). The 35S CaMV plant virus promoter is active in human enterocyte-like cells. Eur Food Res Technol 222: 185–193.

There are multiple well-known mechanisms for cross-resistance to antibiotics of a particular type.[43] Kanamycin is a member of the family aminoglycoside antibiotics. There are approximately 17 different classes of aminoglycoside-modifying enzymes. Some of these inactivate up to four different aminoglycosides. Cross-resistance between kanamycin and other aminoglycosides, e.g. gentamycin and tobramycin, was found to vary markedly between isolates.[44] All of the antibiotics mentioned are used to treat human diseases.
In spite of the belief of many genetic engineers that kanamycin is no longer employed in medical applications, there is evidence that the antibiotic is used extensively for some applications.[45]

Concluding remarks: Where do we go from here?

We have discussed in some detail a handful of selected, unanswered risk questions related to the first generation of transgenic GMOs. There are many more risk issues. Among them are issues of Horizontal Gene Transfer (HGT),[46] the new generations of multitransgenic GMOs for pharmaceutical and industrial purposes,[47] safety questions related to GM vaccines,[48] the new nanobiotechnology approaches,[49] and the applications of small double-stranded (ds)RNAs (which can cause RNAi) for a number of medical purposes.[50] Furthermore, we have the 'questions not yet asked', and we have the problem of whether available methods and regulatory frameworks will be able to pick up and manage the conceived risks once they become reality.
In recent publications it has been demonstrated that the presently used sampling and detection methods may fail to detect GM materials in food and feed.[51] In another article it was demonstrated that HGT events, that potentially carry very serious public health consequences, would not be detected in time for any meaningful preventive actions.[52] In addition, it has been shown that the dsRNA techniques are not as 'surgically targeted' as initially indicated.[53]

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[43]Heinemann, J.A., Ankenbauer, R.G., and Amábile-Cuevas, C.F. (2000). Do antibiotics maintain antibiotic resistance? Drug Discov Today 5: 195-204.

[44]The aminoglycoside antibiotic neomycin was found to cross react with kanamycin B in inhibiting RNase P ribozyme 16s ribosomal RNA and tRNA maturation (Mikkelsen et al. (1999). Inhibition of RNase P RNA cleavage by aminoglycosides. Proc Natl Acad Sci USA 96: 6155-6160).

[45]Kanamycin is used prior to endoscopy of colon and rectum (Ishikawa et al. (1999). Prevention of infectious complications subsequent to endoscopic treatment of the colon and rectum. J Infect Chemother 5: 86-90) and to treat ocular infections (Hehl et al. (1999). Improved penetration of aminoglycosides and fluorozuinolones into the aqueous humour of patients by means of Acuvue contact lenses. Eur J Clin Pharmacol. 55(4): 317-23). It is used in blunt trauma emergency treatment (Yelon et al. (1996). Efficacy of an intraperitoneal antibiotic to reduce the incidence of infection in the trauma patient: a prospective, randomized study. J Am Coll Surg 182(6): 509-14), and has been found to be effective against E coli 0157 without causing release of verotoxin (Ito et al. (1997). Evaluation of antibiotics used for enterohemorrhagic Escherichia coli O157 enteritis-effect of various antibiotics on extracellular release of verotoxin. Kansenshogaku Zasshi 71(2): 130-5).

[46]Heinemann, J.A. and Billington, C. (2004). How do genomes emerge from genes? Horizontal gene transfers can lead to critical differences between species when those genes begin reproducing vertically. ASM News 70: 464-471.

[47]Twyman, R.M. et al. (2003). Molecular pharming in plants: host systems and expression technology. Trends in Biotechnology 21: 570-578.

[48]Traavik, T. (2002).Environmental risks of genetically engineered vaccines. In: DK Letourneau and BE Burrows (eds): Genetically Engineered Organisms: Assessing Environmental and Health Effects. CRC Books, La Boca, Florida (ISBN 0849304393).

[49]Mazzola, L. (2003). Commercializing nanotechnology. Nat Biotechnol 21: 1137-1143; Colvin, V. L. (2003). The potential environmental impact of engineered nanomaterials. Nat Biotechnol 21: 1166-1170.

[50]Hannon, G.J. and Rossi, J.J. (2004). Unlocking the potential of the human genome with RNA interference. Nature 431: 371-378.

[51]Heinemann J.A., Sparrow A.D. and Traavik T. (2004). Is confidence in the monitoring of GM foods justified? Trend Biotechnol 22: 331-336.

[52]Heinemann J.A. and Traavik, T. (2004). Problems in monitoring horizontal gene transfer in field trials of transgenic plants. Nat Biotechnol 22: 331-336; Heinemann J.A. and Traavik T. (2004). Monitoring horizontal gene transfer. Reply. Nat Biotechnol 22: 1349-1350.

[53]E.g. Jackson, A.L. et al. (2003). Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol 21: 635-637, and a number of other recent articles.

We are therefore left with a high number of risk issues lacking answers, adding up to a vast area of omitted research, and this falls together in time with a strong tendency towards corporate take-over of publicly funded research institutions and scientists.[54]
We must, as citizens and professionals, join together to reverse the present situation. Publicly funded, independent research grants need to become a hot political issue. This would be the most efficient remedy for chronically unanswered questions and the corporate take-over of science. In conclusion, we once more quote Mayer and Stirling:[55] 'Deciding on the questions to be asked and the comparisons to be made has to be an inclusive process and not the provenance of experts alone'. Then again, whom should society rely on for answers and advice should the time come when all science resource persons work directly or indirectly for the GM producers?

[54]Mayer, S. and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024; Martin, B. (1999), in Science and Technology Policy Year Book. Washington DC, USA: American Association for the Advancement of Science, www.aaas.org/spp/yearbook/chap15.htm; Graff GD et al. (2003). The public-private structure of intellectual ownership in agricultural biotechnology. Nat Biotechnol 21: 989-995; Heinemann, J.A. and Goven, J. The social context of drug discovery and safety testing. In Multiple Drug Resistant Bacteria (C.F. Amábile-Cuevas, ed., second edition). Horizon Scientific Press, in press.
[55]Mayer, S and Stirling, A. (2004). GM crops: good or bad? EMBO Reports 5: 1021-1024.

Chapter 10

Biodiversity, ecosystem services and genetically modified organisms

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Introduction

Genetically modified (GM) crops have been commercially grown for 10 years. During this time the debate about them and about genetic engineering in general has continued to rage. The general public eagerly follows the developments as well as the arguments; the level of attention is possibly unparalleled since the appearance of the atomic bomb. Some argue that this is the triumph of ignorance, the result of manipulation by environmental protection organizations such as Greenpeace and/or media hype. Sometimes 'risk assessment' is pictured as a strategy to block the spread of growing GM crops. Few ecologists subscribe to any of the aforementioned. The debate about the benefits, risks and overall impact of genetic engineering is complex and so it should be. After all, genetic engineering introduces new combinations of genes that may irreversibly be a part of future evolution, and affect the environment and natural resources. The scale of this issue is thus huge and beyond the short-term scientific and political agendas: it triggers ideological, ethical and religious evaluations. In this chapter, we consider one limited but significant part of this problem circle – the potential environmental impact – and link it to the concepts of biodiversity and ecosystem services.

The overall reason to test GM plants before field release is because humankind's total impact on ecosystem services from previous introductions of new technologies is substantial (Millennium Ecosystem Assessment (MEA) 2005), including habitat destruction, introduction of exotic species, chemical pollution, and global warming, all of which, in themselves and in combination, lead to loss of biodiversity, but also to substantial pressure on all kinds of ecosystems and their services. We have learned from over 100 years of industrial-technological development that all environmentally relevant technologies come with a price – many of which outweigh the benefits in the long run (Harremoës 2002). Consequently, all new potential environmental stressors need to be carefully assessed.

Ecosystem services are ecological processes that operate on vast scales, and we derive substantial benefits from them. Production of goods such as fish and timber, generation of soils and maintenance of their fertility, decomposition, detoxification of wastes, mitigation of climatic extremes, biological control of potential pests, weeds and pathogens, and crop pollination are just some examples of ecosystem services. Their continued functioning is essential for humankind's survival – they cannot be replaced by technology. Until recently, ecosystem services have been treated as inexhaustible, but the global human population size and its use of resources have reached the point where ecosystem services show evident signs of strain.

Agriculture is one of the human activities that have a large 'ecological footprint' (Wackernagel & Rees 1997), meaning that it is a crucial factor in the global ecology. Agriculture is an important driver of environmental quality. In developed countries, there are few farmers (typically < 5% of the population) and they produce food and feed in mostly large-scale, high-input agricultural

systems, including expensive machinery and combustion of fossil fuels. In the developing countries, the situation is different. For example, approximately 70% of Africa's population is engaged in agriculture. Natural processes that underpin agricultural diversity and productivity are both recognized and needed in these regions as most of them have no means to compensate with external inputs.

The concept of biodiversity

According to a recent definition, biological diversity as a concept refers to the variety and variability of living organisms (MEA 2005). Diversity is a multifaceted concept, and ranges from intra-cellular (genetic diversity) to supra-individual (community, landscape and ecosystem diversities) levels (Magurran 2003). Ecologists have long struggled with the concept of diversity and how to quantify it. After decades of intensive search for the best index or formula describing diversity, it was finally realized that there is no single, 'best' diversity description. There exists a 'diversity of diversities' (Juhasz-Nagy 1993), including genetic, physiological, species, functional group, landscape, and ecosystem diversity (Box 10.1). In the interests of preserving biodiversity, we also have to recognize the significance of the processes that create, maintain and further develop biodiversity. In a short-term perspective, this means the ecological processes (i.e. competition, predation, etc.); over the long-term, it includes the process of evolution (Bøhn & Amundsen 2004). Too often, biodiversity is viewed as a static characteristic of communities. However, biodiversity is the emergent outcome of dynamics at ecological and evolutionary timescales.

Box 10.1 Definitions

Genetic diversity: This concept refers to the variability of genes within a species. The total number of genes that can be found in one species is never present in one individual: individuals of the same species contain a lot of identical genes but also many different ones. Genetic variability is the key to the adaptation potential to changing conditions. A species that has lost its genetic diversity is either unable or severely impaired to adapt to new conditions.

Physiological diversity: As genes only provide a 'set of instructions', the realization of this programme, depending on the environmental conditions during development, always results in slightly different physiological outcomes in individuals. They will differ in their physiology: heat tolerance, ability to resist starvation, digestion efficiency, etc.

Species-individual diversity: Communities of living organisms are composed of individuals that are classified into species. Intuitively, the more species there are in a community, the more diverse it is. The minimum diversity in a community occurs when all individuals belong to the same species. A theoretical maximum level of species diversity would be reached when all individuals belong to different species. A characterization of species diversity depends on our ability to recognize individuals as belonging to different species, and to count them.

Functional diversity: Species have different characteristics and are distinguishable, but they may be grouped according to their activity in habitats and food webs. One possibility is to group them by their feeding habits. Plants use inorganic materials and energy (mostly sunlight) to grow, in the process of producing more plant material. They can be classified into the functional group of *primary producers*. Organisms feeding on plants form the *primary consumers*, while those feeding on these are called *secondary consumers*. At the top of some food-chains are the *top predators*, often large animals. Functional groups can be further refined. One aspect of functional diversity is the diversity of such groups themselves (not all of them are present everywhere), while another is to assess the diversity within each group.

Landscape diversity: At a wider spatial scale, different habitats (for example, forests, meadows, streams, marshes, cultivated fields) form landscapes. Both the types and distribution of these compositional elements are important in determining the diversity at this level. For example, if the elements occur in one block each, the landscape-level diversity is considered lower than when the same total area of the composing elements occurs in several smaller blocks. The transition between landscape and ecosystem diversity is not always straightforward.

Ecosystem diversity: Ecosystems can be larger units, composed of several landscapes (but some argue the opposite). An ecosystem is defined as a recognizable, self-sustaining unit, but it is more plausible to consider this a theoretical.

Different biodiversity concepts, as detailed in Box 10.1, range from intra-individual (genetic) to supra-individual (species, landscape, etc.) levels, and all are relevant, depending on *context*. However, it has to be added that the most frequent use of the word biodiversity (sometimes even without definition) implies the species-individual based diversity, i.e. the word 'diversity' means the number of species. In nature, most communities contain a small number of 'common' and a much larger number of 'rare' species. Some diversity indices account for such differences but all diversity representations contain different simplifications. For example, for most diversity indices, the species identity is not important – only the density of the species present is taken into account. Two communities with the same number of species and identical relative densities would have the same diversity value even if there were no common species in them.

The functions of biodiversity

Diversity, in all of its manifestations, is valued for several different reasons. Biodiversity is also important for the functioning of ecological systems (Loreau et al. 2002), but the central question is: just how important? There are different theories to explain the significance of biodiversity for ecological systems. These theories are vigorously studied, hotly debated and not always mutually exclusive (Loreau et al. 2002; Hooper et al. 2005). The main ideas are briefly presented as follows.

1. Biodiversity has a (positive) impact on productivity

Several experiments have indicated that a more diverse ecological community of plants will produce a higher biomass than a less species-rich one (Loreau et al. 2002). The existing evidence supporting this claim is equivocal and has been debated (Hooper et al. 2005). More species can utilize the available resources more efficiently, but there seem to be some key species that have disproportionate influence on this and consequently also on productivity (Wardle & van der Putten 2002). In a more species-rich assemblage, it is more probable that such species can be found. Another hypothesis claims that a more diverse system will experience less year-to-year fluctuations in plant biomass production than a species-poor one.

2. Insurance against change (resistance and resilience)

In terms of energy efficiency, most biodiversity is unnecessary (redundant) for ecological functioning *under stable conditions*. However, elements that seem redundant under one set of conditions may become necessary if conditions change, since the organisms have to adapt. Changing conditions occur naturally, for example by extreme weather conditions, but also due to human activities, such as global warming and introduction of exotic species. It may be hard to separate natural- and human-triggered changes. For example, global warming tends to increase the occurrence of extreme weather events. Whereas resistance refers to the ability to resist change under the pressure of stressful conditions, resilience refers to the ability to return to a previous

state after a disturbance. Both traits are important for continued functioning of ecological systems.

3. Providing ecosystem services
Ecosystem services are linked to points 1 and 2 above. A more detailed explanation of their nature and importance will follow.

Human domination of the Earth
We now recognize that human impact over all of the Earth is substantial, whether we consider land conversion, use of resources, or impact on other species. Today, 25% of the global terrestrial surface has been converted to cropland (Fig. 10.1). The conversion rate is accelerating: more land was converted in the 30 years since 1950 than during the 150 years from 1700 to 1850. More than two-thirds of the area of two biomes (temperate forest; tropical dry forest) and more than half of the area of four others (Mediterranean forests; flooded grassland and savannas; tropical and subtropical savannas and grasslands; tropical and sub-tropical coniferous forests) had been converted by 1990. Our impact on other parts of the globe is also large. For example, 20% of all coral reefs had been exterminated, a further 20% damaged, and 35% of the global mangrove area had been destroyed by 1990 (MEA 2005).

Increases in fertilizer application have followed suit, and biologically available nitrogen in terrestrial systems has doubled, and that of phosphorus tripled since 1960. However, this change is extremely disproportionately distributed, with overuse in industrial countries to the point of polluting water bodies and lack of it in developing countries to the point where agriculture production is severely limited (e.g. Africa). For example, the average application in 1992 of N fertilizer was 323 kg/ha in Western Europe while only 7 kg/ha in Africa (FAO 1993). Nevertheless, at a global level, more than 50% of all the synthetic nitrogen fertilizer ever used has been used since 1985, and 60% of the increase in the atmospheric concentration of CO_2 since 1750 has taken place since 1959 (MEA 2005).

Another limited vital resource is water and we claim more and more of the available freshwater resources. The amount of water in reservoirs has quadrupled since 1960, and today there is 3–6 times more water in reservoirs than in all natural rivers combined (MEA 2005). Water withdrawal from rivers and lakes has doubled since 1960. As a result of combined erosion and river regulation, the sediment load of many major rivers has been substantially altered from pre-human conditions (Syvitski et al. 2005). In some rivers, sedimentation has increased by up to 200% and even large rivers hardly reach the coast. For example, only 10% of the Nile manages to meet the ocean. Increased sedimentation rates have caused death zones in deltas where depositing sediments are often loaded with poisonous chemicals (Syvitski et al. 2005).

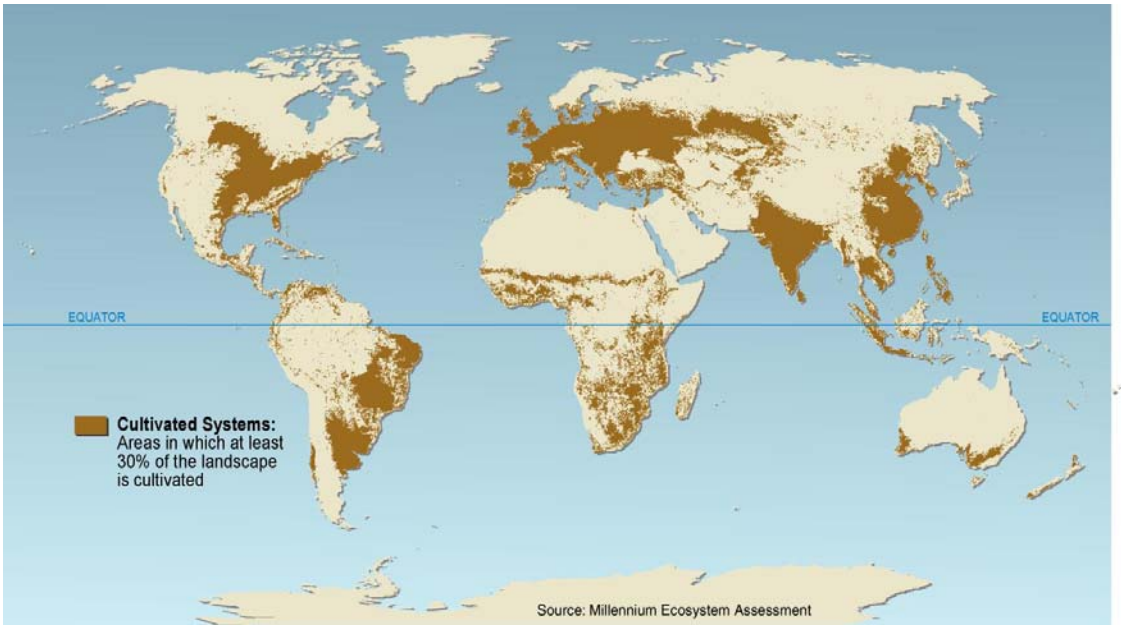

Figure 10.1. The terrestrial areas converted to cropland worldwide. Source: Millennium Ecosystem Assessment, 2005.

Concerns about biodiversity

The impacts of agriculture on resources together with other human activities have had significant impacts on global biodiversity. Introduced species have had particularly broad impact. In historic times, numerous intentional introductions of species deemed useful or merely desirable at new locations have been made. Their effects are often considered beneficial, but we have numerous examples of unwanted, significant negative effects (Baskin 2002), and the number of invasive species is steadily increasing (for an example, see Fig. 10.2). Together with unintended introductions, invasions have become a significant problem, and an element of global change (Vitousek et al. 1997). One significant consequence of this is the increasing homogenization of the distribution of species on Earth (Lövei 1997). The breakdown of biogeographical barriers leads to reduced global biodiversity (Vitousek et al. 1997).

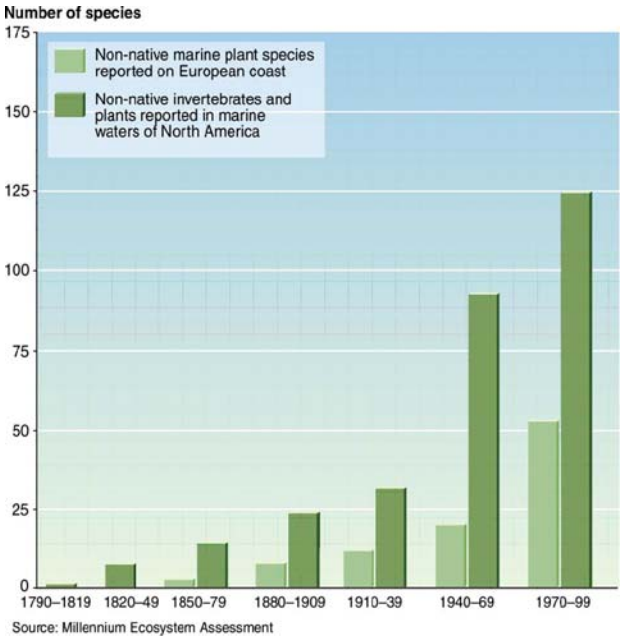

Figure 10.2. The number of non-native species reported from marine habitats in Europe and North America, 1790–1999. Source: Millennium Ecosystem Assessment, 2005.

Further signs of stress in the global biodiversity is that the population size or range (or both) of the majority of species across a range of taxonomic groups is declining (MEA 2005). Currently, estimated species extinction rates are 1000 times higher than background rates typical of the planet's history (Fig. 10.3) (MEA 2005; Lövei 2007). A total of 10–30% of mammal, bird, and amphibian species are currently threatened with extinction (Secretariat CBD 2006).

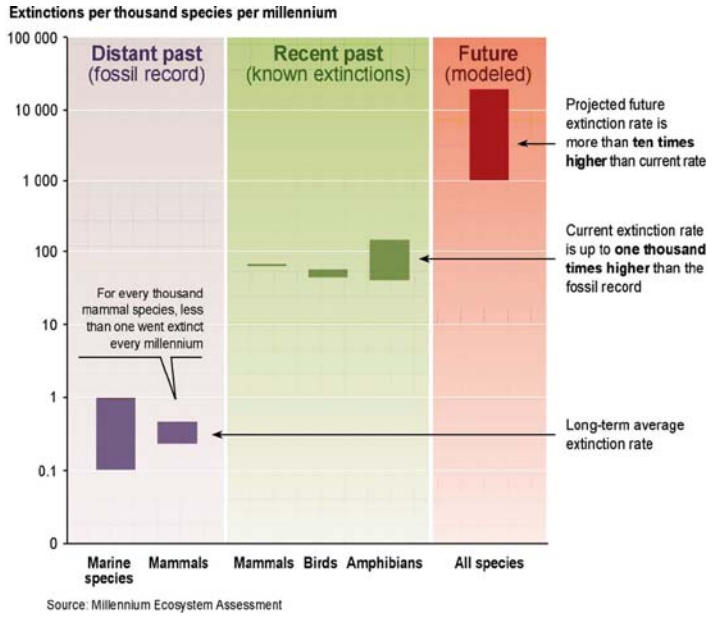

Figure 10.3. Estimated extinction rates: historical, recent and predicted. Source: Millennium Ecosystem Assessment, 2005.

Ecosystem services
Ecosystem services denote ecological processes that humankind benefits from (Daily 1997). These processes operate on vast scales, are irreplaceable, and have been formerly perceived as inexhaustible. Several types of ecosystem services ensure agricultural productivity, including soil formation, decomposition of plant residues, pollination, and natural pest control, to name a few. Several of these are already under pressure and their ability to continue at desired rates is in peril (MEA 2005).
The Millennium Ecosystem Assessment (MEA) recognizes four categories of ecosystem services (Box 10.2).

Box 10.2
Provisioning services are simply used or harvested, and in most cases humans do not do anything to manage them. Provisioning services include the provision (harvesting from the wild) of food, freshwater, medicine, fibre, and timber, energy, or industrial products (e.g. rubber). Genetic resources used for plant breeding also belong to this category.

Supporting services include services that, by their functioning, support the normal functioning of ecosystems. This includes the removal of waste products through detoxification, decomposition, air and water purification, but also soil formation and fertility maintenance, and supporting plant production through seed dispersal, and pollination.

Regulating services provide coastal and river channel stability, moderation of weather extremes, floods and drought, as well as the natural control of pests. Most organisms can occur at high densities but they do not (i.e. they do not become pests). This is due to the activity of natural enemies.

Cultural services provide numerous valuables to humans and human culture. Humankind is psychologically closely linked to nature (the 'biophilia' hypothesis, Wilson 1984). Nature is a constant source of aesthetic beauty, provides cultural and spiritual inspiration, inspires scientific discovery, and endless varieties of recreation.

Why do ecosystem services have to be considered in GM impact assessment?
As described, ecosystem services are essential for agricultural production. As the MEA concluded, humankind already is using many of the ecosystem services in a non-sustainable manner. Any further damage must be avoided. Also, the negative trends in biodiversity and natural resources must be taken very seriously. Consequently, when introducing new technologies today, such as GM crops, their potential impact on ecosystem services must be tested (Lövei 2001). Such testing is even more important in tropical countries, where agricultural producers often depend on ecosystem services more closely than farmers in the developed countries. Modern high-input agricultural practices use several external inputs that at least partially replace ecosystem services (fertilizers, pesticides, irrigation, and even pollination). Irrespective of the questionable sustainability of this practice (Tilman et al. 2002), these external inputs are often not available to farmers in developing countries, hence they have to rely more on natural ecosystem services. As GM crops will be grown outdoors, in contact with surrounding ecosystems, and they certainly have the potential to substantially modify current agricultural practices (Hawes et al. 2003), the environmental impact of genetic engineering on ecosystem services will have to be examined thoroughly (Hails 2002). Box 10.3 lists the most important potential adverse impacts currently discussed and partly investigated.

Box 10.3 Possible environmental impacts of GM crops

At intra-individual (genetic) level:

- damage to genetic resources (particular genes, gene combinations, seeds, varieties, etc.)
- uncontrolled gene flow to other species

At population level:

- species shifts due to altered traits, consciously or accidentally (via unintended gene flow)
- development of secondary pests
- development of resistant populations, curtailing the usefulness of the GM trait
- damaging of protected/endangered species (nature conservation)

At ecosystem level:

- decline in agricultural biodiversity due to the homogenization of the primary producer base (a centralized production of a relatively few, patented events, traits and varieties).

Loss of ecosystem services:

- damaging naturally-occurring biocontrol organisms
- loss of pollination services
- impact on soil organisms involved in recycling of soil nutrients and maintaining soil fertility (can be positive, due to reduced soil tillage, or negative)

For agricultural production systems:

- decrease in pesticide use, soil tillage, environmental contamination
- threatening of GM-free production reducing future choices
- loss or reduction in practices that uphold and develop varieties (i.e. diversity) with adaptations to local environmental conditions
- food or agricultural production in areas where it was not possible earlier (e.g. due to high levels of stress, lack of water, etc.)
- rearrangement of agricultural production systems, in space and time, and its resulting consequences for landscape management

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Incorporating ecosystem services into risk/impact assessment poses several challenges:
The structure and function in relevant ecosystems and food-webs have to be recognized. For example, an ecosystem may contain predator-prey relationships that keep a number of pests under control (i.e. at low densities, so we do not recognize them as a pest). Productivity may also depend on insect pollination services (e.g. cotton).

The significant functional links must be established where structure and function are reasonably well understood. Following the aforementioned example, it may turn out that pollination is much more significant than pest control for productivity in the ecosystem where a GM crop is to be introduced.

Most important species fulfilling identified relevant ecological roles that should be subjected to pre-release testing have to be identified. However, we should not forget that even the most important functions will typically be performed by numerous species. Again, following the

aforementioned example, pollination services may be provided by more than 30 bee species, but the most important could be just one, or a handful of them.

Pre-release testing should focus on these functionally important species. When such species are identified, suitable testing and monitoring methods must be developed for them. If there is no option to identify species responsible for the execution of important ecological services – as, for instance, is the case with most soil microorganisms – the relevant processes must be identified and a potential adverse impact of the GMO tested. There may or may not be suitable laboratory culture systems or field monitoring methods already available for these functionally important species or processes. If such tools are lacking, they should be developed.

Current testing regimes for GM plants

Understanding the importance of ecosystem services and the need to avoid any further adverse impacts on them through the introduction of GMOs begs the question as to what degree current regulatory testing actually addresses the issues raised so far in this chapter and how they are tested. Today, applicants applying for regulatory approval of GM plants follow largely the guidelines originally developed for testing the environmental effects of chemicals (pesticide model). The *strategy used in ecotoxicology testing of chemicals* is to expose single species (standard set) to single chemicals in a hierarchical tiered system. Tests commence with simple inexpensive range finding tests on single species and measure acute toxicological response to a chemical stressor. Further testing proceeds to more expensive higher tiered levels (including some chronic toxicity tests), only if first-tier experiments yield results of concern. In practice, this results in the testing of a standard set of species exposed individually to high concentrations of the toxin.

In the case of a GM plant producing the *Bacillus thuringiensis* toxin (Bt plant), for example microbially produced Bt-toxins are fed directly to testing organisms (bi-trophic exposition) in an experimental set-up originally developed to assess acute toxicity of synthetic chemicals. Acute toxicity measures the physiological toxicological response of an organism after being directly exposed to the isolated test substance within a short period of time (sometimes hours rather than days).

The standard set of species is representative of model ecosystem compartments, such as a generalized aquatic or terrestrial compartment. An algae species is tested as a representative for primary producers in aquatic systems (plants), water fleas (*Daphnia* spp.) as a representative of a primary consumer, and a fish species representing a secondary consumer (i.e. predator). The endpoint measured is mortality after hours or a few days (Table 10.1) (Andow & Hilbeck 2004). Further criteria for their selection as standard organisms are their documented sensitivity to certain groups of chemicals and/or their capability of accumulating high concentrations of heavy metals (e.g. springtails or earthworms). Hence, the concept of toxicity (and ecotoxicity) testing of chemicals is exceeding the notion of a case-specific testing regime related to the given receiving environment. A standard test performed in temperate Europe is (erroneously) considered applicable to tropical Africa, and vice versa.

Table 10.1. Some standardized guidelines for ecotoxicological testing of pesticides and GMOs (OECD 1998).

Test organism	*Test method*	*Duration*	*OECD Guideline No.*
Water fleas, Daphnia	*Acute immobilization/toxicity*	*24-96 h*	*202*

Fish sp. (rainbow trout)	*Acute toxicity*	*24-96 h*	*203*
Fish sp.	*Toxicity to juvenile life stages*	*4-12 wk*	*210*
Eisenia foetida (compost worm)	*Acute toxicity*	*7-14 d*	*207*
Bobwhite quail & mallard duck	*Acute toxicity*	*14-21 d*	*205*
Honey bees	*Acute toxicity (oral & contact)*	*4-24 h*	*New (1998)* *213* *214*

http: ecb.jr.it/testing-methods
www.oecd.org/dataoecd/9/11/33663321.pdf

The pesticide model as a testing guideline for insecticidal GM plants is problematic for a number of reasons. Plants are not chemicals and regulations and scientifically sound testing procedures must account for the differences:

i) In GM plants, the plant-expressed transgene product is an integral component of the plant and coupled to its metabolism. This leads to variable expression levels of the transgene product that is additionally modulated by environmental conditions, including seasonal changes in temperature, soil type, moisture, and light. On the other hand, due to the wide use of universally functioning viral promoters and terminators, the transgene products of most, if not all, currently commercially available GM plants are expressed essentially in all plant parts throughout the entire growing season. When comparing with pesticides, this is equivalent to a long persistence of the pesticidal substance and an almost complete coverage of the plant.

ii) GM plants are capable of self-reproduction. This is a fundamental difference to chemicals. Because of this capability, biological traits and organisms can increase in the environment and potentially spread and exist for unlimited time. In contrast, chemicals cannot reproduce and, thus, their absolute amount will, at best (or worst), remain stable for a long time, but over time will always decline. Most disappear within humanly conceivable time periods due to degradation.

iii) GMOs can actively spread and with them their transgene products will also spread. In addition, all passive mechanisms of spread for chemicals also apply to transgene products released into the environment from the living GM plants (e.g. exudates, leaching from living and dead material). The potential of human-aided spread of seeds, plants and animals (as already realized and exemplified in invasion biology) should not be underestimated (Baskin 2002, see Box 10.4).

Box 10.4 Spread of GM plants: Control or chaos?

Unwanted and uncontrollable spread of GM plants is a highly visible process on a global scale. By the end of 2006, over 100 cases of confirmed, unwanted contamination and 26 cases of illegal releases were registered (mostly by civil society organizations) (see GM contamination register, http://www.gmcontaminationregister.org/). A total of 39 countries on five continents have been affected, almost twice the number of countries that currently grow GM crops. In 2005, there were 7 documented cases of contamination and 8 illegal releases. In 2006, the number of

contamination cases more than doubled to 15. Most prominently, two unapproved GM events were found in rice (a herbicide-tolerant transgene from the USA and a Bt transgene from China) – these were detected at the consumer level (in shipments intended for human consumption). These were possible to detect because the necessary detection methods were available. More problematic is the detection of plants with GM traits that have not yet been commercialized. Several such lines are at the field trial stage, among them many pharmaceutical traits, for which the necessary detection methods are not yet widely available and therefore detection is more difficult. The global, illegal or unwanted spread of transgenes and their products shows a worrying tendency and it is likely that this trend will continue, perhaps even accelerate, over the coming years.

For these reasons, it is extraordinarily more difficult if not impossible to determine the exact exposure concentrations in a given environmental compartment for GM plants as compared to chemicals. In contrast, chemical pesticides (i.e. sprayed in the field) are controlled by the applicator: the timing, the point location, etc. Degradation begins immediately after application and the mode of action is typically acute (also for non-target species). A scientifically sound testing strategy and methodology for GM plants require case-specific risk assessment and must account for the whole transgenic organism. It must also treat a GM plant within an integrated biological system consisting of the plant, the novel trait and the receiving environment. Sub-lethal, chronic effects might be even more important to test for than acute effects, as the mode of action for the toxin is not immediate (it normally takes two days or longer before the 'target' dies).

Selection of test organisms

Even for chemical testing, it is problematic to use test organisms of higher trophic levels because the test substance is often not ingested directly by these organisms but is ingested via one or several intoxicated prey species. These prey species may contain the test substance, or metabolites thereof, in unknown concentrations. From our knowledge of persistent chemicals such as DDT and PCB, we know that they can accumulate and even become more toxic along the food chain. This means they can reach concentrations and toxicity levels that, at the end of the food chain, are multi-fold above the levels originally introduced into the ecosystem (Woodwell et al. 1967). We also know from research on insect-plant interactions, that insects can use toxic proteins in their host plants to turn them into defence mechanisms against their enemies. One example is the monarch butterfly (*Danais plexippus*), whose larvae accumulate an alkaloid from the host plant, milkweed, that makes them unpalatable. We do not know how herbivore species, which are not affected by novel transgene compounds, may be using them against their enemies. These complications make it currently unlikely that a few selected species could universally be used for pre-release risk assessment of GM plants.

Representativeness of test materials

As already mentioned, in toxicological and ecotoxicological testing of pesticidal GM plants, high concentrations of the microbially produced transgene product, e.g. the Bt-toxin, are applied. The significance of such tests is limited because the Bt-toxin expressed in GM plants can be quite different from the microbially derived toxin. For example, the Bt-toxin of the Cry1-class used in the regulatory tests has been derived either from the original *Bacillus* or from genetically modified *Escherichia coli*. After the microbial synthesis, the product is a protoxin of 130 kDa in size which is inactive (Höfte & Whiteley 1989; Müller-Cohn et al. 1996). Before use in the tests, the protoxin is cleaved by trypsin to create the toxic fragment of 65 kDa size. However, in transgenic Bt-plants, fragments of different sizes of the Cry1-class toxins are produced. For example, the Bt-corn event MON810 expresses a 91 kDA fragment, whereas Bt-corn event 176

expresses a 64 kDa fragment (Andow & Hilbeck 2004). From other events, it is known that the Bt-toxins degrade within the plant to fragments of even smaller size (36, 40, 55, 60 kDa) of unknown activity[1](Andow & Hilbeck 2004; AGBIOS 2006). In conclusion, this means that the Bt-toxins expressed in GM plants may vary significantly in size and activity from the test substances used to assess safety, i.e. in standard toxicological and ecotoxicological testing.
In summary, a GM plant is not a chemical. Any environmental testing must therefore account for the difference. Test strategies for case-specific risk assessment of GM plants must include the transgene product, the transformed plant and the environment of deployment as an integrated system. This is even more important in the case of GM plants that do not express a toxin, but have, for instance, an altered metabolism (e.g. herbicide tolerant plants or altered starch composition). In these cases, the adoption of test principles from chemical testing is even less relevant because environmental effects of these GM plants may become evident on other levels altogether. Following the logic for strict toxicity testing, for those GM plants that do not express a novel toxin, no testing would be required at all. This is the case for most herbicide tolerant plants to date. As the ecological impact will arise through the application of registered chemicals, no toxicity or ecotoxicity testing will need to be conducted with these plants.

A proposed new approach for environmental impact testing
Conceptual and methodological uncertainties of studying the ecological effects of GM crop plants on non-target arthropods (insects) have raised several intriguing general problems. What species or ecosystem functions should be chosen to test? By what routes might these species or functions be exposed directly or indirectly to GM crop plant products? How can meaningful scientific hypotheses be constructed to provide rapid assessments of the magnitude of the potential risks? In contrast to toxicological and ecotoxicological methods for addressing these problems, assessment of the impacts of GM crop plants must be case specific and contextualized to the environment in which they will be used. An international project in which two of the authors (Gábor Lövei and Angelika Hilbeck) have been involved, developed an 'ecosystem representative approach' for selecting species and ecosystem function as foci for further testing (Birch et al. 2004; Andow et al. 2006). This approach combines ideas and methods from a 'community approach', which emphasizes analysis of intact biodiversity, a 'functional approach', which emphasizes community reactions, a 'key species approach', which emphasizes the individuality of species, and an 'indicator species approach', which is central in ecotoxicological testing. We used classic qualitative methods of risk assessment formalized in selection matrices and directed questions, which provide transparent summaries of scientific data and expert judgement that then serve as basis for constructing testing hypotheses and designing proper experiments that address the hypotheses.

The process of ranking and species selection in the above-ground functional groups (herbivores, decomposers, natural enemies, and pollinators), allows the identification and prioritization of non-target species for some key ecological groups; it also reflects the current state of knowledge and expertise available, and identifies gaps in knowledge and uncertainties. When analysing the available information to assess the relative importance of parasitoids in maize in Kenya, for example, the information gaps could be recognized, as well as the realization that the two main maize growing regions, the lowland and the Western highlands, have to be considered separately (Table 10.2). It is also important to consider the process of exposure as part of the overall species selection. The species selection can identify missing information, for example the varying expression of Bt-toxin in different plant tissues in the Kenyan example, and is also crucial for the above-ground exposure analysis. An example of an analysis of significance and exposure is presented in Table 10.3.

[1]www.agbios.com/main.php

Table 10.2. An example of the filled-in selection matrix for parasitoids in maize agroecosystems in Kenya, following the system proposed by Birch et al. (2004).

Sub-guild	Species	Occurrence	Abundance	Presence	Linkage	Rank
Lowland, Kenyan coast						
Egg parasitoid	*Trichogramma* spp.	Certain	Medium	All season	Strong	1
Larval parasitoid	*Cotesia flavipes*	Certain	Medium	All season	Strong	1
Larval parasitoid	C.sesamiae	Certain	Low-medium	All season	Strong	2
Larval parasitoid	*Goniozus indicus*	**Not completed**				
Egg & larval parasitoid	***Chelonus curvimaculatus***	Not completed	Short rains?			
Pupal parasitoid	Pediobus furvus	Certain	Low	All season	Strong	2
Pupal parasitoid	*Dentichasmias busseolae*	Occasional	Low	All season	Strong	3
Highland, Western Kenya						
Egg parasitoid	*Trichogramma* spp., native	Likely	Medium	All season	Strong	2
Egg parasitoid	*Telenomus* spp.	**Not completed**				
Larval parasitoid	*Cotesia sesamiae*	Certain	Medium	All season	Strong	1
Larval parasitoid	*C. flavipes*	Occasional	Low	All season	Strong	3
Pupal parasitoid	*Dentichasmias busseolae*	Occasional	Low	All season	Strong	3
Pupal parasitoid	*Pediobus furvus*	Certain	Low	All season	Strong	2

Table 10.3. An example of the exposure analysis assessment as suggested by Birch et al. (2004). The example is plant-feeding arthropods in maize agroecosystems in Kenya.

Species	Feeding category	Significance	Assessment of exposure
			Spodoptera spp.
		Acarid spp.	Leaf feeder
		Locusts	Leaf feeder
		Sitophilus zeamays	Grain feeder
		Prostephanus truncatus	Grain feeder
		Plant- and leafhoppers	Phloem feeder
		Carpophilus spp.	Saprovore
		Honey bee (*Apis mellifera*)	Pollen feeder
		Wild bee spp.	Pollen feeder
		Coccinellid spp.	Pollen feeder, predator
		Forficulidae	Pollen feeder, predator
		Trichogramma spp.	Parasitoid
		Trichogrammatoidea spp	
		Cotesia flavipes	Parasitoid
		Cotesia sesamiae	Parasitoid
		Other predators: ants, anthocorids, chrysopids	Predators

This underlines the role of this approach to identify and assess the significance of knowledge gaps and uncertainty. Rather than only moving on as a 'decision has to be made', significant knowledge gaps will not be overlooked and can trigger specific action, either to stop an assessment procedure, or to initiate specific, targeted research.

The ranking and selection matrix for soil ecosystem functions has a slightly modified format, to rank and select ecosystem functions. Here, key interactions are to be identified in a systematic and transparent way; species and food-webs affected by, e.g. Bt maize, might be studied in a more relevant manner than performed until present.

Conclusions

In this chapter, we suggested that the basis of environmental risk/impact assessment should be the concepts of biodiversity and ecosystem services. Biodiversity is under threat by mainly human activities. Apart from a moral obligation to protect biodiversity, there is also a utilitarian reason, as biodiversity is important for the functioning of ecosystem services. Ecosystem services are vital for our continued existence, but recent summaries have indicated that humankind is using many of them in unsustainable ways. Consequently, it is mandatory that the impact of new kinds of activities, such as growing GM plants, be tested for their impacts on ecosystem services. Ecological systems are, however, complex and often imperfectly known. We have suggested a transparent, knowledge-based assessment procedure by which important functions and the species or groups that are most significant for this function are identified. This provides one way to develop specific pre-release testing and monitoring systems to assess the environmental impact of GM plants. This system also allows for the identification and evaluation of the significance of knowledge gaps, thus making the precautionary approach in risk assessment operational.

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Chapter 11

Invasion of exotic species: Lessons for GMOs?

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Introduction

A number of species that have never occurred in a particular ecosystem may be extremely well adapted to living there. However, the species pool in any ecosystem is restricted by the limitations in species to migrate. Nowadays, as humans increasingly travel and, even more importantly, have their cargo moved from coast to coast, and between continents, quantities of stowaways are also carried. From a captive situation such as in a ballast water tank, or being slipped in through customs by a tourist, some of the translocated exotic species start to thrive after escaping into a new environment. When exotic species expand their territory in new environments at the expense of native species, they are called *invasive* species. The introduction of exotic species may be intended (i.e. bringing reptiles, birds and ornamental flowers into a new environment for specific purposes) or not intended (micro-organisms, spores, eggs, insects, small animals, seeds, etc.). Both groups are challenging to manage.

The introduction of exotic species (non-GM) is ranked as the second most important factor in all large-scale environmental problems. Habitat destruction is ranked as number one; chemical pollution is ranked third and climate change, fourth (Sandlund et al. 1999). Introduced exotic species leads us in the direction of a 'recombination ecology' or a 'global biological homogenisation' (Enserink 1999), with a consecutive loss of native biodiversity. The introduction of (non-GM) exotic species has been going on for a long time, and as usual, we might have something to learn from past experiences.

In this chapter, I present two case studies of introduced exotic species to discuss some of the similarities and differences between non-GM and GM exotic species. I argue that GMOs form a sub-group of the exotic species (Box 11.1). As all exotic species, GMOs may be introduced into recipient ecosystems, they may have secondary spread and they may become invasive. A comparative analysis of similarities and differences may provide valuable insights; as a common starting point, we should all agree that we have to lean on models as long as the empirical data are not (yet) available. This is the case because GM species have not been used extensively in nature for more than a decade. Yet, general Invasion Biology can tell us that the major ecosystem effects will not (or will rarely) be visible within this time frame.

Box 11.1

In Latin, *exoticus* means 'from the outside'. Exotic species (also called alien, non-native, non-indigenous) are species that are observed in ecosystems where they do not naturally belong. This means that they never had the ability to spread by their own means, i.e. by natural migration. By this definition, all GMOs are exotic because they cannot fulfil any criteria of natural migration (from the laboratory), neither can GMOs be said to have a natural evolutionary background, as opposed to native species. Thus, GMOs are modified and introduced by humans.

Case study I: The invasion of vendace in northern Norway

Vendace (*Coregonus albula*), is a highly specialized zooplanktivore fish species with a natural distribution that does not include the northern parts of Norway, Sweden or Finland. However, in the 1960s the species was introduced into tributaries of Lake Inari, Northern Finland (Mutenia & Salonen 1992). In Lake Inari, vendace reached a high population density during the second half of the 1980s (Mutenia & Ahonen 1990), then subsequently swam downstream into the Pasvik watercourse in Norway, where it was recorded in the upstream part for the first time in 1989 (Amundsen et al. 1999). By the early 1990s, the vendace invaded the whole Pasvik watercourse (Fig. 11.1).

The fish communities in the lakes of the Pasvik watercourse were originally dominated by whitefish (*Coregonus lavaretus*). The gradual downstream expansion of vendace in the Pasvik watercourse has facilitated a study of the mechanisms of ecological interactions in a large scale 'natural experiment', comparing several levels in the food web over 15 years.

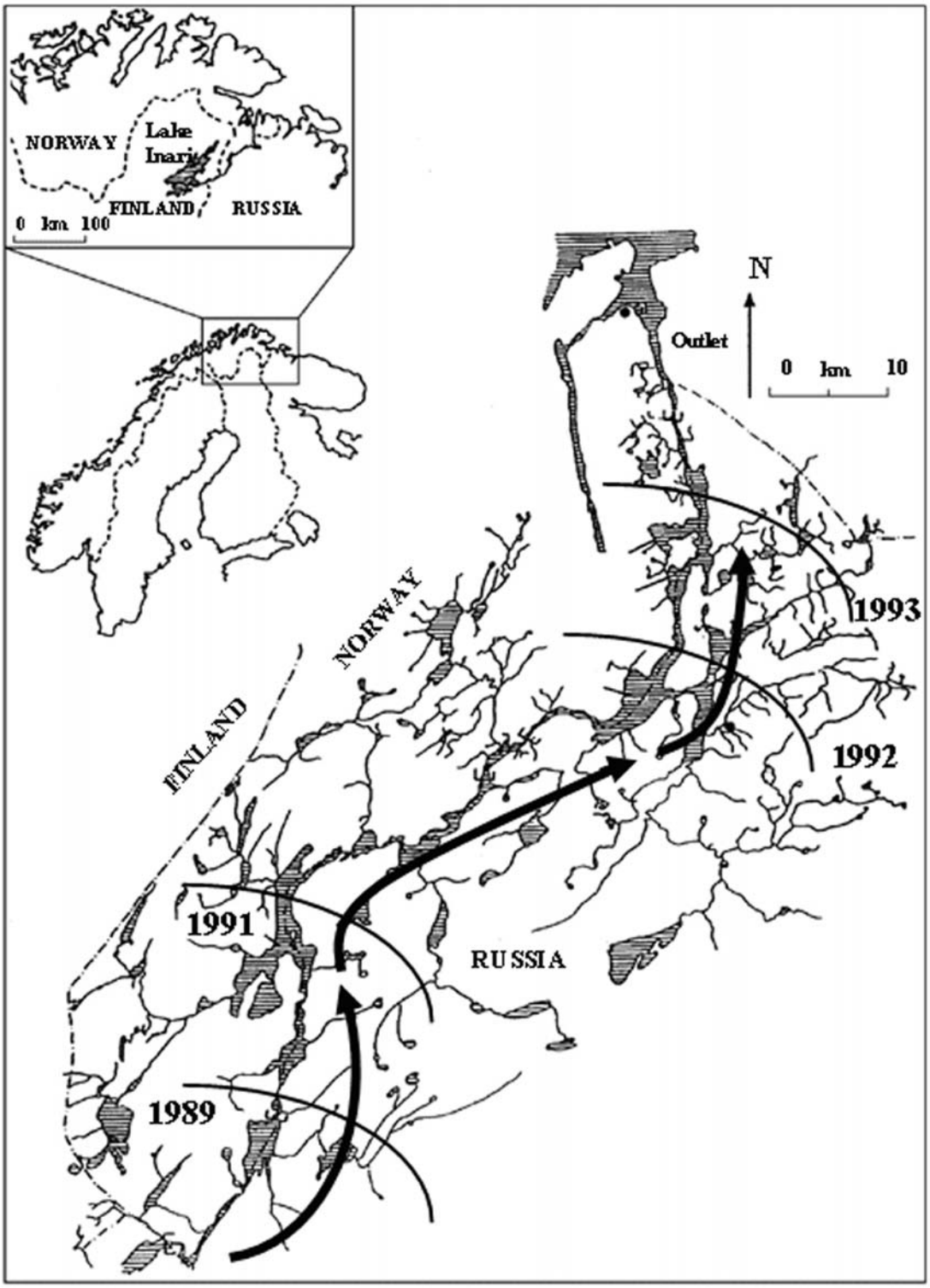

Figure 11.1. Map of the Pasvik watercourse. Arrows show the direction of the vendace invasion and arcs show the year of the first observation.

During this period the vendace proved to be a keystone species (i.e. a strongly interacting species in the food web – see Figure 11.2 for a community overview), with effects on at least three trophic levels:

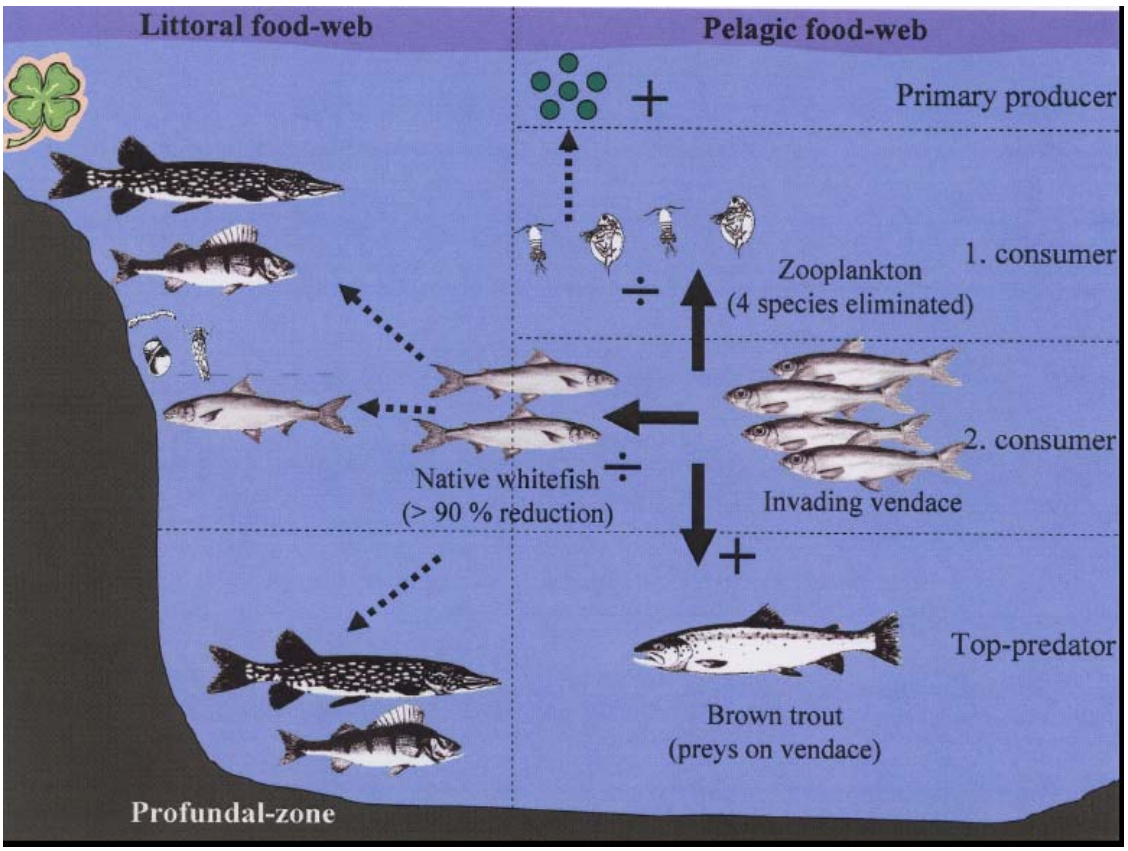

Figure 11.2. Community overview of direct (bold arrows) and indirect effects (dashed arrows) of the invading vendace into the food web of the Pasvik watercourse.

1) As a *predator*, vendace has grazed down the zooplankton community, eliminating the four largest species of zooplankton between 1991 and 1998. In addition, a reduction of body sizes within cladoceran species and a shift towards smaller species in the zooplankton community was observed. These results represent a strong 'top-down' regulation that may limit the food resources of other zooplanktivore fish species. Studies of predation effects on zooplankton communities are usually based on comparisons between water bodies in which a fish predator is present or absent (Brooks & Dodson 1965; Hall et al. 1976). Such studies can be done experimentally or when a predator invades a new area, and may reveal how ecological interactions work, e.g. how exotic species (or GMOs) may alter native ecosystems.

2) As a *competitor*, vendace has competed with the native whitefish for pelagic food resources, the zooplankton. This interaction has lead to a more than 90% reduction in the density of the whitefish. Subsequent to a biological invasion, the processes of interspecific competition, rather than its steady-state outcome, can be studied in the receiving ecosystem (Simberloff 1981; Pimm 1989; Ross 1991). Introduced exotic species thus provide large-scale 'natural experiments' where ecological theory (e.g. competition) may be tested empirically. Biological invasions may also provide unique long-term recording of empirical data (Bøhn et al. 2004). By definition, two

species compete when they negatively affect each other by consuming, or controlling access to, a limited resource (Keddy 1989). Low resource availability and high niche overlap make competition more likely (Giller 1984; Wootton 1998). Interspecific competition may, for one or more of the species involved, lead to altered resource utilization, to reduced density, or ultimately to competitive exclusion and extinction (Gause 1934; Hardin 1960).

3) As a *prey*, vendace has become the most important prey species for the dominant pelagic predator, the brown trout. This means that the feeding behaviour of the brown trout has changed and that vendace replaces the whitefish as the link between the zooplankton and the top predators. In general, the impact of predation on a prey community depends on the habitat-specific density of different predators, and their prey selectivity. When a new prey species is invading, an altered size- and species-selection of prey may be expected directly in the predators. In addition, indirect effects, acting through e.g. competition between prey species in the food web, may change growth rates and thus also the timing of new activities (ontogenetical niche shifts), thereby changing the overall outcome in the community. The trade-off between food acquisition and predator avoidance is a major determinant for the habitat choice of animals (Pyke 1984; Stephens & Krebs 1986). When size-selective predators are present, this trade-off discriminates between age- or size-classes in a prey population, and between species within a prey community (Werner et al. 1983; Persson 1988; Hambright et al. 1991; L'abee-Lund et al. 1993; Brabrand & Faafeng 1993).

There are important links between the different effects that vendace contributes to, and the different components of the food web after its invasion. Altering one level in a food web necessarily impacts the other levels, so that the total effect will depend on a number of indirect effects, in addition to the direct effects. As with the vendace fish invader, direct effects have been observed on three trophic levels (zooplankton, zooplanktivore fish and top-predators). The indirect effects are harder to track and follow. The case study of the vendace shows that invasions have case-specific effects that are extremely difficult to predict.

Ecosystem or food-web effects are difficult to study, interpret and understand due to high complexity. Furthermore, only a very few examples of ecosystem changes due to the impact of exotic species have been studied *during* the period of change, and no researchers at all would pretend to fully understand which mechanisms were responsible for the effects shown, even though the scientific discipline of Invasion Biology has 50–100 years of active research to acknowledge. This situation will not become easier with introduced GM species.

Case study II: Rabbits in Australia

The second case study concerns rabbits in Australia, a case that will be familiar to most readers. In 1859 Thomas Austin imported 24 rabbits from England to Victoria: 'The introduction of a few rabbits could do little harm and might provide a touch of home, in addition to a spot of hunting'[1]. Twenty years later, in 1879, there was still a focus on the advantages of introducing exotic species: 'All birds and animals may be introduced as shall afford sport and amusement without doing injury to the Agriculturist and Gardner' (Strahan 1992).

However, by 1890 the situation was very different from what had been imagined. Farmers had to abandon their properties in the face of rabbit plagues in some places. The view of reality and the focus had shifted from a positive potential to a dramatic pest. In the following decades the rabbits, in combination with sheep and cattle, grazed down Australian landscapes. Plants and trees, which

[1]http://www.agric.wa.gov.au/programs/app/barrier/history.htm

keep the surface of the soil intact with their roots, were grazed down and removed, and severe dust storms appeared. Entire homesteads were buried by dust, people died from being buried in dust storms, communities had to reorganize on semi-arid land. There were also adverse health effects, such as blindness. All of this exemplifies indirect and unintended ecosystem effects. Rabbit control through means of shooting, trapping, poisoning, and fencing has proven to be ineffective on a large scale. In 1951, a virus disease (myxomatosis) was introduced to the rabbits in Australia. This reduced the numbers of rabbits from ~600 million to ~100 million in a couple of years. Later, this kind of control turned out to be complicated by the evolution of resistance, resulting in a continuous 'arms race' between rabbits and scientists. Immediately after the introduction of myxomatosis, a return of endangered plant species was observed. The rabbits are estimated to have cost Australian agriculture approximately USD 300 million per year (at least 2% of all agricultural production). The total costs to the nation are twice as high, USD 600 million dollars per year, according to the Australian CSIRO (Commonwealth Scientific and Industrial Research Organisation[2]).

High densities of rabbits still inhabit the southern part of the whole continent and Australia continually struggles with soil erosion. This is the number one environmental issue facing the country, due to overgrazing by rabbits and other feral species. The cost of lost productivity due to loss of land is incalculable.

Lessons from introduced species – similarities and differences between non-GM and GM exotic species?

Not all exotic species establish after introduction. In fact, the 'tens rule' (Williamson 1996) states that approximately ten per cent of all introduced species succeed in establishment. Further, approximately ten per cent of the established species become pests. This means that approximately one per cent of all introduced exotic species have some sort of serious negative consequences in the receiving ecosystem.

The 'tens rule' may be difficult to apply directly over to GM exotic species. One reason for this is that fitness-relevant traits, such as growth rate or resistance to a limiting factor (predators, parasites, diseases), are often directly modified. This is discussed later in the chapter.
Exotic species (both non-GM and GM) can be divided into two categories: i) those that need support, e.g. by agricultural means such as ploughing, fertilizer, etc. in order to survive, and ii) those that are free ranging and would be readily spread into the environment. The distinction is not always clear. We should remember that all agricultural fields are also part of the 'ecological theatre' in which the 'evolutionary play' is continuously being played (Lövei 2001). Many agricultural plants are also used in areas where the same species, or close relatives, live in the surrounding environment. As all GM organisms have been defined as exotic, the issues of co-existence and horizontal gene transfer between closely related species will fall under the umbrella of Invasion Biology. However, there is no parallel to this issue from classical Invasion Biology. This chapter thus deals mainly with exotic species that have the ability to spread into the environment.

Similarity – Introductions are followed by secondary spread (invasions)
The spread of exotic species, whether they are GM or not, occurs in at least two stages: the first is the active transport made by humans (the introduction). This stage is often unintentional and beyond human control, e.g. in ballast water in ships, in GM-contaminated seeds of maize, etc. The second stage is the secondary spread made by the species itself (spread of pollen,

[2]http://www.csiro.au/communication/rabbits/qa2.htm

microorganisms, running animals, and swimming fish belong to this category). The latter stage is completely beyond human control, but depends on the rate of spread of the exotic species and its ability to establish in still further environments. The vendace invasion serves as an example of how these two stages of spread may be separated in time by several decades.

Similarity – Unintended ecosystem effects after long time delays
Both of the aforementioned case studies show that the sum of ecological harm comes from direct and indirect effects over an extended period of time. For the vendace, the time delay between the first introduction and the observed ecosystem changes (in the Norwegian part of the watercourse) was approximately 35–40 years, due both to the secondary spread of the species and the building up of consequences through linked ecological interactions, i.e. competition from the invading vendace forced the native whitefish to change habitat, and in the new habitat a high density of predators fed on the small-sized and relegated whitefish individuals. For the rabbits in Australia, a time delay of approximately 20–30 years occurred before people realized that the rabbits represented an irreversible large-scale plague.

The time lag before effects are observed is an important but difficult fact to handle in decision making. A major difficulty with political decision making is to tackle the trade-off between rapid profit and long-term negative consequences. This is a matter relevant to most environmental problems. However, for biological pollution, i.e. reproducing organisms that may be increasingly harmful over time, there are, in addition, risks of inaction. Here, action means that society acts to prevent the introduction of a potentially harmful species, or eradicates it early in the process of establishment. As one prominent invasion biologist Daniel Simberloff (2003) puts it: 'because of their population growth and dispersal abilities, introduced species are one target of resource management at which it is often better to shoot first and ask questions later'.

Unfortunately, many examples from Invasion Biology show a shift from the expectation of progress and benefit, due to the introduction of an exotic species, to the realization of the spread of a growing pest. This is regularly a one-way shift. Biological pests hardly ever shift back to beneficial species but instead last for the unforeseeable future (for example, this is the case with the rabbits in Australia). Whereas it may be completely natural to have a naïve first attitude to what is new and unknown, we should realize that risks and the harm of self-replicating biological organisms are not like any other 'invention'. A 'successful' invasive species cannot be taken back, and the harm to the receiving ecosystem regularly increases over time. Therefore, all biological material should be treated with precaution and humility.

Similarity – huge resources are needed to understand and study complex ecological interactions
To rightly evaluate the ecosystem consequences of an exotic species a long-term perspective is necessary. Often it will also be necessary to follow the consequences on several trophic levels, which necessitates a diverse competence. Further, the complex structure of food webs makes studies difficult and sometimes inconclusive, especially in species-rich ecosystems. As an example, the amount of resources required to study the vendace invasion, in a fairly detailed manner through a period of change, is quite considerable. People included in this 15 year long study include, from Norway, one university professor, two PhD students, seven Masters students and one university field course over five years. In addition, there have been seven Russian and three Finish researchers involved in the study. An estimate of the financial input adds up to approximately USD 1 million. Large samples of fish (15,000) and zooplankton (50,000) have been necessary to reach conclusions on the ecosystem changes.

Similarity – Irreversibility
Some of the human impacts on natural ecosystems are possible to reverse, meaning that stopping unwanted development, like chemical pollution, may lead to a restoration of the system. Even though it is correct to say that ecosystems never return exactly to their original state (since all living systems continue to evolve) they may return more or less to their pre-disturbed state or quality. Examples of reversible impact factors include DDT, acid precipitation, nuclear emissions, organic and inorganic pollutants, etc. When it comes to spread of living organisms, we may learn a lesson from Invasion Biology: Invasive species may be eliminated just after their introduction only under some (unlikely) specific conditions. And it is almost impossible to get rid of them later.

By the same token, GMOs that often carry single or multiple enhanced fitness traits will have a huge evolutionary potential if spread into the environment. Hence, they must be considered as potentially irreversible elements of the future environment and evolution. What this will ultimately mean, e.g. for ecosystem interactions and biodiversity, is open to speculation. The environment and the ecosystems function on a level of complexity that we rarely can cope with in terms of precise scientific understanding. When, in addition, we know that introduced invasive species represent irreversible events, we should act with precaution.

Difference – Public invisibility
An important difference between introduced (non-GM) exotic species and GMOs in the environment is the public invisibility of GMOs. Humans are terrestrial mammals and it is easy for us to recognize and react to introduced exotic species and their effects: we are able to see rabbits, dust storms, plant eradications, and exotic fish species. In contrast, the public will not be able to distinguish a GM from a non-GM organism in the environment. No outside examination will reveal the modified genetic origin of a maize plant, a tilapia or salmon fish as they will look more or less identical. The same is true for hybrids between GM- and non-GM organisms. Furthermore, microorganisms and naked DNA are invisible (to the human eye). The invisibility of GMOs, or the difficulty in easily distinguishing them from conventional organisms, is causing huge difficulties for handling and management, and has triggered a resource-demanding enterprise in tracing and controlling GMOs.

Difference – Scale of introduction
GMOs are intended for industrial production, which means they will be introduced on a large scale and on a continuous basis (e.g. for fish farming). In contrast, non-GM exotic species are usually introduced without purpose in small numbers e.g. in ballast water tanks in ships.

Difference – GMOs often have modified fitness parameters
I will now go into some more detail, by using examples, about specific traits that are modified in GMOs: increased resistance to controlling factors and enhanced growth. What do these traits mean in nature?

GMOs with increased physiological tolerances, or GMOs that are able to resist predators, parasites or any kind of disease are expected to perform better in nature, simply because stress from factors in the environment are released. Resistance to controlling factors implies increased survival. GMOs may thus reduce grazing, predation, parasites, diseases, and other cues in the environment. Whereas these may be valuable traits in a contained system, we have to remember that both agriculture and aquaculture represent open systems that interact with neighbouring ecosystems. We should therefore evaluate the potential invasiveness of GMOs on a case-by-case basis. Increased tolerance or resistance represents an expansion of the fundamental niche of a species, which likely leads to an expansion of its geographical distribution (increased spread).

This is due to the fact that limiting environmental factors, both biotic and abiotic, restrict the distribution of organisms. An expansion of the fundamental niche of a species is also expected to influence the species' ecological role through increased competitive ability within its natural geographical range.

For example, GM fish are often modified to have increased growth, usually resulting also in a larger maximum size, by inserting growth hormone genes. The effect of introducing growth hormone genes can be remarkable; transgenic coho salmon have been shown to be eleven times heavier (on average) compared to control fish over a period of fourteen months (Tymchuk et al. 2005). Growth is a fundamental fitness trait that is linked to a number of species interactions in food webs. High growth rates and large size may translate to strong competitive ability and increased predation on lower trophic levels. Transgenic coho salmon are shown to outgrow non-transgenic salmon when food availability is low, as is usual in nature, and also to invariably contribute to the dominating individuals in competition trials with non-transgenic individuals (Devlin et al. 2004). Altered growth also means altered ecological interactions with most other species. This is well known from studies of fish. Fish have indeterminate growth, meaning that the adult size is flexible and not fixed. This fact is already taken advantage of by GM techniques, as we have seen.

The perch shown in Fig. 11.3 has three more or less separate and different ecological roles as the size of the fish changes (perch have ontogenetic niche shifts). Small perch eat mainly zooplankton in the pelagic habitat, while medium-sized perch eat benthic invertebrates and large perch are piscivorous. In nature, this means that perch functions as three different species in practice. A GM fish experiencing rapid growth would in nature potentially alter such fine-tuned evolutionary equilibria.

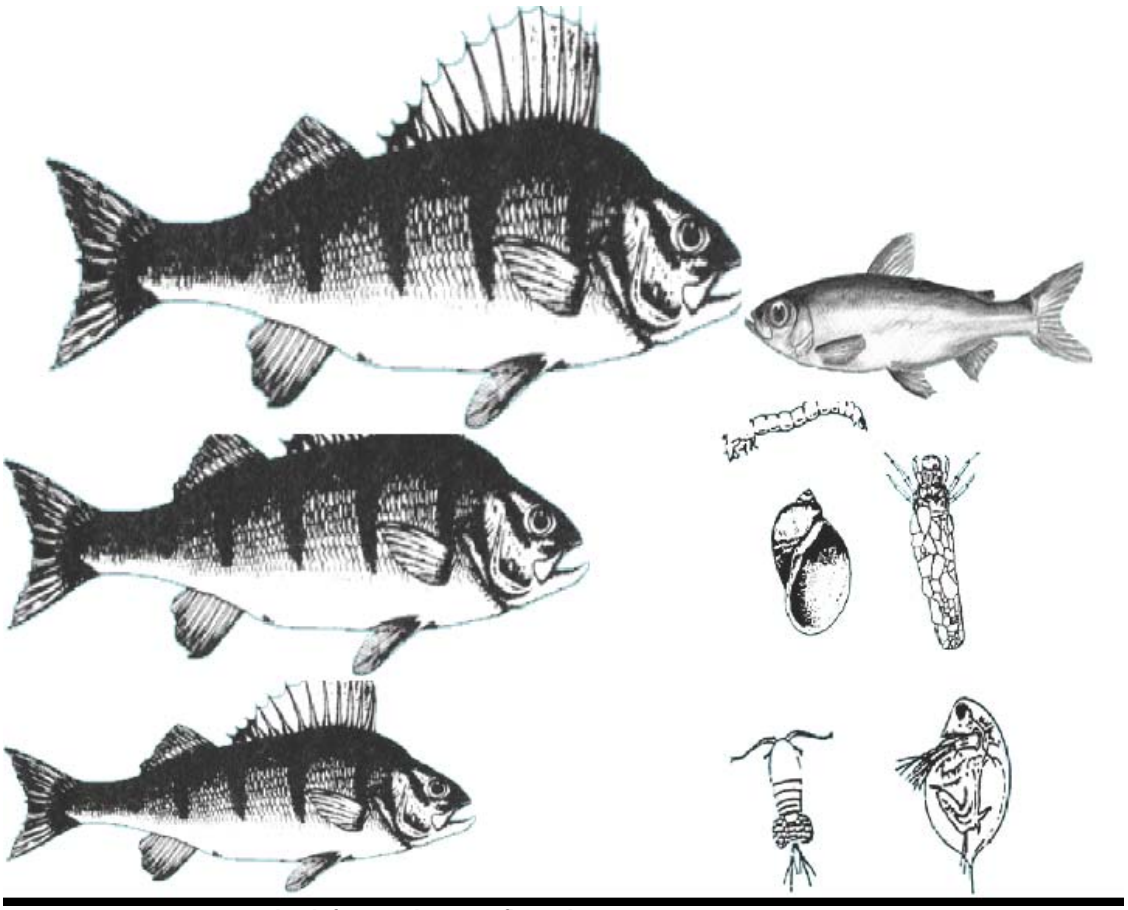

Figure 11.3. Ontogenetic niche shift in perch (Perca fluviatilis).

Higher growth rates may also affect behavioural and life history traits in fish. High growth rates may also be likely to increase the fecundity and the maximum swimming speed; the fish may mature earlier and become more dominant on spawning grounds. However, the viability of GM fish may be lower compared to non-GM fish. The transgenic Japanese medaka, expressing growth hormones, studied by Howard et al. (2004), may serve as an example. The GM fish was 83% heavier and the males mated three times more compared to the non-GM males. However, the GM offspring survival was 30% lower than the non-GM offspring. The sum of these differences (as demonstrated in a mathematical model) leads to two interesting effects. The first is that due to more mating, the GM fish will take over the population, and secondly, due to the lower offspring survival, the population goes to extinction (Howard 2004). The phenomenon of being invaded by malfunctioning individuals is called a 'Trojan Gene Effect'.

Also with plants, the introduced transgene may increase the fitness of the organism in the environment: transgenic Bt-sunflowers, expressing Bt-toxin that reduces grazing by insects, are shown to hybridize with unmodified sunflowers in the environment (Snow et al. 2003). The hybrids also express Bt-toxin (at a lower level). In areas where insects graze on the sunflowers, the hybrids are shown to be superior seed producers, with up to 55% more seeds (Snow et al. 2003). Under these conditions, GM sunflowers would gradually spread into wild populations and take over due to higher fitness. According to Snow (2002), 'It is currently impossible to prevent gene flow between sexually compatible species in the same area. Pollen and seeds disperse too

easily and too far to make containment practical. This makes the need for environmental studies all the more urgent'.

To sum up the adaptive value of many transgenic organisms, many or all affected traits have short-term positive fitness consequences: higher growth rates, increased disease resistance, reduced age at maturation, higher number of offspring, expanded environmental tolerance. Thus, the traits that are advantageous for industrial production are strongly overlapping with what makes a species invasive in nature. There may be metabolic or other costs involved in producing these transgenic traits, and these should be studied under controlled and contained conditions. However, to extrapolate data from the laboratory to complex ecosystems is extremely difficult, also because many organisms (e.g. fish) behave differently in the laboratory as compared to in nature (due to genotype by environment interactions) (Devlin et al. 2004). A summary of similarities and differences between non-GM and GM species is given in Table 11.1.

Table 11.1. Summary of similarities and differences between non-GM and GM exotic species.

	Similarity	**Difference**
Introductions followed by invasions	Both have secondary spread (invasions).	
Ecosystem effects after long time delays	Effects often appear several decades after introduction.	
Huge resources needed to understand complex ecological interactions	To evaluate ecosystem consequences of exotic species, long-term studies on several trophic levels may be required.	
Irreversibility	After release, exotic species (whether GM or non-GM) can rarely be eliminated from the environment.	
Public invisibility		Whereas non-GM exotic species often are easily recognized, GMOs will often not be distinguished from a non-GM organism in the environment – they will look identical.
Scale of introduction		Whereas many GMOs intended for industrial production will be released on a large-scale, non-GM exotics are mostly released unintentionally and on a small-scale.
Modified fitness-parameters		GMOs have a number of traits that may be altered towards higher fitness. Advantageous traits for industrial production overlap with what makes a species invasive.

Conclusion

From Invasion Biology we have learned that a minority of introduced species have caused huge ecological and economic problems, after time lags of decades. A rather naïve and short-sighted perspective has prevailed in the history of humankind; species have been introduced with a focus on profit, and not on their potential damage. For a long time, however, the introduction of exotic species was limited by the poor ability of humans to travel across large distances. Recently, this ability has been dramatically increased, and many well-adapted exotic species (invasive species) have caused large-scale ecological harm to native biodiversity. Unfortunately, we may *not* have learned the most important lessons of Invasion Biology and may end up in the same situation again, with unwanted introductions of exotic GM species. Many exotic GM species even have fitness advantages compared to species in the natural environment. The performance of these should be thoroughly tested in contained semi-natural conditions to explore their potential invasiveness. The pressure to commercialize GMOs should not be at the cost of thorough independent scientific testing (Fig. 11.4), nor at a scale relevant for foodwebs and ecosystems.

Figure 11.4. Technology seems to be running ahead and risk assessment and risk management are dragged passively behind.

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Chapter 12

Vertical (trans)gene flow: Implications for crop diversity and wild relatives

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The purpose of this chapter is to present an overview of the potential evolutionary consequences of (trans)gene flow, focusing on crop plants. From a scientific standpoint, the challenge is to determine and gather all of the relevant scientific knowledge possible, identify uncertainties and known gaps of knowledge, and use this information to design a context-specific framework to guide the safe use of a particular technology. Likewise, understanding and minimizing the potential safety impacts of GMO crops requires identifying the relevant issues and information – not only genetic and biological information, but also socio-cultural and legal dimensions as well. In this case, I will introduce rudimentary concepts of gene flow, discuss the current state of knowledge, assumptions and future needs in biosafety research. The objective is to contextualize the scientific issues to help understand the issues for developing a sound scientific assessment of the potential implications of vertical transgene flow for crop biodiversity, weed and target resistance evolution, and food security. From this, a series of critical questions and needs emerge, and can be added to discussion and decision making within the realm of a particular country, crop, and/or policy regime. Other emerging issues, such as the impacts on human health and environment, are discussed in Chapters 14 and 10, respectively, and are outside the scope of this chapter. Note that this chapter is intended to give only a basic introduction to the subject, yet provides references to key literature in the field for further reading (for extensive reviews on the subject, see Ellstrand et al. 1999; Ellstrand 2001; Eastham & Sweet 2002; Gepts & Papa 2003; Messeguer 2003; Snow et al. 2004).

In Section 1, I will introduce the basic concepts in biology of gene flow. Section 2 will be dedicated to discussing the potential evolutionary significance of transgene flow from a) crop to wild relative, b) crop to landrace, and c) crop to crop, each of which have their own set of emergent socio-cultural, political and economic considerations. These will be illustrated by recent research and actual transgene flow events. In Section 3, I will discuss some of the means of tracking transgenes. Section 4 contains a discussion of some critical gaps in scientific understanding and uncertainties that should be communicated to policy makers, and the general public, for making informed decisions on the safety of transgenic crops. In the fifth and final section, I discuss some questions that may be useful in consideration of policy and risk assessment concerning GMOs with respect to crop biodiversity and food security issues.

1. Overview of vertical gene transfer (gene flow)

1.1 What is gene flow?

Gene flow is the movement of genes from one population to another, conferring new traits – the biophysical characteristics of the organism – to individuals of the recipient population. This happens by *cross-pollination* (also called *hybridization*), that is, the pollination of members of one population or genetic pool with that of another. The outcrossing of genes is said to be 'vertical' as the genetic information is passed 'down' from parents to offspring. This is contrasted with *horizontal gene transfer* (discussed in Chapter 13), where the acquisition of genes is passed over, i.e. 'horizontally', from one organism to another by means other than inheritance. Vertical gene flow often results in *introgression*, the establishment of *alleles* (gene variants), or wholly new genes (as is the case with transgenes) in the recipient population.

Therefore, vertical gene flow is restricted to organisms that can mate with one another and make offspring. In the case of crop plants, which are domesticated forms of wild plants, a high degree of compatibility can therefore exist between the crop and wild and weedy relatives. Gene flow can be from crop to crop (or landrace), from crop to wild relative, and even from wild relative to crop plant. Gene flow has been a natural, and in some cases desirable, part of evolution and speciation in flowering plants (Anderson 1961; Reiseberg & Wendel 1993; Ellstrand et al. 1999). Thus, gene flow per se is not the main concern, but rather the *types of genes*, and the level of genetic heterogeneity or homogeneity (genetic diversity) that is spread through gene flow and its effect on recipient populations, are the relevant issues. Whether flow of new genes or gene variants results in a change in *fitness*, i.e. the ability of the organism to survive *and* produce viable offspring (either positively or negatively), has been a central focus in population biology. It should be clearly pointed out that considerations of gene flow discussed here are not unique to transgenic crop varieties, but are relevant for all commercial crops. The introduction of wholly new genomic identities into recipient populations from commercial traits should be equally scrutinized, but is outside the scope of this chapter.

If commercial crops have been exchanging genes with related species for some time, why are transgenic varieties of particular concern? With transgenics, completely novel traits are passed on that could dramatically affect the fitness of individuals receiving the given gene in a population. Thus, the commercialization of transgenic plants has sparked widespread interest in the potential evolutionary significance of transgene flow. The central question is how transgene introgression may impact fitness in the new transgenic hybrids, and consequently, the significance for maintaining important crop genetic diversity for future crop breeding.

1.2 Under what conditions does gene flow occur?

Hybridization and subsequent gene flow depend on a number of biological and ecological conditions. First, the sexually compatible plants need to be growing within sufficient pollen or seed dispersal range of the transgenic crop. In many cases, there is no overlap between crops and wild/landrace relatives, and they do not pose a concern, yet crop to crop gene flow often is a concern. The possible dispersal range of reproductive propagules (i.e. pollen) is dependent on many different climactic (including wind, humidity, temperature, etc.) and biological factors (height of plant, size of propagule, natural outcrossing rates, etc.), but human dispersal can also broaden this range. Second, in order for gene flow to occur, there must be an overlap in *phenology* (flowering and fertility times) between the transgenic crop and recipient population. Flowering times may be affected by ecological and or biological factors in some circumstances, leading to partial or total reproductive isolation among neighbouring populations. Third, any mating between a transgenic crop and a landrace or wild relative must produce fertile and viable offspring. Reproductive barriers to introgression are strong, especially where *ploidy number* (genomic copy number) differs between domesticated and wild crop relatives (Jenczewski et al. 2003).This may only occur in limited scenarios. Plants that normally are only self-compatible, i.e. have the capacity to only mate with itself, also represent a type of reproductive barrier. Fourth, the offspring of the new transgenic-hybrid plant must also be viable and fertile to some extent, and a lack of survivors means that any potential gene flow would cease at this point. Yet even a low level of fertility can lead to fully viable populations in subsequent generations, as would be the case with *backcrossing* (mating 'back' or again, with the parent population) into the wild progenitor populations.

When these four conditions are met, transgene flow is likely. In some of these cases, offspring will have reduced fitness, or produce sterile (unviable) seeds. In other cases they will have improved vigour (Singh et al. 1995; Hauser et al. 1998) and fitness, yet the advantages may reduce or reverse over time. Thus, there must be a minimum level of fertility in order for the

recipient population to maintain the transgene(s) and survive to the next generation. Lastly, it should be mentioned that the dispersal of seeds themselves can also be an agent of gene flow. The movement of seeds can occur in a range of ways, mostly by human activities, such as transportation (Figure 12.1), or by wind or wild animals.

Figure 12.1. A maize plant growing on the side of the highway outside Guadalajara, Mexico. This plant presumably arrived as a seed fallen from a transport truck. (Photo: D. Quist, 2002)

1.3 In what species or kinds of crops could transgene flow occur?

Almost all of the world's most important crop plants are known to hybridize with wild relatives. At least 44 cultivated crops have demonstrated the capacity for hybridization with wild and weedy relatives, including 12 of the 13 most widely cultivated crops (Ellstrand et al. 1999), and 11 of the 20 most important US crops, including sunflower, radish, sorghum, canola, squash, rice, wheat, sugar beet, lettuce, poplar, strawberry, and bentgrass (Ellstrand 2003). As discussed, gene flow to wild relatives and landraces will depend on the availability of such species near the area of cultivation (Messeguer 2003). Crop to crop gene transfer often occurs where transgenic and non-transgenic crops are planted in close proximity. Many of these crop plants are primarily

outcrossing species, including maize, canola (rapeseed), tomato, sorghum, wheat, sugar beet, alfalfa, cucumber, radish, and strawberries (NRC 2000).

2. (Trans)gene flow and its potential evolutionary consequences

So why might changes in plant fitness (its ability to survive and reproduce) resulting from transgene flow be significant? Effects on fitness are largely dependent on the nature of the genetically engineered traits, and the external and internal factors that influence their expression (Gepts & Papa 2003; Jenczewski et al. 2003). As approximately 97% of all transgenic crops involve insect resistance or herbicide resistance, these are the main traits under consideration (yet the history of unintended transgene flow events, and the coming generation of plant-made pharmaceuticals are perhaps signs of things to come). In the case of insect resistance transgenes, levels of pest pressure in wild or landrace populations may be lower compared to crop populations, reducing the selective value of the trait. Herbicide resistance genes might exhibit an energetic cost on the hybridized plant that would have no value if the herbicide is not applied (but alternatively, great value if it is). In the case of stress-tolerant transgenic crops (drought tolerance, salt tolerance, etc.), with traits that allow survival in a broader range of ecological conditions, hybridization is likely to increase fitness and invasiveness.

Hence, whether transgenes from a source population will establish in wild or landrace sink populations will depend on a number of independent and interrelated factors – genetic, ecological and even human management variables. Identifying the most important components to survival is not straightforward, and must be considered within the ecology of transgenic hybrids. Variation in fitness is also likely across hybrid generations. With such little knowledge on the behaviour of transgenes in unintended and new genomic and ecological backgrounds, prediction of real-world effects is particularly challenging.

One principal concern of transgene flow is the loss of potentially useful crop genetic diversity in the recipient population (whether other crops, landraces or wild relatives). *Outbreeding depression* (the reduction of fitness from hybridization) can lead to a decrease in allelic diversity by extinction of members of a diverse gene pool that are less adapted to survive because of the particular introgressed transgenic trait. This is loss of diversity through negative selection. On the other hand, when transgene hybrids have an increased fitness, and can survive into the next generation, *genetic assimilation* (loss of unique genetic identity through continual hybridization and backcrossing) will have a homogenizing affect on the recipient population, also leading to a less diverse gene pool. Thus, both instances can have negative effects on genetic diversity. The magnitude of these selective forces within the new genomic and ecological background of the recipient population will largely determine the rate of evolutionary change in the recipient population (Gepts & Papa 2003).

So how can we predict the outcomes of transgene flows on a recipient population? Population matrix models have been suggested as useful ways to estimate this risk (Parker & Kareiva 1996; Bullock 1999). However, the magnitude and evidence of effects is idiosyncratic, and may take years to develop (Ellstrand & Hoffman 1990). Few direct studies have been conducted to measure the fitness effects of transgenes in wild populations (Linder 1998; Linder et al. 1998; Snow et al. 2001; Spencer & Snow 2001; Gueritaine et al. 2002; Snow et al. 2003). Of these, many were conducted under ideal agricultural conditions, where water and nutrients were not limiting, and interspecific competition was low, rather than stress conditions often faced by low- or unmanaged populations. Further, many studies seeking to understand persistence of transgenes in natural populations have only studied the first hybrid generation. Some investigators have questioned the value of such estimates in early hybrid generations (Linder et al. 1998), as variation in fitness

may occur across generations due to recombination and selection (Hauser et al. 1998). Models to quantify such changes over subsequent hybrid generations have been useful to help predict potential outcomes of such events through time (Lavigne et al. 1998).

A useful model to study survivorship after gene flow is *migration-selection balance*. This model demonstrates (Lenormand 2002) that in crop to crop, or crop to wild gene flow, even *negatively selected traits* (traits that decrease the plants' ability to survive) are still likely to be maintained (in balance) in the recipient population. Whether this allele is maintained or not depends on the level of gene flow to the population. If there are sufficient rates of gene flow of the negative selected allele, a threshold value will be reached, leading to *fixation* (permanent maintenance of the allele in the population). In this case, the sub-optimal allele would predominate purely by magnitude of gene flow coming into the population.

Given the importance of introgression for the evolution of land plants, and the ubiquity of gene flow between crops and wild relatives, the impacts on native genetic diversity is a broad concern (NRC 2000; Pilson & Prendeville 2004; Snow et al. 2004). Some investigators downplay these risks, assuming that if transgene flow produced offspring of low fitness, the transgene would not survive in the population at all. Yet, research contradicts this assertion. Theoretical studies suggest that introgression rates of genes from one population to another can be quite rapid even when the fitness advantage is small (Barton & Dracup 2000), or when there is a high frequency of transgressive hybrids (Reiseberg & Wendel 1993). A modelling study conducted by Haygood et al. (2003) demonstrated that crop alleles can be rapidly fixed in a recipient population when the migration frequency exceeds the selection threshold, even when they have a negative impact on fitness. Their study expands on how *demographic swamping* (reduced fitness in the hybrid's offspring populations) can facilitate genetic assimilation just where high rates of gene flow occur from agricultural populations. In this situation, gene flow that reduces fitness will become stable in the population when the migration rate of the alleles exceeds the level of selection, leading to reduced population size and perhaps local extinction. Further, extinction through hybridization is a valid concern not only when it involves transgenic plants, but in any situation of non-native biological or genetic invasions (see Chapter 11 on invasives) where hybridization may increase a plant's invasiveness (Ellstrand & Schierenbeck 2000).

2.1 Types of transgene flows and their implications

With the now decade-long history of GMO commercialization, the world has already witnessed a number of cases of transgene flow, from crop to wild relatives, crop to landrace and crop to crop. Within each type of transgene flow, a host of environmental, agronomic, cultural, and intellectual property concerns emerge in conjunction with the biological and evolutionary considerations of gene flow. While research has made some progress, there is still much to be learned.

2.1.1 Gene flow from crops to wild and weedy relatives

Transgene flow, generally regarded as undesirable and hence often regarded as 'transgenic contamination', presents a number of management challenges with the formation of transgenic hybrids in sexually compatible weed species (Darmency 1994; Snow & Palma 1997). Hybridization may give distinct selective advantage over non-hybrids in a population, particularly where certain herbicides are used to control these weeds – and can allow the hybrids to become more invasive in natural and agricultural habitats (Ellstrand 2003). Increased weediness of some wild relatives also augments their invasive potential into new environments whereas resistance to insect damage is inherited from insect-resistant crops. Gene flow from crops to wild relatives has been linked to the evolution of weediness in seven out of the thirteen most important crop plants (Ellstrand et al. 1999).

A good example of transgene flow between a crop and its wild relative is that of transgenic oilseed rape (also called canola) *Brassica napus*, and its wild relative *B. rapa*. Early research suggested that hybrids between oilseed rape and the weedy *B. rapa*, would be minimal, due to gene flow barriers and low survival (Crawley et al. 1993). However, later research by Mikkellsen et al. (1996) and Hall et al. (2000) have shown wide dispersal of herbicide tolerance genes in weedy *B. napa*. Gene flow has subsequently been shown to persist for many years (Pessel et al. 2001; Simard et al. 2002). This has led to a number of distinct challenges for weed management near agricultural lands.

Another example involves the escape of transgenes from glyphosate-resistant (a herbicide) bentgrass (*Agrostis stolonifera*) in the United States. Reichman et al. (2006) detected transgenic hybrids with weedy *Agrostis species* some 3.8 km downwind of transgenic field trials, in federally-protected grassland. The ecological consequences of such outcrossings are uncertain, yet any decrease in genetic diversity would lead to a change in community structure with the introgressed regions. As a result, in 2007 a federal judge ordered a temporary halt in new approvals of GM field trials, citing an inadequate environmental review of the potential environmental impacts.[1] The ruling requires that future GM trials in the US must undergo more rigorous environmental reviews.

Whether or not any resulting gene flow has an evolutionarily significant effect on wild and weedy relatives must be tested carefully. Few studies have directly addressed crop to wild transgene flow in the field (Linder & Schmitt 1995; Linder et al. 1998; Bartsch et al. 1999; Spencer & Snow 2001; Gueritaine et al. 2002; Snow et al. 2003). Researching the impacts is difficult, as the selective value of a transgene in a wild population may be different within its ecological and biological context, where a host of factors (including epistasis, genetic drift, etc.) may influence the magnitude of evolutionary impact. Nonetheless, cases such as with the aforementioned creeping bentgrass signal the need for more intensive research in this area.

2.1.2 Crop to landrace gene flow

Gene flow between modern crops and *landraces* – the genetically diverse domesticated, local, farmer-selected cultivars – has been an area of concern since the early inception of modern plant breeding. Many landraces are still being cultivated within their areas of origin, and hence, local farmers play an important role in the maintenance of in situ diversity and conservation (Gepts & Papa 2003). Landraces act as important sources of genetic diversity – the genetic stock that plant breeders must rely on for future crop improvement. For this reason protection of this diversity has been a concern of international crop research centres, international agencies, and national governments alike. The loss of this diversity involves not only food security considerations, but also cultural notions of patrimony and locally-derived genetic resources.

Centres of crop origin and diversification therefore both play crucial roles for future crop breeding. Figure 12.2 details some centres of origin for some of the world's most important food crops.

[1]http://www.centerforfoodsafety.org/GTBC_DecisionPR_2_7_07.cfm accessed 10 February 2007

Figure 12.2. Centres of origin and diversification for major crops. Other geographic areas may as well contain important sources of genetic diversity for these crops. (Modified from Crop Genetic Resources: An Economic Appraisal/EIB-2, Economic Research Service/USDA, 2002).

A number of important transgene flow cases have been reported in centres of crop origin and diversity. Perhaps most widely known is the case of transgene introgression of maize in Oaxaca, Mexico (Quist & Chapela 2001; 2002). The substantial attention paid to reports on the status of transgenes in Mexican maize (Quist & Chapela 2001; NAFTA-CEC 2002; Alvarez Morales 2002; Quist & Chapela 2002; Cleveland et al. 2005; Ortiz-Garcia et al. 2005) has not translated into follow-up empirical studies on the evolutionary significance of transgenes in maize landrace populations. Given the occurrence of transgenic introgression events in Mexico, concerns have emerged over similar events taking place in other important crop plants, including rice and soya in China (Huang et al. 2003). The impending commercialization of GM rice has been met with considerable concern over gene flow to wild and weedy rice relatives (Lu & Snow 2005), and to non-transgenic commercial varieties. Given these events, and the uncertainties over the significance of transgenic hybridization, the introduction of transgenic crops in their centres of origin and diversification represents a broad concern with socio-economic and agricultural implications. Some of these impacts, particularly evolutionary implications, may be irreversible. For these reasons, transgenic introductions in centres of origin and diversification merit special consideration.

The issue of intellectual property rights (IPR) on crop cultivars adds another dimension to the issue of transgene flow. While IPRs are in conflict with the age-old practice of seed exchange amongst local farmers who use landraces, the introduction of identifiable transgenic technologies opens up the possibility that legal action could be taken against local farmers by the patent holders.[2]

While there has been greater attention paid to gene flow to wild relatives, there has been very little scientific study, descriptive or experimental, over the potential impacts of transgene

[2]See the case of Percy Schmeiser, a canola farmer from Canada (http://www.percyschmeiser.com/)

introgression in landraces. Clearly, establishment of transgenic hybrids in landrace populations is undesirable, given the high level of uncertainty as to their effects and incidence of gene movement. Policies that limit the planting of transgenic varieties in centres of origin have been widely recommended (NRC 2000; Eastham & Sweet 2002; Gepts & Papa 2003). Yet well-intentioned policies have been largely ineffective to date.

2.1.3 Crop to crop gene flow

Crop to crop gene flow, as previously mentioned, is a broad concern in areas of GM and non-GM cultivation or use of offspring's seeds. A number of 'gene spill' events of transgenics 'contaminating' non-transgenic crops, resulting from cross pollination (Friesen et al. 2003; Mellon & Rissler 2004), and sometimes seed mixing (Mellon & Rissler 2004) have been recorded. Transgenic introgression of conventional crops has its own share of biological, socio-economic, policy, and intellectual property concerns.

Of the biological considerations, the most significant is loss of non-transgenic genetic varieties, many of which are 'heirloom varieties' (landraces) of important crop diversity. It is important to note that this is also an issue with non-transgenic commercial hybrids, where the process of domestication of crops has led to genetic bottlenecks in virtually all crops analysed to date (Doebley 1992; Gepts 1993). This has the effect of limiting the genetic stocks available to farmers and breeders.

Socio-economically, many of the same concerns mentioned for landraces also exist with crop to crop transgene flow. A number of cases of inadvertent contamination of the food supply – particularly in the USA – with varieties not approved for human consumption have made recent headlines. Cases such as the Starlink corn contamination in 2000 (Kaufman 2000) and rice in the US with multiple transgenic varieties,[3] are just a few examples of inevitable gene flow. Nations that do not accept (certain) GMO products have been forced to ban the import of grains or foods from these countries, causing a loss of markets for farmers and food distributors. Contamination events of organic crops can affect the premium value and genetic stocks of the crops for the affected farmers. Quite clearly, the unintended spread of transgenes has been a result of cultivation and seed distribution systems that were never designed for segregation of particular crop varieties. Human error and negligence of laws are also often to blame. Lastly, patent infringement lawsuits might be brought against farmers affected by transgene flow, as previously mentioned.

As a result of the many documented cases of transgene flow, robust monitoring programmes have been an important initiative for many countries, especially those with policies limiting GMOs in their food supply. Hence, tracking transgenes has not only biological but political implications.

3. Tracking transgenes

An essential initial component of understanding the ecological and environmental impact of transgene flow is first documenting the movement or presence of transgenes in a population, food shipment, or processed food item. This involves employing molecular methods to detect the synthetic transgenic DNA constructs, or target marker proteins introduced into the gene-modified commodity (Holst-Jensen et al. 2003; Nesvold et al. 2006).

[3]http://www.guardian.co.uk/gmdebate/Story/0,,1884523,00.html and http://www.aphis.usda.gov/publications/biotechnology/content/printable_version/ia_ge_rice.pdf

Successful monitoring and surveillance of transgenes in the environment or food shipment is reliant on a number of factors. First, one must be able to detect the transgenic sequences or proteins (see Chapter 33). Therefore *a priori* knowledge of the genes one is looking for is essential. Further, the gene sequence or protein one is targeting in the monitoring efforts must be intact and/or expressing. In addition, the sampling regime, limit of detection and reproducibility of results can further effect the outcome of any monitoring efforts, usually leading to false-negative results (Holst-Jensen et al. 2003). Hence, any sampling for GMOs is likely to underestimate the presence and/or frequency of GM DNA in a sampled population. Thus, not detecting a transgene in a sample population is no guarantee that the population is transgene free (Heinemann & Traavik 2004). Only with a careful multifaceted monitoring strategy can the accuracy and precision of our monitoring efforts be reasonably assured. Agencies dedicated to the detection of transgenes, such as the European Network of GMO Laboratories (ENGL) in the EU, have devised validated methods for the detection of transgenic DNA from approved GMOs in the European community. Thus, tracking transgenes is difficult, but not impossible.

4. Research needs, gaps in knowledge and uncertainties in gene flow assessments

In the first years following the commercialization of genetically modified organisms, the primary research focus has gone into developing detection systems and monitoring to account for unwanted GM DNA is foodstuffs and crops (as discussed). This has been motivated largely by policies of low or no GMO components in grain and foodstuffs in some countries, and has been a driving force in the science of GMO-related research. The salient question is the significance of gene flow when it occurs. Ecological studies of transgene flow have shed significant light on many of the unanticipated or unintended effects of transgenic biology, and have highlighted the need for robust science as the driving force behind risk assessments. Where ‘early warnings’ are identified (Harremoes et al. 2002), there is a need for careful consideration where lasting effects might otherwise be mitigated. The importance of context should not be lost on transgenic biology, where the behaviour of transgenes and their proteins might be very different within different biological (organismal) or ecological backgrounds.

While a much greater degree of risk science on transgene flow to date has focused on the direct ecological implications of specific transgenes, investigations into the ecological and evolutionary significance of transgene flow for genetic diversity in centres of origin are lacking. The case of transgenic maize in Mexico is one clear example of where such studies are urgently needed (Garcia et al. 1998; Quist & Chapela 2001; NAFTA-CEC 2002; Cleveland et al. 2005). As a result, many critical gaps in understanding remain on gene flow potential and barriers, including sexual compatibility, hybrid viability and fitness for many crop species.

Part of the difficulty in such studies is the lack of *a priori* predictive power given the likely variable behaviour of the transgene in new ecological and genetic backgrounds (Gepts & Papa 2003). Transgenic plants, like most commercial crop varieties, are designed for use within very specific environmental and cultural conditions of the agricultural field over one generation. They were never intended for new genomic or ecological backgrounds, or for use over subsequent generations that occur with gene flow. Much research has focused on the notion of fitness of a transgenic hybrid population to be substantially equivalent to transgenic crops within the intended agricultural setting. Conceptually, one must consider that the setting of the transgenic organism may grossly affect the effect or impact it may have within a particular milieu. For example, pest and competition pressures may be different depending on ecological setting, affecting fitness of the population much differently outside its intended agricultural context, such that equivalence of outcomes cannot be assumed. Further, hybridization into new genetic backgrounds may have a range of effects on the fitness of the recipient population. These responses may include a

metabolic cost decreasing its fitness, to a hybrid vigour increasing its costs. Outcomes may not be consistent across generations, growing ranges, climatic fluctuations, or stress pressures. A further consideration is the lack of understanding of the fate and stability of transgenes across generations (McCabe et al. 1999; Quist & Chapela 2001; Svitashev et al. 2002; Wilson et al. 2006) and post-translational silencing (Matzke et al. 2000), non-Mendelian inheritance, *pleiotropic* or *epistatic* effects (i.e. unintended changes in phenotype by the transgene introduction or interaction with other genes) that are important considerations for assessing gene establishment, expression, and hence fitness effects. Further, other levels of biological organization within the plant (transcriptome, proteome, metabolome; see Chapter 8) may also have direct impacts on fitness of gene flow. Another consideration is that the dominant currency of gene flow research as genes conferring traits assumes that all genes transferred will be protein-coding genes. This fails to consider the vast array of non-protein encoding DNA and RNA derivatives that are also implicated in the transfer of genetic information and the outcomes from one population to another (Mattick 2003).

Thus, the evolutionary implications of hybridization and introgression from crop to crop or crop to landrace/wild populations where it actually occurs are dependent on a number of factors, where the fitness effects cannot be predicted *a priori* to GM crop release, and may change over hybrid generations. Therefore, studies must be conducted on a case by case basis within any given context (country, environment, GMO, etc.) where relevant scientific questions can be addressed.

5. Practical considerations for policy and risk assessment on gene flow

5.1 Strategies for mitigating transgene flow

The knowledge gained from transgene flow studies has been useful in developing appropriate measures to limit gene flow from transgenic plants. A number of strategies have been outlined to document and minimize gene flow from transgenic sources.

Given the uncertainties over the ecological and evolutionary impacts of gene flow, the means to minimize potential gene flow are active areas of investigation. Most of these will involve temporal and spatial isolation of the transgenic crops from potential gene flow scenarios. Containment and confinement strategies span the range from the physical (Morris et al. 1994; Staniland et al. 2000) to the chemical (Schemthaner et al. 2003) to the molecular (Daniell 2002). No single strategy is failsafe, and overlapping approaches will be necessary to adequately ensure minimal transgene escape, yet must also be investigated for their own biosafety.

5.2 Context-specific considerations

The country, crop, and/or transgenic trait under consideration may be relevant to policy decisions on transgenic crops. For example, gene flow to landraces and wild relatives of maize may be an issue for a country such as Mexico, but not for Canada. Certain types of transgenic products may also trigger policy implications if they may impact sensitive non-target biodiversity. Foremost is a robust detection and monitoring system, whereby specific information on the marker DNA sequences, molecular characterizations and background knowledge on gene flow potential will all be important in any biosafety policy on transgenic crops. Lastly, beyond the possible ecological and economic implications of gene flow, the possible socio-economic costs of unintended gene flow must also be taken into account in any policy decision or risk assessment (Gepts & Papa 2003).

6. Conclusions

Emerging knowledge over the importance of the ecological, genetic and political backgrounds of GMO introductions is bringing new insights into the complexities surrounding the use of GMOs in agriculture. There is still much to be learned. Quite clearly, GMOs represent a new challenge in the management of agriculture where external costs and potential consequences must be duly measured along with and contrasted with any potential benefits. This is even more critical with the emerging use of crop plants to manufacture bioactive compounds, such as pharmaceuticals, that have an even greater risk magnitude. Given the scope, irreversibility and uncertainty surrounding the impacts of transgene flow, a critical analysis of the biological, ecological and social ramifications needs be thoroughly examined to arrive at sound policy decisions. This requires asking the right questions – the relevant types of 'what if' risk questions—regarding the GMO under consideration within the right social, political and agroecological dimensions.

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Chapter 13

Unintended Horizontal Transfer of Recombinant DNA

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DNA is usually transferred over generations following the normal reproduction pathway of the organism involved (e.g. sexual reproduction/inheritance by descent). This process is called *vertical gene transfer* and an example is pollen flow between the same or related plant species.[1] Thus, vertical gene transfer is the normal mode in which DNA is shared among individuals and passed on to the following generations. DNA can, however, also more infrequently spread to unrelated species through a process called *horizontal gene transfer* (HGT). HGT, sometimes also called lateral gene transfer, occurs independently of normal sexual reproduction and is more common among single-celled organisms such as bacteria. HGT is a one-way transfer of a limited amount of DNA from a donor cell/organism into single recipient cells (Figure 13.1). Examples of HGT are the spread of antibiotic resistance among bacterial species, gene therapy in humans, and *Agrobacterium*-infection in plants. HGT of recombinant DNA from GMOs to bacteria is a potential biosafety concern (Nielsen et al. 2005). In this chapter we introduce the main biosafety aspects of unintended[2] HGT processes as they relate to the use of recombinant DNA, as follows:

1. Introduction to some biosafety aspects of recombinant DNA

Genetically modified organisms (GMOs) often contain recombined genes (transgenes) collected from different species to enable the expression of new traits. Most commercialized GMOs harbour < 5 protein-encoding transgenes assembled into unique genetic combinations and regulatory contexts that provide new functions to the host organism. The intended horizontal transfer and recombination of genetic material across species barriers is thought to be of little concern by many scientists active in genetic engineering, as genes are considered to be mechanistic entities or modules that can function equally well in many organisms, regardless of

[1]Pollen transfer between related plant species is less frequent than within species, and is also called outcrossing or hybridization. Note that hybridization processes still follow the normal ways of plant reproduction and are therefore vertical gene transfer events. The participating plants contribute c.50% each to the DNA composition of the seeds, in contrast to HGT events where most often much less than 1% of the genome of one organism is transferred to another.

[2]This chapter focuses on the likelihood of unintentional HGT. Intentional HGT, i.e. the insertion of defined DNA fragments into the target organism, is the basis for all genetic engineering and production of GMOs.

their historical and evolutionary context. This reductionistic understanding of genes as functional modules acting more or less independent of their organismal background and genetic networks underlies also the way risks of potential subsequent horizontal transfer of recombinant DNA to unintended recipients are presented and addressed in the biosafety assessment of GMOs.

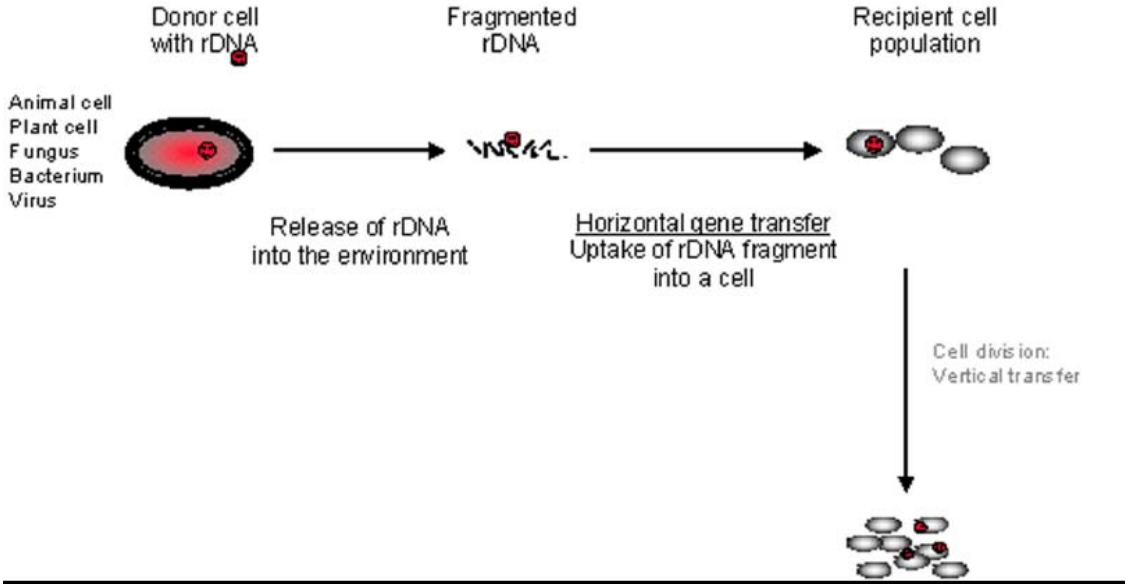

Figure 13.1. A schematic representation of horizontal gene transfer. A donor cell (of any origin) can release DNA (the presence of a particular gene is indicated with a red dot in the figure) that can persist in the environment. The subsequent uptake of DNA fragments by exposed recipient cells is called HGT. Such HGT can occur deliberately, e.g. by gene therapy in humans, and genetic engineering of plants. Bacteria have several processes that can facilitate HGT, including transformation, conjugation and transduction.

The prevailing gene-centric perspective on GMO production is also shaping the approaches to, and understanding of, biological-mechanistic consequences of unintended HGT events.[3] The health and environmental impact of potential unintended HGT from GMOs is a debated concern and risk scenario (Nielsen et al. 1998; 2001; 2005; van den Eede 2004). For instance, whereas *vertical* spread of recombinant DNA from GMOs (e.g. GM plants) to conventional crops, landraces and to some wild relatives has been documented in several studies (see Chapter 12), no studies have conclusively proven *horizontal* spread of recombinant DNA from GMOs into naturally occurring host tissues or bacteria. The reason for the absence of observations of horizontal transfer of DNA from GMOs is currently debated and can be due to:

- Lack of receptive host cells or bacteria, conducive environments, or available recombinant DNA in a given environment (e.g. the gastrointestinal tract, agricultural fields).
- Lack of a selective advantage of the horizontally transferred recombinant DNA so that rare host cells or bacterial transformants never surface in investigations working with limited sample sizes.

[3]We recognize that an implicit utilitarian value set frames the presentation of the biological aspects of unintended HGT of transgenes in this chapter. Nevertheless, we acknowledge a non-consequentialist view on HGT processes: that any unintended HGT of a man-made, recombined gene construct with traits derived from many unrelated organisms represents an unacceptable violation of nature. This latter argument may be seen an ethical objection. However, most gene constructs used in GMOs today could not have arisen by natural genetic processes or traditional breeding within the timescale of modern civilization. Ethical concerns related to the novel origin, genome and biochemical composition of GMOs are, however, also founded in a comparative perspective taking into account the long-term complex processes underlying the evolution and composition of extant organisms.

- Lack of funding, and hence, conducted and published studies that have examined the process with a reasonable effort and detection limit.
- Lack of motivation among scientists to investigate such HGT processes due to the many levels of conflicts of interest and highly vocal opinion leaders in the field.[4]
- Lack of methods preventing an investigation of HGT processes with a sensitivity that is relevant to somatic cell dynamics or bacterial evolutionary processes.

As outlined in Nielsen (2003a), some commonly occurring characteristics of recombinant DNA in GMOs can make their transgenes more likely to be taken up and expressed in unintended host or bacterial cell recipients than the majority of the genes present in naturally occurring higher organisms (Table 13.1). Given the many specific characteristics of transgenes exemplified in Table 13.1, it is clear that the argument that 'native plant genes are not observed in bacterial genomes, therefore plant transgenes will have the same constraints and, hence hypothesized occurrence of HGT processes from GM plants should be dismissed' is not relevant.

Here, we briefly present the state of knowledge concerning horizontal transfer of recombinant DNA from GM plants into human cells or into bacteria present in the gastrointestinal tract or in agricultural fields. We discuss knowledge gaps and describe various types of uncertainty embedded in the prevailing biological paradigms underlying the evaluation of HGT processes in biological risk assessments.

2. Recombinant DNA introduction and potential impact in various environments

The large-scale approval, cultivation and consumption of GM commodity crops will necessarily lead to the release and, to some extent, persistence of recombinant DNA in the environment. DNA is continually released from living organisms (e.g. crop plants) shedding tissues or cells or from their decaying debris. The release of DNA is therefore not specific to GMOs and the effect thereof should be seen in the context of DNA released from other organisms present in the same natural system (e.g. by conventional agriculture).

All living cells harbour long DNA molecules. In higher organisms, some of the DNA is broken down (fragmented) within the host during controlled cell death (apoptosis). In contrast, in single-celled organisms such as bacteria, DNA breakdown is mainly facilitated by nearby organisms with specific enzymes (called nucleases or DNases) that facilitate the degradation process. Thus, released DNA is routinely and continually degraded and recycled into nutrients in all ecosystems. Yet, evidence obtained both from DNA sequencing of whole organismal genomes and laboratory studies of DNA exchange between organisms demonstrate that some, often minor fragments of DNA, can be integrated into the genome of the exposed recipient organism (Ochman et al. 2000; Rosewich & Kistler 2000; Nakamura et al. 2004; Thomas & Nielsen 2005).

[4]A rapid transition from a scientific debate to personal attacks and attempts to discredit the researcher may soon follow if 'unwelcome' paradigm-challenging results are published. Hence, potential threats to a further scientific career development are to be considered prior to initiating risk-focused studies.

Table 13.1. Characteristics of recombinant DNA that may alter the likelihood of horizontal transfer, expression and stabilization in unintended hosts.

Modification	Recombinant DNA has an altered likelihood of mediating:
Use of bacterial gene constructs and vector sequences	- Recombination with prokaryotic genomes because the bacterial genes and mobile elements (vector sequences) have high sequence similarity to commonly occurring bacteria.[a]
Functional assembly into a single genetic unit	- Transfer of entire novel multi-gene encoded traits because only a single transfer event is necessary for a recipient to acquire a functionally optimized genetic trait complex. The trait may have previously been distributed across the donor genome (with a lower likelihood for simultaneous multi-gene transfer), or the trait was absent from the evolving species/lineages.
Introduced changes in gene expression and protein composition	- Expression of the modified traits in novel hosts, if horizontally acquired, because broad host range promoters (derived from microbial pathogens) are used to drive the expression of the engineered trait. Codon and promoter modifications may also change the expression levels and protein characteristics (e.g. mRNA processing and editing, post-translational modifications) affecting protein composition, function, stability, and location in some unintended recipients.
Insertion of a transgene construct into an unrelated genome	- Host-specific differences in the gene expression and regulation systems between the transgene's original host and the modified recipient host, can lead to unpredictable changes in the global gene regulation in the new host and in the transgene's transcription level and mRNA modifications, the translation process and composition of the translation product, altered post-translational modifications, and hence protein stability, activity and degradation.
Removal of introns from cDNA cloned genes	- Expression of the modified traits in a broader set of species and domains because intron processing (specific to eukaryotes) is regarded as a main barrier for functional assembly and expression of eukaryotic genes in bacteria.
Insertion of transgenes into organelles	- Increased exposure rates (relative to nuclear-inserted genes) to unintended recipients due to high transgene copy number in organelles, recombination (homology-based) and functional expression of the modified traits in unintended bacterial recipients because organellar genomes resemble bacteria in overall genome organization and regulation.
Large-scale release of modified gene constructs	- The large-scale and continual cultivation, processing and consumption of GMOs may result in a very low frequency horizontal gene transfer event becoming statistically likely. Empirically derived HGT frequencies obtained in laboratory-scale models are therefore of little use to understand the occurrence and impact of HGT in field scales.[b]

[a] De Vries et al. 2001; Bensasson et al. 2004
[b] Heinemann & Traavik 2004; Nielsen & Townsend 2004; Pettersen et al. 2005

The uptake process of DNA molecules into the cytoplasm of a cell is considered to be random and independent of the DNA's subsequent biological utility. Most foreign DNA taken up and integrated into the genome of an organism will have a deleterious effect due to its interference with the host cell biology and genome structure (Elena et al. 1998; Doerfler 2000). HGT processes thus resemble mutational processes, that is, they may occur by chance and repeatedly over time, but a very low proportion of the HGT events will confer a benefit, and be retained in the host over time (Heinemann & Bungard 2005). For multi-cellular organisms, HGT events occurring in somatic (i.e. not germ-line cells) will be lost when the organism dies. In contrast, HGT events occurring into germ-line cells or single-celled organisms such as bacteria will be passed on to the following generations. Predicting the long-term survival and competitive ability (*fitness*) of the transformed host organism is therefore essential to understanding whether the transformant cells will expand in numbers or eventually die out.

The potential impact of unintended HGT of recombinant DNA from GMOs to exposed organisms must be seen within the broader picture of naturally occurring processes, including i) the continual large-scale release of genetically diverse DNA molecules from a broad range of naturally occurring or introduced species in a given environment, ii) the infrequent and random HGT events occurring naturally in the same environment that the GMO will be released into, and iii) the extremely low likelihood that any DNA taken up will improve the fitness of the exposed host organism. Within the aforementioned naturally occurring HGT context one can ask biosafety relevant questions such as:

Will recombined DNA released from GMOs have an altered and increased capacity to be transferred to, and change the fitness of, exposed host cells and bacteria?
Can the likelihood of this HGT process and the subsequent population genetic trajectories of the transformed cell be accurately predicted?

Do the currently available scientific literature and empirically-founded knowledge base on HGT processes allow a scientifically-robust impact assessment to be made?

Some scientists would argue that a hypothesized low frequency HGT event is irrelevant from a GMO risk perspective, others may argue that the HGT issues are case- and transgene specific, requiring a more detailed understanding of the natural selection context of each GMO case. Common to all biosafety viewpoints is that they are founded on expert *opinion*, familiarity with the gene donor and inference, rather than conclusive empirical *evidence*. The latter is unachievable given the limited understanding of the complexity of host cells and microbial communities exposed to GMOs.

Familiarity with the gene donor as a starting point for safety assessment is important. For instance, a GMO-specific and credible risk hypothesis can be difficult to design and test if the protein-coding regions of the recombined DNA ('the transgene') are already present naturally in the same environment as the GMO is being introduced to. If the recombined DNA sequences (present in the transgene) are also present naturally, then the HGT risk aspect would be narrowed to the potential biological effects caused by the recombinant DNA's altered genome location, context and regulation. Identifying and understanding the effects of the novel genetic compositions in GMOs are thus key elements in HGT risk assessment. Risk assessments based on absence of effects due to a predicted low frequency of HGT events are invalid, given the minor (non-linear) relationship between gene transfer frequencies and environmental impact (Pettersen et al. 2005).

We encourage a shift in the focus of the further development of GMOs to the use of intragenic and genomic modifications; that is, to limit the genetic modification to within the genome of an organism without the introduction of recombined DNA from several unrelated species. Doing so may alleviate many of the current HGT concerns (Nielsen 2003b). The interest in developing an intragenic approach is currently limited by a prevailing gene-centric approach to GE (that assumes a gene's biological performance is independent of genome context) and a lack of in-depth understanding of the regulation and traits in the genomes of organisms that are of commercial interest.

2.1 Human exposure to foreign DNA
Humans are continually exposed to DNA in inhaled organisms (e.g. bacteria, viruses, pollen etc.), from a broad variety of food sources including the microorganisms present in food, via microorganisms normally present in and on humans, and infectious agents entering the body.

Thus, the human body has mechanisms to protect host cells, and utilize and degrade or remove foreign DNA molecules.

For instance, free bacterial DNA in the blood triggers immune system reactions (Stacey et al. 1996; Cohen 2002). It is estimated that humans ingest 0.1 g to 1 g of DNA per day (Doerfler 2000). Moreover, DNA is also released continually in the gastrointestinal tract from dead microorganisms and shed intestinal cells. The quantity of any recombinant DNA ingested will be a minor fraction of the total DNA consumed per human per day. Transgenes are considered chemically equivalent to any other gene present in food (Jonas et al. 2001) (with the possible exception of transgene-induced epigenetic modifications and protein interactions). Therefore, risk hypotheses of an unintended impact of recombinant DNA are mainly focused on the novel genetic composition of the recombinant DNA and not the overall chemical structure.
In the following sections, the presence of DNA in food, and its subsequent degradation in the intestine are briefly discussed. We then consider potential uptake of food-derived DNA into host intestinal cells or tissues, or into exposed bacterial cells present in the gut or in agricultural settings.

2.1.1. DNA in food
DNA molecules of broad size ranges are present in large numbers in all raw and unprocessed food sources. Depending on the extent of processing, various fractions of DNA molecules of a reduced size may be present in the consumed product. The proven persistence of DNA molecules in raw or many types of processed food is crucial for the identification of GMO ingredients (see Chapter 33). The broad application of sensitive PCR technology has thus exemplified the widespread occurrence and persistence of DNA molecules in various food sources, including processed food such as corn chips and chocolate (Rizzi et al. 2001; 2003; 2004). However, the PCR protocols applied for GMO detection routinely target small DNA fragments, typically 100–400 nucleotides long. This size range is less than the length of a single transgene with a complete protein coding sequence. Thus, the overall concentration and distribution of DNA of a size that enables entire protein coding genes to be horizontally acquired from various food sources by host cells or bacteria remains largely undetermined. Many studies have demonstrated the persistence of DNA in food, for instance in canned food, whole seeds, cracked seeds and meal of canola, wet sugar beet pulp, cereal grains, and silage (Bauer et al. 1999; Chiter et al. 2000; Einspanier et al. 2001; Duggan et al. 2003). Processing often decreases the size of DNA, and such molecules can be undetectable in extensively processed food (Pauli et al. 2000; Kharazmi et al. 2003). See Nielsen et al. (2007) for a more extensive review of DNA in various environments. Table 13.2 lists several major knowledge gaps related to the general state of knowledge of the fate of DNA in food and during digestion.

2.1.2. DNA stability in the digestive tract
Most free DNA molecules entering the digestive system undergo substantial degradation by enzymes attacking DNA (nucleases, DNases), released from the pancreas and by bacteria present in the intestine (Wilcks et al. 2004). In addition, the low pH of the stomach may chemically modify the DNA molecules. Remaining DNA fragments are excreted in the faeces with variation in the degradation efficiency between mammals. For instance, Chowdhury et al. (2003a; 2003b) reported that maize DNA could be detected in pig faeces. Few studies have been conducted on the digestion of food-derived DNA within the 6–8 m long digestive tract of adult humans. One study by Netherwood et al. (2004) reported that whereas some DNA fragments survived passage through the small bowel, transgenes could not be detected in the faeces of human volunteers feed GM soy products.

In general, studies of the degradation of DNA in the gastrointestinal tract face many methodological challenges. Ingested food contains DNA present within tissues and cells or as complex biochemical mixtures in heat- or mechanically-damaged cells. Therefore, each food source, preparation conditions, and host physiology will determine the DNA degradation efficiencies in the digestive tract. Most studies on DNA stability in the digestive systems of mammals have used purified DNA and may therefore not capture the impact of various food components, treatments and locations on DNA degradation and stability (Martín-Orúe et al. 2002). Whereas it is generally acknowledged that DNA molecules in food are substantially degraded upon digestion in animals, there are many knowledge gaps related to the specific circumstances leading to survival of smaller DNA fragments during digestion (Table 13.2).

Table 13.2. Knowledge gaps in the understanding of the fate of (recombinant) DNA in food and the GIT.

Location / process	Lack of detailed biological understanding of:
DNA in food	- The amount, size distribution, stability and degradation dynamics in various types of raw food sources. - The effects of various types of processing and subsequent storage. - The protective or degradative role of cellular/nuclear proteins, the cytoplasmic content and cell membranes/walls. - The combined effects of the above in complex food sources.
Food-derived DNA in the GIT	- The amount, size distribution, stability, and degradation dynamics in various compartments of the GIT as a function of food source, food mixtures and prior processing. - The specific degradation mechanisms active and their relative role. - The relationship between degradation mechanisms, degradation rate and DNA availability to epithelial or bacterial cells. - Quantitative DNA exposure rates to epithelial or bacterial cells. - Intra- and interspecies host variation in the above parameters.
HGT of DNA in the GIT to host cells	- The DNA uptake mechanisms, transport pathways and degradation mechanisms in host tissues and cells. - The quantitative aspects of DNA uptake from the GIT into the bloodstream of mammals. - The cellular locations of DNA after uptake, the potential transcription, and the elimination mechanisms active. - The overall uptake process such that sensitive methods and models can be developed to adequately address the fate and possible biological effects of DNA taken up into host cells from the GIT.
HGT of DNA in the GIT to intestinal bacteria	- The proportion, size distribution, location and nature of DNA complexes exposed to bacteria in various parts of the GIT. - The diversity, function, variability, and population dynamics of the microbiota in the GIT of mammals. - The species distribution of, and tempo-spatial variability in natural transformation of bacteria present in the GIT. - The host, microbial and food factors influencing uptake of feed-DNA into bacteria. - The overall uptake process such that sensitive methods and models can be developed to adequately address the occurrence of, the relevant recipient bacterial species, and the possible biological effects of bacterial DNA uptake in the GIT.

Revised from Nielsen et al. 2005. HGT: horizontal gene transfer, GIT: gastrointestinal tract.

3. HGT of recombinant DNA to eukaryotic cells (e.g. human cells)

The uptake of food-derived DNA into host intestinal cells or tissues has been raised as a potential concern related to the introduction of GMO-based food sources. As discussed, such exposure must be seen in relation to the broad variety of DNA naturally present in food, and hence, whether specific qualitative or quantitative genetic changes are present in the GMO that would create a higher risk/impact of DNA exposure from this source.

Experimental data are readily available that support the notion that intestinal cells of the host will be exposed to DNA molecules present in food (see the following). The potential transfer of transgenes from GM food into epithelial cells of the gastrointestinal tract can thus be hypothesized to take place but experimental studies have not yet shown such transfer to occur. The lack of such observation is likely due to the fact that the total surface area of the small intestine (microvillus) alone is more than 40 m^2, with approximately 100,000,000,000 mucosal cells. Rare gene transfer events into a few of these cells are practically impossible to detect with currently available methods. In risk assessment, such hypothesized HGT events are considered to have little effect on the host because intestinal cells are shed from the lumen wall continually. The life span of mucosal cells of the small intestine is 1–2 days, and less than 10 days for most epithelial cells in the human gastrointestinal tract.

Humans eat natural food products that when combined contain > 1 million genes, some that would likely cause adverse effects if inadvertently inserted and expressed in human cells. The high general genetic diversity of DNA that enters and undergoes degradation in the intestinal system is astonishing. For instance, a simple meal consisting of chicken and two vegetables will contain a genetic diversity of more than 1 million different unique (non-overlapping) DNA fragments of 1000 bp and more than 10 million unique (non-overlapping) DNA fragments of 100 bp. Assuming a normal diet will consist of at least 50 different food sources over a limited time period, the routine exposure to DNA fragments with different compositions is between 50 to 500 million. This rough calculation does not take into account the highly diverse DNA leaking from microorganisms (eaten or present in the intestine). Thus, it can be concluded that humans are continually and naturally exposed to a genetic diversity ranging from between 50 million to 5 billion different and unique DNA compositions in the size range of 100–1000 bp. Given the high variety of DNA compositions already present in conventional food sources, few, if any, specific and testable hypotheses have been put forward that suggest commercially-used transgenes would elicit more adverse effects if horizontally acquired by intestinal cells than their conventional counterparts.[5]

Whereas potential events of uptake and integration of food-derived DNA into exposed lumen (epithelial) cells remain unidentified, many studies have shown that food-ingested DNA can pass luminal cells in the gastrointestinal tract, and be detected in the bloodstream and tissues of mammals. Specific examples are feed-derived DNA taken up from the gastrointestinal tract and detection in leucocytes, spleen, liver, and kidneys in mice (M13 DNA), in the brain, eyes, liver, and heart of the offspring of mice (plasmid DNA), detection in the liver and spleen of mice following feeding with soybean leaves (Schubbert et al. 1994; 1997; 1998; Hohlweg & Doerfler 2001), and detection of fragments of plant DNA in muscle, liver, spleen, and kidneys in chicken and cattle (Einspanier et al. 2001) It has been estimated that approximately 0.1% to 1% of dietary DNA is absorbed from the gastrointestinal tract (Nielsen et al. 2005a; 2006). A precise measurement of this process is complicated because absorption from the gastrointestinal tract takes place over several hours and absorbed DNA undergoes continuous transport, degradation

[5]This argument assumes that there are no genome positional effects, epigenetic modifications or protein associations specific to the transgene that will affect its stability and likelihood of HGT.

and elimination. Nevertheless it is clear that DNA in food may reach the bloodstream and be exposed to and localized to various host cells and tissues. Some infrequent horizontal transfer events can thus be hypothesized to take place. Thus, the genetic composition of transgenes must be assessed in the 'worst-case-scenario' of being inadvertently taken up into the body from the gastrointestinal system.

This gene-centric assessment may still be ignorant of yet to be identified effects of higher order genome structures and chromosome modifications of importance for the HGT potential and subsequent inheritance. It can be concluded from Table 13.2 that the many gaps in the general biological understanding of food DNA limits the scientific basis and quality of the current risk assessment of HGT processes in this environment. The final risk assessment may therefore often be founded on expert opinion, experience and inference, rather than an in-depth understanding of the biological fate of food DNA in the gastrointestinal tract.

4. HGT of recombinant DNA to prokaryotic cells (e.g. bacterial cells)

HGT of transgenes into pathogenic, beneficial or environmental microorganisms, resulting in potential unanticipated (absolute and relative) fitness effects, has been voiced as a potential biosafety issue. As discussed so far in this chapter, a broad range of DNA compositions is continually released from decaying organic matter. Microorganisms are responsible for the majority of organic matter decomposition and therefore also DNA degradation. Thus, microorganisms present in the human gastrointestinal tract and in agricultural environments experience continual exposure to DNA released from themselves and the organisms in their immediate surroundings.

DNA fragments exposed to bacteria will most often be utilized as a nutrient source (Nielsen et al. 2007). However, in rare circumstances, foreign DNA may also be integrated into the bacterial genome (Dröge et al. 1998; Davison 1999). Many experimental observations show that bacteria can integrate DNA molecules from their environment at measurable frequencies in the laboratory. The mosaic genetic composition of bacterial genomes also strongly suggests that horizontal transfer of chromosomal DNA has shaped their composition over evolutionary timescales (Ochman et al. 2000; Feil & Spratt 2001). However, the comparative analysis of bacterial genomes identifies HGT events that are evolutionary stable and have occurred over a time span of million of years. Comparative DNA analysis does not provide information on the gene transfer frequency itself or provide a historical account of the diversity of prior DNA exposure into the bacterium in question (Pettersen et al. 2005). Thus, it remains unclear to what extent chromosomal DNA from unrelated higher organisms is taken up into bacterial cells under natural conditions over the time course of modern agriculture.[6]

Experimental studies do not suggest bacteria integrate foreign unrelated chromosomal DNA at measurable frequencies over the limited time span (hours to days) and population size examined in laboratories (De Vries et al. 2001; Nielsen et al. 1998; 2005). A high uptake frequency is also unlikely because bacteria are continually exposed to a high diversity of DNA compositions in their environments, and unchecked uptake of DNA would quickly reduce the fitness of the bacterium and soon become lethal (Elena et al. 1998). Thus, an advantage of carrying the horizontally transferred DNA is assumed necessary to cause a biologically significant

[6]The spread of antibiotic resistance genes in clinical bacterial communities demonstrates that strongly selected genes can spread between bacterial species and communities within a short time. Although most of these resistance genes are localized on mobile genetic elements, these events demonstrate that genes can spread rapidly between microbial species when they confer a strong selective advantage to the new host.

amplification and impact of the transfer event (see Figure 13.2). It is therefore suggested that biosafety risk assessments question, determine, and identify qualitative changes in the transgenes of GMOs that would make them likely to:

Transfer horizontally, establish, and be expressed in exposed bacterial recipients.
Increase the fitness of transformed bacteria more extensively than any other transforming DNA source present in the same environment, so that altered bacterial population size or habitat utilization can be expected.

For example, many of the commercially introduced first-generation of plant transgenes are derived from soil microorganisms. Thus, microbial communities are in some cases already exposed to naturally occurring counterparts to these protein encoding genes (Nielsen 2003a; EFSA 2004; Nielsen et al. 2005b) although the combinations of associated regulatory elements are unique. The introduction of similar protein coding genes from recombinant sources to soil is therefore often inferred in biological risk assessments to cause little additional environmental impact, if a HGT event occurred (Nielsen 2003a; EFSA 2004). The HGT risk of some of the commercialized GM commodity crops currently cultivated may thus be confined to the altered genetic locations, context and regulation, and overall gene copy number concentrations. See Nielsen et al. (2005) for a further discussion on some risk considerations related to the use of antibiotic marker genes in GM plants.[7]

The novelty of the transgenes inserted into GMOs is likely to increase in the future due to development of novel gene constructs (synthetic and artificial bifunctional and multifunctional proteins) obtained through gene fusions, reshuffling and *de novo* construction of novel protein encoding domains (Nielsen, 2003b). For instance, GM plants producing novel pharmaceuticals or chemicals are in development and have already been tested in field trials. Specific, reasonable and testable hypotheses can be put forward that some of these novel plant varieties may release recombinant genes that will cause a selective advantage if taken up by exposed bacteria. Thus, HGT of recombinant DNA into bacteria will become a bigger biosafety issue in the future if the current directions in GMO production are continued. The current genetic modification approaches have little focus on the gene sources and the cellular context of the recombinations made.

[7]A precautionary-based decision to phase out antibiotic resistance plant marker genes has been made in the EU (EFSA 2004; Nielsen et al. 2005). Such a decision also exemplifies the gaps in the knowledge of resistance development in bacteria. Some of the antibiotics to which the plant marker genes encode resistance are among the most widely used in the world. Thus, whereas resistance genes to these antibiotics are known to be distributed also in non-clinical environments, they are still not a part of the majority of the antibiotic treated population of clinically troublesome bacteria. We have currently no predictive understanding to identify the specific environments, locations and conditions that will lead to the acquisition of resistance in previously sensitive bacterial populations. In the absence of such knowledge, it is impossible to accurately predict the contribution of, and long-term impact of, plant marker genes to overall resistance development in bacteria. It is also noteworthy that most emerging bacterial pathogens arise from positive selection of single HGT events. Thus, most HGT events that have had an ecological impact are not a proportional result of a high DNA exposure or HGT rate. The lack of a direct relationship between exposure/bacterial uptake, and a subsequent biological population scale impact suggest that qualitative aspects and the selection present for a given HGT event are the most important contributor and predictors of risk, and that DNA exposure or HGT rates is of little informative value (Pettersen et al. 2005).

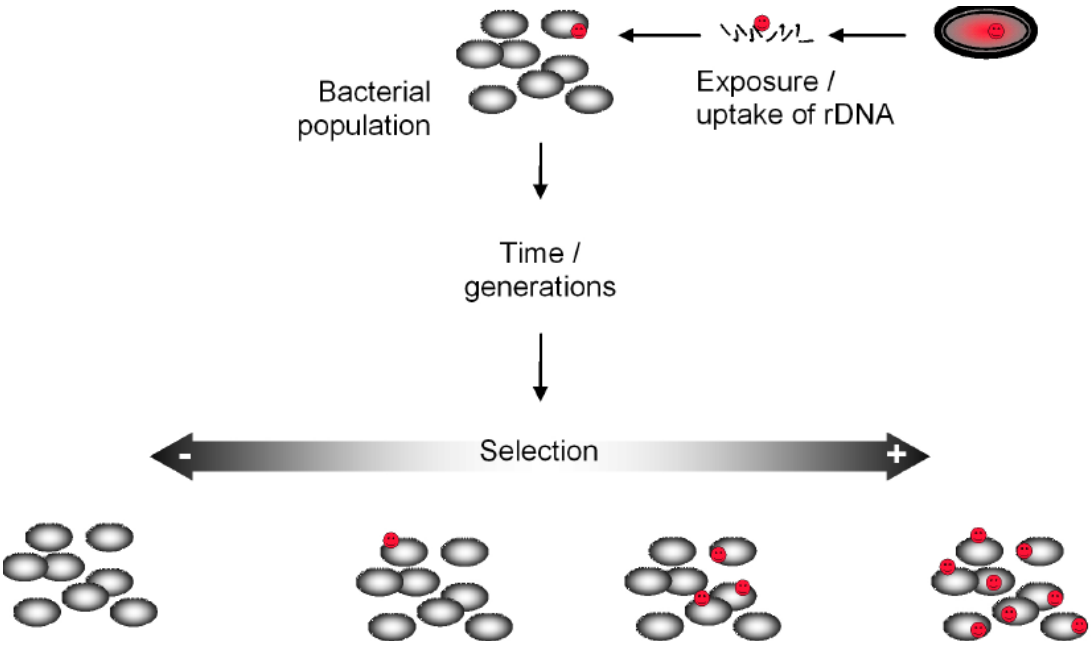

Figure 13.2. Schematic illustration of the fate of a horizontally acquired gene (red dot) over time. As shown, depending on directional selection, loss maintenance or amplification of the transformant population will occur. If the acquired gene has little effect on fitness of the transformed bacterium, random processes will determine the survival and distribution of the transformant population (this process is called genetic drift). Because most bacterial populations consist of high numbers of individuals, rare transformants will in most cases disappear from the bacterial population by chance, unless they confer a clear fitness gain to their host. Such disappearance is explained by the fact that only some members of a bacterial population will contribute to the next generation with daughter cells.

5. Concluding remarks

There are a number of knowledge gaps relating to the fate of DNA in the environment and if, when, and how exposed cells and bacteria will take up and incorporate such DNA. Knowledge gaps are themselves not indicative of harm, but are the driving motivation for new hypothesis formation and data collection. Discrepancy between the regulatory agencies' need for exact information on HGT processes and the iterative, dynamic process of knowledge formation create a situation with no clear scientific answers or regulatory or consumer consensus.
Assumption-based reasoning and a variety of information sources of variable quality have been used to aid in the assessment of potential HGT of recombinant DNA. The basis for the current risk assumptions consists of:

Laboratory test results submitted by the GMO developers.
Experimentally collected laboratory data available in the peer-reviewed literature.
Published and/or communicated historical and comparative experiences and observations of HGT processes in similar biological systems.

Submitted or conducted expert evaluations of the outcomes of conceived worst-case scenarios.
Public trust in, and scientific consensus, confidence and support of HGT risk assessment conducted by regulatory bodies depends on the quality of the data used and how uncertainty has been addressed, acknowledged and communicated (see Chapter 6). Public trust also depends on the value sets underlying scientific expert opinion formation and to what extent the consumer

adheres to the same values. The current lack of standards in HGT research that can guide hypothesis construction, choice of models and methods, and data interpretation and presentation result in sometimes heavily contextualized and motivationally biased research communications. Thus, the regulatory agencies have a challenging job separating facts from opinions, keeping in mind that even the experimental study design may bias the study to lead to a certain outcome. HGT processes occurring in nature are still not well understood and many years of further study and biological knowledge accumulation are required before precise predictions can be made on the effect or absence of effects of introduced, novel recombinant DNA. The acknowledgement of broad empirical knowledge gaps contrasts with some of the risk conclusions (the absence or presence of a HGT risk outcome) made by perhaps overly confident researchers drawing on poor data sets on HGT processes. A transparent communication of the current scientific understanding of HGT processes, the data basis applied for risk assessment, and the knowledge gaps addressed, are necessary to build public confidence in the regulatory process and to direct further HGT research on transgene ecology.

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Chapter 14

Potential Health Effects of Foods Derived from Genetically Modified (GM) plants – What are the issues?

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Abstract

In the European Union, the acceptance and regulation of GM crops/foods is based on the safety data which the biotech companies provide for EFSA (European Food Safety Authority) and not on the results of the EFSA's own investigations. The situation is worse in the USA where there is effectively no regulation and the commercialization of GM crops/foods is based on the flawed concept of 'substantial equivalence'. This, without stringent quantitative criteria can only serve at best, as an indication of comparability, but at worst, it can be misleading. It is therefore imperative that each GM crop is subjected to, as a minimum, the following:

- comparison of the composition of the GM- and isogenic lines with up-to-date analytical techniques, such as proteomic analysis (2D electrophoresis and mass spectrometric analysis of components)
- full biochemical, nutritional and toxicological comparison of the in planta expressed transgene product with that of the original gene used for the transformation
- microarray analysis of all novel RNA species in the genetically modified plant
- molecular examination of possible secondary DNA inserts into the plant genome
- full obligatory metabolomic NMR, etc. analysis of the transformed plant
- assessment of the variation of known toxins of GM plants grown under different agronomic conditions
- determination of the stability to degradation by acid or pepsin or other proteases/hydrolases of GM products, foreign DNA, including the gene construct, promoter, antibiotic resistance marker gene, etc. in the gut of animals in vivo
- with GM lectins, including the Bt-toxins, estimation by immunohistology of the presence/absence of epithelial binding in the gut
- investigation of the nutritional, immunological, hormonal properties, and allergenicity of GM products using the transgene product isolated from the GM crop and not with recombinant material from *E. coli*
- short- and long-term independent biological risk-assessment tests, first with laboratory animals, followed by human clinical studies of all GM crops/foods themselves and not just the transgene products. This chapter describes a suggested protocol for the testing of GM crops and foods derived from them.

Introduction

The basic tenet of the biotechnology industry engaged in the production of genetically modified (GM) crop plants and foods is that no 'credible' evidence exists that GM crops damage the environment or that GM foods harm human/animal health. Accordingly, they are as safe as their 'substantially equivalent conventional counterparts' and need no safety testing. The general acceptance of such a view could, of course, save a great deal of money for the biotechnology industry that otherwise would have to be spent on very expensive environmental- and health risk assessments of their GM products.

However, practically all recent reviews that have critically assessed the results of GM crop/food safety research data published in peer-reviewed science journals have come to the conclusion that, at best, their safety has not yet been adequately established, or at worst, that the results of risk assessment studies, particularly (but not exclusively) those carried out independent of the biotechnology industry, have raised important safety concerns which have not been properly settled. Thus, one review concluded that the most pertinent questions on environmental safety of GM crops have not yet been asked (Wolfanberger & Phifer 2000). A more recent update (Snow et al. 2005) came up with a long list of important questions that regulatory authorities should ask before any GM crops are released into the environment. Unfortunately, few of these questions have been addressed in the biotechnology companies' submissions to the regulatory authorities. The situation is not much better with the results of studies in which the potential health effects of GM foods have been investigated. Thus, an early review (Domingo 2000) found only eight peer-reviewed papers published on the potential health aspects of GM food. Pryme & Lembcke (2003) reported a rather curious aspect of the results of health risk assessment studies using laboratory animals. It appeared that most independently funded research scientists who performed animal testing of GM crops reported some potential health problems, while the results of the studies sponsored by the industry indicated none. Further reviews confirmed the scarcity of GM risk assessment research, particularly research carried out independent of the biotechnology industry. Thus, there were just over a dozen academic research papers on the health aspects of GM crops published by 2003 (Pusztai et al. 2003) and this number had increased to approximately 20 by 2005 (Pusztai & Bardocz 2006).

A report by the Canadian Royal Society stated that without in-depth biological testing of GM crops, 'substantial equivalence' is a fatally flawed concept and regulation based on it exposes Canadians to potential health risks of toxic and allergic reactions. Neither did the British Medical Association accept that all that GM crops/foods are safe, and therefore no testing is needed. In their report (The Medical Research Council 2000, recently updated) it was stated that 'any conclusion upon the safety of introducing GM material into the UK is premature as there is insufficient evidence to inform the decision making process at present'. It is, therefore, not surprising that the majority of British consumers think that GM foods are unsafe. As there is no demand for them most supermarkets in the UK have phased them out. Most consumers in Europe demand, as a minimum, the labelling and rigorous, transparent and independent safety testing of all GM foods.

Most GM crops are grown in America, the bulk in the USA. It is therefore regrettable that effectively there is no regulation in the USA that would guarantee their safety. The food regulatory agency in the USA, the Food and Drug Administration (FDA), almost totally relies on voluntary notification by the biotechnology companies that they carried out their own safety assessment of the GM crops they want to release commercially and found them to be safe. The FDA has no laboratory of its own and never, in fact, underwrites the safety of GM crops/foods. It only accepts the assurances of the biotechnology companies that their product is safe. This, in most instances, relies on a safety assessment that is based on the poorly defined and not legally binding concept of substantial equivalence.

However, similarity in composition is no guarantee that GM food is as safe as conventional food. Thus, the content of proteins, lipids and carbohydrate components of a BSE cow (a cow suffering from a condition known as bovine spongiform encephalopathy) will be similar to that of a healthy cow but, obviously, these two cows cannot be regarded as substantially equivalent for consumer health. True, compositional analysis is an obligatory starting point in risk assessment but it cannot be its endpoint. Whether GM food is toxic or allergenic cannot be decided on the basis of chemical analyses but only by biological testing with animals.

Furthermore, the biotechnology companies try to claim as much 'confidential business information' concerning their risk assessments as possible, and therefore most of the time these are unavailable in full for public or independent scrutiny or even for some national regulatory bodies.

Present state of GM food science

One of the most important reasons for the present scarcity of GM safety data is the lack of funding for basic physiological and nutritional studies of the possible health effects of GM foods on consumers. The attitude of the industry is that GM foods are safe and therefore there is no need for independent risk assessment studies. Thus, it is not surprising that ten years after the commercialization of the first GM crop, the FLAVR-SAVR tomato, there is still no generally agreed protocol for the risk assessment of GM products.

Although the EU has recently made an attempt to present a safety testing protocol for GM foods (Kuiper et al. 2004), the only previous independently funded research to set up a blueprint for GM risk assessment was the GM potato study carried out in Scotland between 1995 and 1998. Even though a blueprint for GM risk assessment based on this study was presented at an OECD meeting in Edinburgh in 2000 and subsequently published (Pusztai 2002), neither this nor the EU protocol has been generally accepted and put into practice. Accordingly, if there is any risk assessment carried out at all by the biotechnology companies this is usually an ad hoc study to suit their requirements. In the case of the more rare independent investigations into the possible biological effects of GM foods, the results obtained are non-binding on the regulatory authorities. Our database on the likely biological effects of GM foods is woefully inadequate. This is not surprising, because from the published results of one human clinical trial and a few animal studies published to date it is impossible to establish reliable and reproducible factual conclusions that are fully supported by the experimental evidence. Neither is it much help that data obtained by the biotechnology companies are seldom published and therefore these results are unavailable for most scientists. In the few cases when the industry's own risk assessment results have become public knowledge and they revealed statistically significant differences between the GM- and non-GM crop/food, the GM biotech industry denied that these differences had any biological significance. When independent scientists find such differences they are vilified.

The complexity of GM foods makes their biological testing difficult even when funding for such studies can be obtained. Thus, any protocol that may be devised must take into account that, in addition to the generally recognized importance of testing for the direct effects of the expression of the transgene, its insertion into the plant genome via a gene construct may also cause significant, indirect and unintended physiological effects by disturbing the functionality of the plant's own genes (Ewen & Pusztai 1999a; Schubert 2002, Freese & Schubert 2003; Wilson et al. 2004) and special testing methods are needed to recognize these. The number of copies of the construct inserted and their location in the plant genome (positioning effect) is also of importance.

Although the presence and consequences of such unintended effects in GM foods has long been ignored by the GM biotechnology industry, their importance is now beginning to be recognized by the regulatory agencies. Indeed, testing for these is now recommended in the Codex Alimentarius guidelines (Haslberger 2003).

Unfortunately, most currently used methods to detect unintended changes in GM products are largely inadequate. Positioning effects in plants often occur with both conventional crossbreeding and genetic engineering and empirically selecting for the desired trait and discarding the potentially harmful ones, usually to eliminate their unwanted consequences (Haslberger 2003, Pusztai & Bardocz 2006). However, it may be difficult to have appropriate selection criteria for

establishing which trait is harmful or beneficial. As it is only possible to compare the known properties and constituents of GM and conventional plants but not to look for, and even less to analyse, unknown newly created components, the limitations on our selection criteria are severe. Reliance based solely on chemical analysis of macro/micronutrients and known toxins is at best inadequate and, at worst dangerous, even when new and more sophisticated analytical methods are used, such as mRNA fingerprinting, proteomics, secondary metabolite profiling, and other profiling techniques (Kuiper et al. 2003). However, and most importantly, there is an urgent need to develop a protocol for experimental investigations using comprehensive toxicological/nutritional methods which will equally be applicable to scientifically examine the veracity of the claimed benefits of genetic manipulation and screen for its unintended and potentially harmful consequences for human/animal health. As the first contact point of exposure to any foods/feeds, including that which has been genetically modified, is the gastrointestinal tract (GIT), the first task in any proper risk assessment protocol should be to establish the consequences for the gut of short- or long-term exposure to diets that contain such foods/feeds (Ewen & Pusztai 1999a; Pusztai 2002). It is also important to point out here that any risk assessment protocol must take into account that it is not only the biological effects of the transgene product(s) that need to be unravelled but also the direct and indirect effects of the DNA vector constructs.

Alimentary tract as the first target of GM food risk assessment

To show by chemical methods the presence of new toxins/allergens in GM food products is, at best, difficult. In contrast, the presence of even minute amounts of unexpected but harmful potent bioagents in GM foods could be more easily established from their possibly disproportionally large effect on health. Thus, exposure of individuals to biologically active transgenic proteins can have major effects on their gastrointestinal tract. As most proteins are immunogenic their consumption may trigger immune/allergic effects both in the mucosal immune system of the gut and the body. It is also likely that, in addition to the effects on the gastrointestinal tract, the size, structure, and function of other internal organs will be affected, particularly in young and rapidly growing humans or animals. According to some recent unconfirmed reports, the dietary exposure to GM foods may also have harmful effects on reproduction. In addition, the risks will also have to be investigated as to whether measurable amounts of the transgenic DNA constructs in GM crops/foods survive in a functionally active state/size in the gastrointestinal tract of the human/animal ingesting them, and whether they can incorporate into the genome of the cells of their gut and body organs and what will be the consequences, if any, for the individual. The GM risk assessment protocol presented in the following outlines a gradual, step-by-step course of investigation by reliable and up-to-date methodology that addresses all these possible effects. These steps must be regarded as a minimum before any foods/feeds based on GM crops should be allowed into the human/animal food chain.

Suggested protocol for GM crop/food health risk assessment

Before any new GM crop could be made potentially safe transgenes must be identified and selected in preliminary model studies. The main criterion of the selection should be that the selected transgene and its protein product must have no toxic effects on humans or animals when given orally. However, the process of selection must be taken a step further by verifying that the selected transgene does function in the GM plant as intended. The transgene product must therefore be isolated from the GM plant and show unequivocally that its chemical and biological properties are the same as those of the gene product expressed in the original source from which the transgene was taken. It is absolutely essential that all safety studies be carried out on this isolated transgene product and not on *E. coli* recombinant surrogates.

In the GM safety studies performed by the biotechnology industry great emphasis is laid on the assertion that, according to their in vitro tests, all transgene products rapidly break down in simulated intestinal proteolytic digestion tests. Obviously, should a transgenic protein quickly break down to amino acids and small peptides in the alimentary tract its toxic effects or allergenicity could not be more than minimal and thus the safety of the GM crop should apparently be assured. However, in contrast to the protocols used in the biotechnology industry's safety assessment, true proteolytic digestibility must be established in the gut in vivo and not in a test tube in vitro. Clearly, one of the most important differences between the digestion of a protein in the alimentary canal and in a test tube using only pancreatic proteases is that in vivo, the binding of the transgene product to the intestinal wall and/or to the food matrix reduces the availability of the transgene protein (particularly in the case of the widely used transgenic lectins, such as the various *Bacillus thuringiensis*-, Bt-toxins) to the action of the proteases. Thus, an in vitro assay may give a false assurance of safety. In addition, as the structure, conformation and stability of a transgenic protein expressed in and isolated from *E. coli* is very different from that expressed in GM plants, no scientifically valid conclusions may be drawn from the results of experiments in which the assessment of the digestibility of a plant transgenic protein is attempted with an *E. coli* recombinant. Plants and eukaryotic bacteria are eons apart on an evolutionary scale and therefore no bacterial recombinants may be used in tests aimed to establish the true properties of transgenic proteins expressed in GM plants even though they are coded for by the same DNA.

Chemical composition

One of the first steps in any proper risk assessment protocol should be the characterization of the GM plant using well-authenticated and up-to-date methods of chemical analysis to estimate the contents of its major and minor components and to compare their amounts to those of the corresponding parent line. Although the results of such analysis and comparison can also be used to establish whether the GM and non-GM plants are 'substantially equivalent', first and foremost, this is an obligatory step that will allow us to carry out further biological risk assessment tests. However, for such a comparison to be scientifically valid large numbers of the GM- and the isogenic lines grown side-by-side and harvested at the same time are needed to be tested for the measurement of their major and minor constituents in parallel by classical and new analytical methods (proteomics, finger-printing, DNA/metabolic profiling, microarray analysis of all novel RNA species, full molecular biological examination with particular attention to the possibility of secondary DNA insertions into the plant genome, obligatory metabolomic NMR analysis of the transformed plant, stability of expression of foreign DNA, including the gene construct, promoter, antibiotic resistance marker gene, etc.).

Nutritional/toxicological testing with animals

As outlined, GM crops/foods will need to be examined in obligatory short- and long-term nutritional/toxicological tests with laboratory animals under controlled conditions. The intention is to find out whether there are any toxic effects in the animals fed on diets containing GM foods that would make the progression to human clinical trials unsafe. The animal tests are therefore designed to establish the effects of the GM crop/food on growth, metabolism, organ-development, immune and endocrine functions (Pusztai & Bardocz 2006), with particular emphasis on how diets based on GM food will affect the structure, function and bacterial flora of the animal gut. As the normality of these functions determine the development of young animals into healthy adults, the absence of significant differences between the health statuses of animals fed on GM- and non-GM diets may possibly indicate that the GM crop is not unsafe, at least in animal nutrition.

Diet

It is of paramount importance that the conditions of nutritional testing are rigorously standardized. Thus, all diets must be *iso*-proteinic and *iso*-energetic (i.e. contain the same amounts of protein and energy) and are fully supplemented with vitamins and essential minerals. The composition of the control diet containing the parent line should be as close to the GM diet as possible. Diet formulation is therefore – particularly when there are significant compositional differences between the GM- and its corresponding non-GM parent-line crops (e.g. see data for GM potatoes in Table 14.1) – not an easy task and supplementation with pure ingredients may be necessary to make good the compositional differences. In a second control diet, the parent line should be supplemented with the gene product isolated from the GM crop whose concentration should be the same as in the GM crop. All crops/foods should be fed both raw and after heat-treatment.

Table 14.1. Compositional values for 'Desiree' potato tubers and two GM lines expressing the snowdrop (*Galanthus nivalis*) bulb lectin, GNA (Pusztai 2002).

Constituent	Parent line	GM lines	
		Line 71	Line 74
Protein (% w/w)	7.2[a]	7.2[a]	5.6[b]
Lectin (μg/g)	6.7 (0.4)[b]	7.9 (<0.1)[a]	5.8 (0.8)[c]
Trypsin inhibitor (mg/g)	3.4 (<0.1)[a]	3.1 (0.1)[b]	2.7 (0.1)[c]
Chymotrypsin inhibitor (mg/g)	2.7 (0.1)[a]	2.6 (0.1)[a]	2.2 (0.1)[b]

The plants were grown side-by-side in field tunnels. The values are means (sd) of analyses of at least four determinations of each constituent independently carried out by two workers. Values with different superscripts are significantly different ($p<0.05$).

Experimental protocol

Groups of young rapidly growing animals (5–6 in each group) closely matched in weight (less than ± 2% w/w), housed separately, should be strictly pair-fed these diets in short- and long-term experiments. Both males and females should be tested. The progress of the animals should be closely monitored, urine and faecal samples collected throughout the experiment and the nutritional performance of the animals and the nutritional value of the diets assessed by Net Protein Utilization (NPU), and with measurements of nitrogen- and dry weight balances and feed utilization ratios. The animals should be weighed daily and any possible abnormalities observed. Blood samples should be taken before, during and at the end of the feeding experiments for immune studies (immune responsiveness assays (Table 14.2), Elispot, etc.), hormone assays (insulin, CCK, etc.) and determination of blood constituents. At the end of the experiments the animals should be killed, dissected, and their guts rinsed and the contents saved for further studies (enzyme contents, GM products, DNA, etc.), gut sections taken for histology, the wet- and dry weights (after freeze-drying of the tissues) of organs recorded (Table 14.3), and the organs subjected to compositional analyses. All these data could be used to comprehensively characterize the health and metabolic status of the animals and the behaviour of the GM fed animals could be directly compared with that of the controls. The results could then be evaluated by appropriate methods of statistics.

If any of the effects of the diet containing the GM crop on the rats is significantly different from that of the non-GM parental line control diet, the inclusion of the GM crop in food is unsafe and therefore not recommended. If the effects of feeding rats with the parent line control diet are significantly changed when this is spiked with the isolated transgene product, the *transgene is*

unsafe. Most importantly, if the effects of the diets containing the GM plant and the parent line control spiked with the gene product differ, the harm is likely to be due to the use of the particular construct vector or caused by an unintended and unforeseen effect of the *transgene insertion or position* in the plant genome. Accordingly, this method of gene transfer and the resulting GM crop is unacceptable. Thus, further research is needed to find other, more precise and safer methods of genetic modification.

Table 14.2. Results of lymphocyte proliferation assays in rats fed for 10 days on diets containing raw GM-, control/non-GM potatoes, or control/non-GM potatoes supplemented with the gene product, GNA, *Galanthus nivalis* agglutinin (Pusztai 2002).

	μg Con A/well				
Diet	0.3	1.0	3.0	6.0	9.0
Parent	10.3 (13.4)	16.0 (18.5)	4.4 (4.9)	1.9 (1.0)	1.6 (1.6)
Parent + GNA	2.5 (4.3)	2.6 (3.5)	2.0 (3.6)	1.1 (0.5)	0.9 (0.6)
GM	1.5 (0.9)		1.7 (1.1)	1.0 (0.4)	1.6 (1.1)
1.6 (1.5)					
Significance (p<)					
Parent vs					
Parent+GNA	ns	p<0.05	ns	p<0.05	ns
Parent vs GM	p<0.05	p<0.05	p<0.05	ns	ns

Rats were fed on different diets for 10 days. At the end of the experiment blood samples were taken and subjected to standard lymphocyte stimulation assay with Concanavalin A (Con A) as the mitogenic signal. The results are expressed as stimulation indexes vs control. Values are means (sd) and significance was assessed by Student t test.

Table 14.3. Relative dry organ weights of rats significantly affected by feeding with diets containing raw or boiled GM potatoes and/or parent potatoes spiked with the gene product (GNA, *Galanthus nivalis* agglutinin) (Pusztai 2002).

	Raw potatoes			Boiled potatoes
Diet	Pancreas	Jejunum	Prostate	Liver
Parent	0.68 (0.08)	0.62 (0.06)	0.24 (0.08)	3.78 (0.14)
GM	0.81 (0.05)	0.72 (0.07)	0.16 (0.02)	3.28 (0.21)
Parent + GNA	0.70 (0.08)	0.67 (0.04)	0.18 (0.02)	3.40 (0.28)
Significance (p<)				
Parent vs GM	0.01	0.03	0.05	0.001
Parent+GNA vs GM	0.03	ns	ns	ns

Rats were fed with the diets for 10 days. The values of relative dry organ weights (g organ weight/100 g dry body weight) are means (sd), n=6, by multivariate statistical analysis.

Differences in nutritional performance useful for diagnosis of harm

Organ weight changes are useful indicators of metabolic events after feeding laboratory animals with diets containing GM foodstuffs, particularly if followed up by histological examinations as part of the safety assessment of GM crops. Assessment of potential deviations in the normal development of key organs is of great diagnostic value, as shown in one of our GM-potato rat feeding studies. Sections of the various compartments of the gut taken for histology (Ewen & Pusztai 1999b) (Figure 14.1) indicated a strong trophic effect of the GM potatoes on the rats' small intestine and, to a lesser extent, on their stomach. This hyperplastic gut growth was of particular significance because the jejunum was not enlarged when the parent line diet was

supplemented with the gene product, GNA (*Galanthus nivalis* lectin), confirming previous observations which showed that the gene product had negligible growth factor effect on the jejunum, even when included in the diet at a several hundredfold concentration in comparison with that expressed in the GM potato lines (Pusztai et al. 1990). This was, in fact, one of the main reasons for selecting the gene of the natural insecticidal GNA for the genetic transformation of potatoes (Gatehouse et al. 1996) to make them pest-resistant but nutritionally safe.

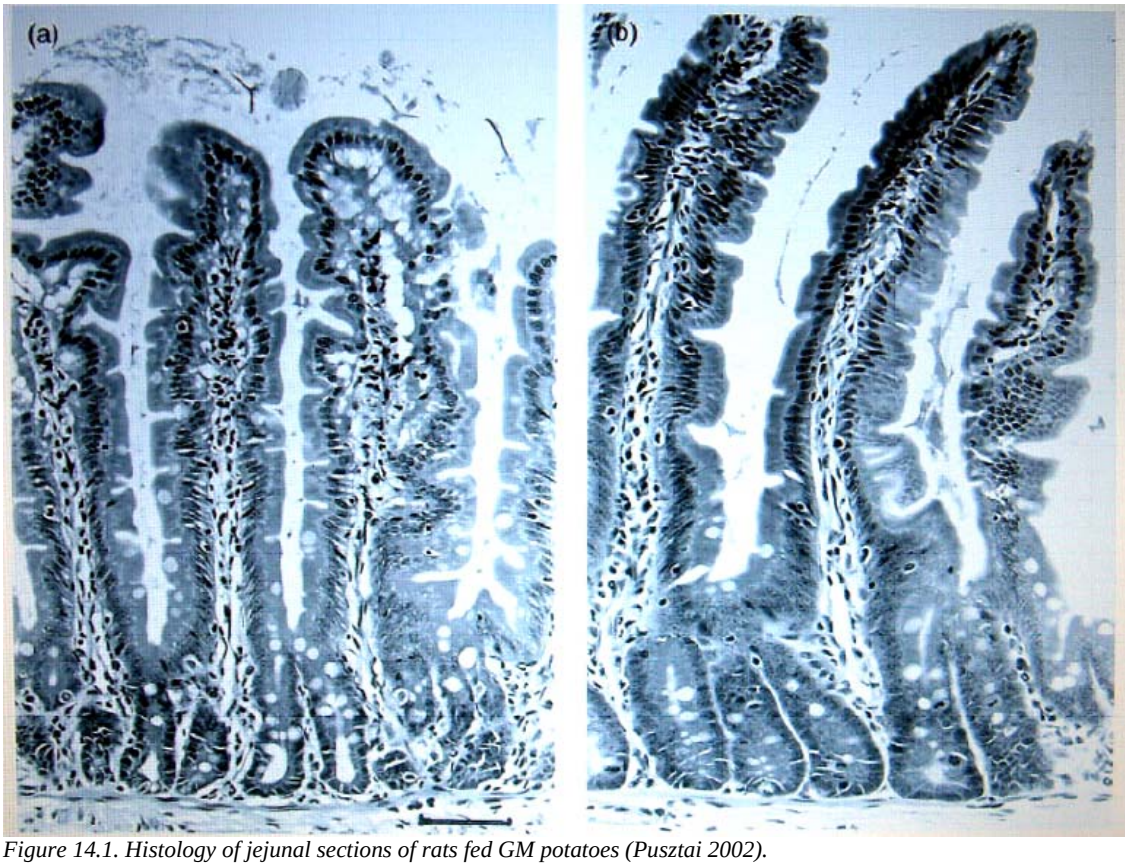

Figure 14.1. Histology of jejunal sections of rats fed GM potatoes (Pusztai 2002).
Jejunal crypt length and cells exhibit marked enlargement after feeding rats a diet of raw GM potato for 10 days, (b) in comparison with that of rats given a parental line potato diet (a). The villus length is similar in both but intraepithelial lymphocyte cell counts appear to be increased on GM potato diet. (14 mm bar = 100 μm).

As similar hypertrophic and other similar changes in gut ultrastructure in the ileum of mice fed GM potatoes expressing *Bacillus thuringiensis* var. *kurstaki* Cry 1 toxin gene or the toxin itself were shown in a different study (for reference see Pusztai et al. 2003), GM potatoes of different origins may have common trophic effects on the gut. Changes in the ultrastructure of other organs, such as the liver, pancreas, etc., on feeding with GM crop containing diets, as shown by the work of the Malatesta group (for references see Pusztai & Bardocz 2006), may also be taken as a first indication of possible harmful effects that should make follow-up studies mandatory. Changes in blood cells and blood protein levels in GM-fed animals may also suggest serious health problems, including disturbances in erithropoiesis, blood protein synthesis and the immune system. Thus, measurement of immune responsiveness could be a useful follow-up study when blood cell counts show significant differences in lymphocyte numbers that may point to one of the potentially serious hazards of the ingestion of GM foodstuffs (e.g. see our GM-potato studies, Table 14.2). This is a particularly useful method because it is in general clinical use and could therefore be easily carried out with humans. Although no hormone assays were performed on rats

fed GM or non-GM diets in our GM potato study, the consistently strong pancreatic growth stimulated by GM potato diets in the feeding studies suggests that this possibly was the result of the release of CCK (cholecystokinin) or some other humoral growth factor from the duodenum by an unknown growth/proliferative signal only found in the GM potatoes. Again, GNA (*Galanthus nivalis* lectin) could not be responsible for this because it does not stimulate the enlargement of the pancreas when fed to rats in its original source (Pusztai et al. 1990).

The measurement of circulating insulin levels after ingestion of GM diets would also be a good indicator for possible disturbances in the general metabolic state of the animals, particularly as insulin assays can be easily done on humans. Changes in blood basophile counts may also suggest possible problems of allergenicity that need to be followed up by more dynamic studies. Although the recommended decision-tree approach is a useful start to look at the allergenic potential of the GM crop, the criteria used in this, such as the lack of structural similarities of the GM protein to known allergens, the lack of glycosylation, small molecular size, or the in vitro digestibility of the GM protein, etc., are not sufficiently decisive to exclude the possibility that the GM protein is an allergen. The development of delayed hyper-sensitivity reaction found recently in GM peas expressing the kidney bean α-amylase inhibitor gene has demonstrated that proteins that are not known to be allergens in the original plant source can develop allergenic reactivity when their genes are transferred to other plant species by genetic engineering, even in the case of closely related species (Prescott et al. 2005). Finding immune-reactive antibodies to GM proteins in blood circulation, particularly of IgE-type, in humans or animals should, of course, be strong evidence for the occurrence of immune/allergenic reactions. Although there is at present no satisfactory animal model for allergenicity testing of GM proteins, immunization studies in brown Norway rats (*Rattus norvegicus*) show some promise.

Problems and perspectives

Compositional studies and animal tests are but the first steps in GM risk assessment. Next, long-term, preferably lifetime-long metabolic, immune and reproduction studies with both male and female laboratory and other animal species should also be conducted under controlled conditions. However, setting up proper protocols for these is a task that has not been accomplished yet. If none of the short- or long-term risk assessment tests on animals show harm, only then could the safety of the GM food be further tested in double-blind placebo-controlled clinical studies with human volunteers. However, it should be pointed out that most clinical studies rely on volunteers in a reasonably good state of health even though any possibly harmful effects of GM foods are expected to be more serious with the old, young and the diseased. Thus, even the results of human clinical investigations may not be representative for the whole population, particularly when it is considered that, according to some estimates, up to 40% of the population may suffer from some sort of disease of the gastrointestinal tract. It also has to be taken into consideration that because it is an irreversible technology once a GM crop is generally grown on the land and foods based on these are released into the human food chain and included in animal rations, its removal or recall will become nearly impossible.

Effects of transgenic plant DNA

In addition to the changes in protein/metabolite profiles and the possible formation of new toxins and allergens in the plant resulting from the unanticipated effects of transgene insertion and the destabilization of the recipient genome and the interference with the expression of the plant's own genes, the effects of transgenic plant DNA should also be considered. Thus, it is essential in any risk assessment protocol to determine in humans/animals ingesting GM foods whether appreciable amounts of the DNA vector construct used for developing the GM plant survive in

the gut in functional form, whether they are taken up and integrated into the genome of the individual, and what, if any, effects the foreign transgenic DNA will have on them.

GM DNA safety studies in the gastrointestinal tract

The tasks in these safety studies should follow closely the principles outlined for GM proteins and will be described in detail separately in a separate chapter in this book. Here, only the main principles of a possible GM DNA risk assessment are outlined.

The first task is to trace the GM DNA used for the development of the GM crop, such as the Bt toxin-expressing maize lines, through the intestinal tract, measure the proportion of the construct DNA surviving in functional form, establish by appropriate methods whether it is absorbed by the gut epithelial cells or by gut bacteria and integrated into the genome of these cells and whether they will express the transgene. Next, it has to be shown whether the GM DNA is absorbed into the systemic circulation and taken up by cells of body organs. In addition, it has to be investigated whether the GM DNA can pass into the placenta in pregnant females, foetus and brain, and, if so, what the biological consequences are.

In these investigations, special emphasis should be laid on whether parts of the DNA constructs, particularly the promoter, such as the cauliflower mosaic virus 35 s (CaMV 35s) are taken up by the gut and have biological effects. Obviously, as discussed in previous sections, it is of particular relevance whether the Bt toxin expressed in the GM plant has any harmful effect on the gut, body organs and the immune system. When an antibiotic resistance gene is used in the DNA construct as a selection marker gene, one of the most important questions that the risk assessment protocol will have to answer is whether this antibiotic resistance gene can transform gut bacteria in vivo. This has become highly pertinent since it was shown that functional DNA constructs used in the development of GM soybean survived in sufficient quantities in human volunteers and were found to be taken up by the bacteria in the gut (Netherwood et al. 2004 and also see Pusztai & Bardocz 2006).

Final general considerations and conclusions

In the absence of safety studies, the lack of evidence that GM food is unsafe cannot be interpreted as proof that it is safe, particularly as all well-designed GM safety studies published to date and carried out independently of the biotechnology industry have demonstrated potentially worrisome biological effects of GM food as referred to in this paper and recently documented by Smith (2007). Unfortunately, the regulators have largely ignored these.

In the light of these problems one can ask whether the future of the present generation of GM crops/foods rests on solid scientific foundations. If not, as it appears, the question is whether it is it needed at all, particularly as according to the FAO apparently there is sufficient food for feeding the world population, providing that it is evenly and properly distributed. It is possible that GM foods may be needed in future but should such a need arise we ought to first find more reliable and safer genetic transformation techniques for the development of GM crops. However, even then, their safety must be rigorously tested with biological methods, as without proper, transparent, inclusive, and independent testing the sceptical public is unlikely to be convinced of their safety and accept any present-day or future GM foods.

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Chapter 15

DNA vaccines: Mechanisms and aspects of relevance for biosafety

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1. Introduction

DNA vaccines represent a new approach to protect against infectious disease, and hence may improve human and animal welfare, reduce antibiotic usage and reduce the spread of pathogens. Edible and injectable DNA vaccines hold prospects for rapid immunization against a variety of diseases that are difficult to eradicate with traditional vaccines and antibiotics. Other potential uses of DNA vaccines include treatment of cancer, autoimmune diseases and allergies. DNA vaccines have several attractive benefits: low cost, ease of production and improved quality control, heat stability, identical production processes for different vaccines, and the possibility of producing multivalent vaccines (Kwang 2000). On the other hand, there is a limited scientific understanding of the mechanisms underlying uptake, persistence and degradation of DNA vaccines following their injection into humans or animals. The main areas of uncertainty are related to the immunological impact, tissue distribution and persistence after injection and whether the DNA vaccine can leak into the environment.

In this chapter, we summarize the uses, mechanisms, immunological parameters, and environmental issues associated with the introduction of DNA vaccines, and identify areas where more research is needed.

2. The use of DNA vaccines

A DNA vaccine consists of a bacterial plasmid with a strong viral promoter, the gene of interest (a gene encoding the immunostimulatory protein), and a polyadenylation/transcriptional termination sequence. The plasmid is grown in bacteria, purified, dissolved in a saline solution, and then administrated by direct intramuscular injection of naked DNA (in ng and μg amounts) to activate protein expression in vivo and to ultimately induce an immune response and disease protection.

The advances in the field of DNA vaccines in recent years have been profound. In 2003, the US Centers for Disease Control (CDC) expedited delivery of an experimental veterinary DNA vaccine developed by the CDC and manufactured by Aldevron (Fargo, ND) (Bouchie 2003). The target for vaccination was the wild Californian condor and the purpose was to protect this endangered species from becoming infected with the West Nile virus. In Canada, an Infectious Hematopoietic Necrosis Virus (IHNV) DNA vaccine (Apex-IHN®) developed by Aqua Health Ltd. (Canada), an affiliate of Novartis, was cleared for marketing by the Canadian Food Inspection Agency on 15 July 2005 (Novartis media release 19 July 2005). Currently, a number of experimental human DNA vaccines have entered phase 1 clinical trials. However, the biosafety aspects have not yet been thoroughly investigated, and it may be expected that these aspects will become increasingly important when the vaccines enter the regulatory approval process prior to commercial use of DNA vaccines.

3. The immune system and immune responses by DNA vaccination

Both mammalian and fish defence systems include, roughly defined, leucocytes, and their products most often localized in lymphoid organs, such as the thymus, spleen and kidney. In addition, several other tissues harbour defence cells and proteins (e.g. liver, skin, intestines, and gills). The immune system contains both adaptive and innate defence mechanisms that eradicate pathogens in a concerted manner. Within minutes after infection, the innate defence is activated, whereas two to three weeks are required to eradicate the pathogens by mechanisms of the adaptive defence (Fig. 15.1).

The innate defence mechanisms involve 1) cell-derived defence factors (e.g. defensive peptides, complement components, reactive oxygen radicals, interferons and receptors), and 2) leucocytes such as monocytes, macrophages, dendritic cells, scavenger endothelial cells, and granulocytes (Fig. 15.2). The expression, amount and activities of the cell-derived defence factors may increase upon activation of innate defence. Almost all organs and tissues in humans and animals contain cells and components of innate defence.

The adaptive defence system, specific for an infectious agent, concerns the immune response that involves: 1) cells and specifically recognizing molecules mediating eradication of e.g. virus infected cells, and 2) the production of reactive antibodies that bind to antigenic determinants against pathogens and foreign substances. These processes mainly involve antigen presenting cells (APC; i.e. macrophages and dendritic cells) that are a partner in both innate and adaptive arms, T- and B cells (Fig. 15.2). The adaptive machinery of defence also creates memory cells that, upon reactivation, induce rapid immune responses and is the rationale for vaccinology.

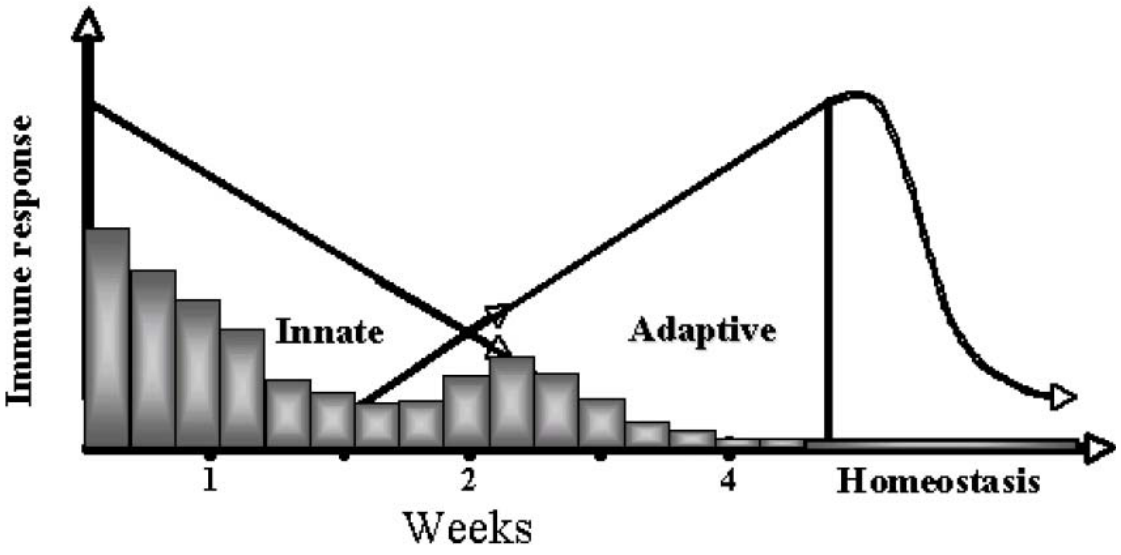

Figure 15.1. The innate immune response is immediate in nature and involves cellular and soluble (humoral) antimicrobial factors and is contributing to the eradication of pathogens. In the case of surviving pathogens (e.g. for 7–14 days), adaptive immune defence mechanisms may bring about final destruction of pathogens whereby homeostasis reoccurs. Bars show pathogen load in the host.

3.1 Persistence and uptake of DNA vaccines after injection in animals

It has been reported that immediately after injection of plasmid DNA intravenously most of the DNA is rapidly degraded (Hashida et al. 1996). The products from the degradation are either used as nutrients or excreted in the urine. Interstitial (extracellular) and cellular nucleases have been reported to be responsible for DNA degradation in mice (Hashida et al. 1996). In preliminary experiments, almost 100% of plasmid DNA is degraded in salmon blood within one hour. The degradation is most probably due to nuclease (DNAse) activity, since known nuclease inhibitors block degradation. Despite rapid breakdown in blood, a minor fraction of intact DNA vaccine remains in the muscle tissue at the injection site, together with fractions of blood-transported intact DNA in organs such as the kidney and liver (Tonheim et al. 2007).

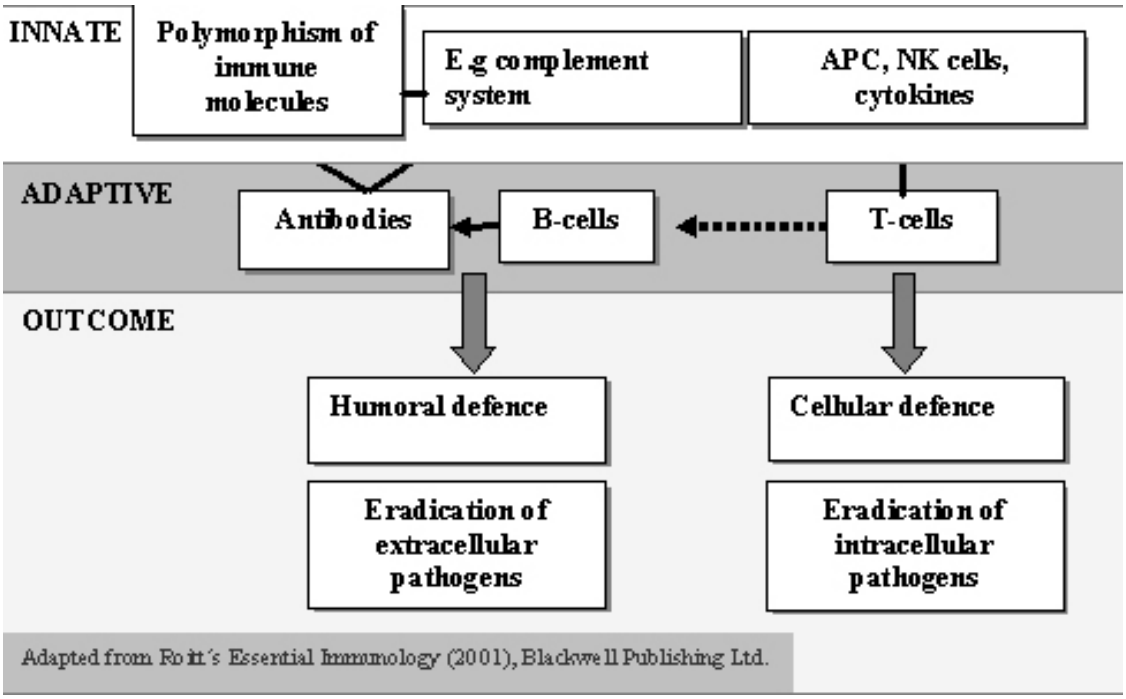

Figure 15.2. The concerted action of the innate and the adaptive immune defences.

The outcome of an immune response depends on the infectious agent, host cell activation, and the cytokine (signalling molecules) profile generated by the leucocytes. In general, humoral immunity (generation of antibodies) will resolve infection caused by extracellular pathogens (e.g. many bacteria) whereas cellular activation may cause eradication of intracellular pathogens by actions of e.g. enzymes, oxide radicals and membranolytic substances. Of importance is the interplay between innate, adaptive, humoral and cellular defences. For instance, both complement and antibodies may facilitate antiviral effects and activated cells (cellular defence) may degrade extracellular bacteria. To increase the efficacy of eradication of pathogens many defence molecules show hyper-variability, in which single nucleotide polymorphism (in genome) induces mutation in the amino acid sequences of proteins. Some of the mutations may cause increased affinity to pathogenic structures, thereby causing higher probability of pathogen scavenging. DNA vaccination causes activation of innate, adaptive, humoral and cellular defences. It is highly acknowledged that the DNA vaccine induced activation of the cellular defence is utmost important in fighting viral pathogens.

A prerequisite for expression of the immunostimulatory protein is that the DNA vaccine (purified plasmid DNA) is taken up by the host cells, transferred to the cytosol and eventually transported to the nucleus before any expression occurs. Several passive and active mechanisms have been described concerning receptor binding and/or uptake of DNA. The uptake processes are described as uptake by endocytosis (phagocytosis (cell eating) and pinocytosis (cell drinking)).

Pinocytosis is utilized essentially by all cell types and occurs by multiple pathways, i.e. clathrin-mediated endocytosis, caveolin-mediated endocytosis, chlatrin- and caveolin-independent endocytosis, and macropinocytosis (Belting et al. 2005). Macrophages, granulocytes and dendritic cells carry out phagocytosis (e.g. of dead cells, bacteria, large molecular complexes), and they are found in many parts of humans and animals. In particular, macrophages, residing in close connection to the bloodstream, are highly phagocytic, thus functioning as an important element in the reticuloendothelial system – together with scavenger endothelial cells. These scavenger cells are responsible for the highest uptake and degradation of plasmid DNA (Kawabata et al. 1995; Takagi et al. 1998). The liver is the main scavenger organ in mammalian species, whereas the kidney and heart have this function in fish.

The endocytic pathway normally confers total degradation of DNA – especially if the DNA is transported to the end-point terminal (lysosomes). However, tiny amounts of DNA may escape the endocytic compartments and degradation. This DNA may be trafficked through the nuclear membrane into the nucleus where transcription occurs.

Transport vehicles (carriers) have been commonly used to increase the efficacy of transgene production and are used in conjunction with DNA vaccines. Such vectors include polyplexes (positive charged cationic polymers, such as poly–L/D-lysine), lipoplexes (cationic lipids: Liposomes) and molecular conjugates (cell receptor ligands conjugated to DNA) (Medina-Kauwe et al. 2005). Their main advantages are that they confer DNA condensation, inhibit DNAses and facilitate endosomal escape of DNA by endosomal membrane association. In spite of these advantages, low-level transfection, relative to viral delivery of DNA/viral infection, often occurs since there are many obstacles to overcome to mediate efficient gene transfer. In conclusion, intracellular trafficking including endosomal escape and cytosolic processes mediating nuclear import of DNA vaccines are issues that warrant further research.

The nuclear membrane filter excludes intact plasmid DNA larger than *c.*40 kDa and the DNA is thus retained in cytosol (Median-Kauwe et al. 2005). Active nuclear transfer mechanisms must be present to facilitate transgene expression mediated by large DNA fragments or intact DNA. Such processes are ‘catalysed’ by nuclear importins or nuclear localization signals (NLS) that are proteins and peptides respectively, that help large molecules to reach the nucleus (Medina-Kauwe et al. 2005). Further, there are reports that describe transgene production, for instance both after intravenous, intraperitoneal and intramuscular injection of naked luciferase-coding plasmid DNA in rainbow trout (e.g. firefly luciferase (enzyme)) in distant organs such as the kidney and spleen (Romøren et al. 2004). More experiments addressing the tissue distribution versus transgene production are needed to elucidate molecular mechanisms of DNA persistence and stability of the expression of the immunostimulatory gene product.

3.2 Immune responses to DNA vaccination

After DNA vaccination, two main immune responses evolve in a time-dependent manner, the first being an immediate response generated by innate defence mechanisms and the second being a late specific response with production of specific antibodies and activation of a cytotoxic response (cytolysis of cells expressing the transgene on their cell membrane) (Fig. 15.1). It has, for instance, been reported that the immediate response following DNA vaccination in fish with a

rhabdo virus (VHSV) DNA vaccine also confers protection against other viral diseases and is thus not pathogen-specific (Lorenzen et al. 2002). Furthermore, genes important in both cellular and humoral defence have been reported to be significantly up-regulated one to three days after DNA vaccination using a VHS-G plasmid construct, whereas the number of differentially expressed genes at days seven and twenty-one have decreased considerably (Byon et al. 2005). This is also in line with the suggestion that pDNA containing the G-protein confers a strong effect on the immune system at early time-points. The long-term effect of pDNA on the immune system is, however, not known.

3.2.1 Innate immune response to DNA vaccines

Bacterial DNA, invertebrate DNA and DNA from some viruses differ structurally from vertebrate DNA because they contain increased frequencies of CpG dinucleotides (Bird 1986). It has been reported that toll-like receptor 9 (TLR9) recognizes such CpG motifs (Hemmi et al. 2000). TLR9 binding of DNA and subsequent intracellular activation induces production of cytokines and type I interferons that augment the host fighting viral infections. It is suggested that CpG containing DNA vaccines (plasmids produced in bacteria) confer an immediate and efficient anti-viral effect – as observed by Lorenzen et al. (2002).

3.2.2 Adaptive immune response to DNA vaccines

After DNA vaccination, host cells may produce the antigen of interest and these antigens may be endocytosed by antigen presenting cells (APC). Peptides of the endocytosed antigen will be presented on MHC class II molecules to T cells leading to production of antibodies by plasma cells. Administration of DNA vaccines has proven to be an effective means for generation of humoral immune responses (antibody production) specific for the encoded antigen(s) (Russell et al. 1998; Fernandez-Alonso et al. 2001; Nusbaum et al. 2002; Verri et al. 2003). A combination DNA vaccine, consisting of multiple discrete plasmids encoding several different antigens of a pathogen, may be employed to induce a broader spectrum of immune responses. This would be effective for vaccination against viruses that undergo antigenic variations (Lee et al. 1996; Wang & Nicholson 1996; Kibenge et al. 2001). Although there may be high vaccine efficacy (increased survival), the potency (amount of specific antibodies generated) may be relatively low compared with traditional vaccines. To obtain increased potency of the DNA vaccines, one may apply higher doses, a prime boosting regime or co-administration of plasmids encoding cytokines or co-stimulatory molecules.

3.2.3 Cytotoxic T cell responses and DNA vaccines

Both viral infection and DNA immunization induce intracellular expression of antigens that may be presented on MHC class I molecules (Dijkstra et al. 2001) which, in turn, activate TCR/CD8+ T-lymphocytes to lyse the 'infected' cells. After DNA vaccination, CTL responses and subsequent cell lysis may eliminate 1–5% of the muscle cells that have been transfected and express, for example, viral antigens on their surfaces. An intramuscular injection of any solution will cause tissue damage, wound repair and tissue remodelling. However, the destruction of 1–5% of muscle cells after DNA vaccination would be unlikely to have clinically significant effect on the performance of the injected muscle. The damaged muscle cells will be replaced by the migration and fusion of satellite cells within existing myotubes as part of normal cellular turnover. It is suggested that the magnitude of cellular turnover caused by DNA vaccination is not higher than by viral and bacterial infections (Donnelly et al. 1997).

4. The need for research on the effects of DNA vaccination

Before distributing any genetically modified DNA constructs (e.g. DNA vaccines) into a new location/ecosystem, important questions and knowledge gaps concerning environmental and

health effects need to be addressed. A number of hypothetical effects, both beneficial and harmful, have only modest scientific support. There are three main issues that need to be resolved:

- Knowledge gaps related to the biology of uptake, the tissue and organ distribution and persistence of the DNA vaccine in the host organism.
- Knowledge gaps with regard to potential unintended physiological effects on the host organisms, including unwanted immune response.
- Knowledge gaps arising from unintended release of the DNA vaccine into the open environment as a result of the expected human error in large-scale vaccination processes or from the vaccinated host organism itself.

4.1 Knowledge gaps related to uptake, distribution and persistence of the DNA vaccine
Preliminary experiments have revealed that organs and tissues rich in leucocytes have accumulated intact DNA (plasmid DNA) for more than one month in salmon after intraperitoneal (ip) and intramuscular (im) injections (Myhr & Dalmo 2005). For instance, in sea bream, intact plasmids were found at the injection site two months after intramuscular injection (Verri et al. 2003). Similar findings have been described for rainbow trout (Anderson et al. 1996). Further, it has been shown that not only muscle cells but also cells in tissues very distal to the injection site (muscle) have expressed the transgene after plasmid injection (Romøren et al. 2004). Moreover, it has been shown that glass catfish have been expressing a transgene as long as two years after injection (Dijkstra et al. 2001). These reports illustrate that plasmid DNA can persist in fish for long time periods after the initial injection. Undoubtedly, there is an urgent need to analyse the longevity of DNA vaccines with respect to immunological parameters, the risk of gene transfer to the host's genome or intestinal bacteria, or other exposed organisms after release of plasmid DNA (excreted into the gastrointestinal tract of the vaccinated host).

4.2 Knowledge gaps with regard to potential unintended immune response
Concerning unintended long-term effects of plasmid DNA on the immune system, no experiments have been conducted to address this issue in any animal species so far although modern tools for gene expression analysis are available. It seems that the immediate short-term elevation of the expression of certain immune genes is normalized within three weeks after DNA vaccination, as reported for Japanese flounder (Byon et al. 2005; 2006). To our knowledge, no microarray analysis on samples obtained from DNA vaccinated mammalian species has been performed.

4.3 Potential effects of unintended environmental release of DNA vaccines
If the transgenes are released into the environment after vaccination, DNA products could be distributed unintentionally over vast areas, and have potentially mediating effects in a range of organisms after horizontal gene transfer. DNA is more resistant to immediate breakdown in the ecosystems, both terrestrial and aquatic ecosystems (Heinemann & Roughan 2000) and after uptake from the gastrointestinal tract micro-organisms, than previously assumed. There are, however, few published studies investigating the stability, horizontal transfer and uptake of released DNA constructs in terrestrial and aquatic systems, including in fish and mammals.

5. Legal implications of the usage of DNA vaccines

At present, scientific uncertainty concerning the risks of introducing DNA vaccines creates disagreements about which legal frameworks should be applied for risk assessments in approval procedures. For instance, scientists and policymakers in Norway and in the EU disagree about how to regulate DNA vaccines (Foss & Rogne 2003). Central to this discussion are the regulatory definitions of 'medicinal products' and 'genetic modification' and which regulatory system to involve. In the United States, the US Food and Drug Administration has asserted that genetic

constructs distributed to animals fall under the legal definition of a drug substance. This corresponds with regulations in Europe; the European Agency of Medicinal Products authorizes pharmaceuticals based on modern biotechnology through a centralized procedure. However, as a part of the European procedure, national GMO authorities are involved in evaluating environmental risks of both the medicinal products and the animals receiving them. For instance, the Norwegian Directorate for Nature Management has stated that a DNA-vaccinated animal is to be considered as genetically modified (GM) for as long as the added DNA is present in the animals This may have implications for the need for labelling and traceability. Accordingly, the current limited scientific understanding of the fate of DNA vaccines, such as host distribution and persistence, has clear policy implications.

6. Conclusions

DNA vaccines hold promises for protection against a range of diseases caused by viruses and intracellular bacteria, for which there at present are no efficient vaccines based on either live, attenuated viruses or vaccines containing recombinant viral antigens. However, the present lack of biological understanding of the health and environmental effects of distribution of DNA vaccines creates a challenge with regard to their perceived safety and regulatory basis. Targeted studies of specific knowledge gaps, as identified here, must therefore be incorporated into the vaccine research and development agenda; encouraging broad and long-term thinking.

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Chapter 16

Models of Science and Policy

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1. Introduction

In this chapter we will focus on the role of science in the development and implementation of policy. Specifically, we will present and briefly discuss a number of conceptual *models* that describe the relationship and interface between science and policy regulating environmental issues. These models come with their particular underlying assumptions, strengths and limitations, and no single model can be said to offer the universal solution to the challenges ahead, neither with respect to biosafety issues nor to complex environmental issues in general. Nevertheless, we argue – along with a growing literature on these problems (see for instance Wynne 1992; Funtowicz & Ravetz 1993; Nowotny et al. 2001) – that a rethinking of the relationship between science and policy (and indeed politics) is called for. In the modern tradition of European Enlightenment, the relationship between science and policy was thought to be simple in theory, even if complicated in practice: science informs policy by producing objective, valid and reliable knowledge. To develop a policy was thus a matter of becoming informed by science and then, in a second step, to sort out diverse values and preferences. We call this the modern model. A crucial feature of this model is that it captures the modern notion of rationality. We could say in a simplified manner that, within the Enlightenment tradition, rational actors act within the modern model and choose those policy options that, according to the scientific evidence, best meet their preferences.

In theory, the modern model is easy to justify, to the extent that it is often taken for granted. Its justification, however, presupposes a number of assumptions that only rarely are expressed in full. First, it is assumed that the available scientific information is really objective, valid and reliable. When there is considerable scientific uncertainty, such as when the facts are highly uncertain, or when experts are in strong doubt, the modern model is no longer the unique rational design choice for the relationship between science and policy. The same would apply in the case where there are conflicts of interest, such as when the experts are themselves stakeholders. Second, the modern model assumes not only that uncertainty can be eliminated or controlled, but also that the scientific information can be complete in the sense that it tells the policy maker everything that is necessary to know in order to decide for the common good: there is only one correct description of the system, and it is to be provided by science. If there are several descriptions of the system, they might be combined and reduced into one all-encompassing scientific description. In other words, the modern model assumes that the system and the problem at hand are not *complex.*

The problem is that most important real-life environmental and health issues display both complexity and scientific uncertainty, posing serious challenges to the modern model. Basically, there can be three reactions to this challenge. The first is denial: to pretend that the challenge does not exist and keep using the modern model as it is. The second is accommodation: to try to adjust

[1]The views expressed are those of the author and do not represent necessarily those of the European Commission

the modern model to confront the challenges of uncertainty and complexity. The third is to search for innovative, more radical departures from the modern model. Each of these possibilities will be briefly discussed in this chapter. It is only fair, though, that we admit that our main interest lies in the articulation of potential radical alternatives. We believe that recognition of irreducible scientific uncertainty and complexity in environmental and health issues necessitates a fundamental departure from the modern model, revisiting its definition of knowledge as well as of governance. Knowledge is not only produced by science, and governance is more than deducing action from facts and preferences. Our reasons for believing so will be presented in the following.

2. Theoretical Framework: Sources of Uncertainty and Complexity in the Biosafety Issue

As noted in the Introduction, many authors and strands of thought currently point towards the inadequacy of the Enlightenment tradition to meet emergent challenges, and the need to rethink the relationship between science and governance (including policy and politics). Beck (1992) has discussed how modern societies routinely produce not only goods but also *bads*, in the form of risks, due to the adverse and often unanticipated effects of progress. The accumulated magnitude and unequal distribution of these risks gradually become more severe and more apparent with the passage of societies to the post-industrial stage, to the extent that it becomes a key feature of our time, which Beck calls *second modernity*. Nowotny et al. (2001) emphasise the emergence of transient innovations research (so-called *Mode 2*) at the expense of the established university disciplines and their celebrated academic (Mertonian) ideals. In their view, the emergence of Mode 2 research is a logical response to ongoing developments in the economy and technology and the inadequacy of university disciplines to deal with these problems. In their work on *post-normal science*, Funtowicz & Ravetz (1990; 1993) have analysed how the presence of irreducible uncertainty and complexity in environmental and technological policy issues necessitates the development of alternative problem-solving approaches and interfaces between science and policy, in which uncertainty is acknowledged and science is consciously democratised. Finally, in Lyotard's (1984) description of the post-modern condition, many thinkers have found inspiration for the investigation of the colonialist and intolerant aspects of the Enlightenment tradition that imposes its standards and models of science and governance upon all other cultures.

It is not unlikely that there is a certain core of cultural critique common to all of the aforementioned theoretical strands, although we would expect that each of them would produce slightly different insights when deployed on a given topic. This means that although we will not discuss the biosafety issue from the perspective of, for instance, Beck's theory of reflexive modernisation in this chapter, we would like to encourage others to do so as this might stimulate supplementary relevant insights. The point of departure of our analysis, then, is that of *post-normal science*, based on the recognition of complexity and scientific uncertainty. Hence, we will briefly address different types of uncertainty and complexity, which are inherent in the biosafety issue.

In line with Funtowicz & Ravetz (1990), we may distinguish between *technical*, *methodological* and *epistemological* uncertainty. Technical uncertainty is a matter of questions such as 'How many digits are reliable?' while methodological uncertainty is the uncertainty related to the choice of research methodologies and methods. In terms of statistics, it is a matter of significance and confidence. Epistemological uncertainty – *episteme* signifying knowledge in Greek – is referred to by questions such as 'What can be known about this phenomenon?' and 'How do we know that we know?'

To show that there is ample uncertainty in the biosafety issue, little more is needed than a glance at the Cartagena Protocol on Biosafety (CBD 2000). For instance, in Annex III (Risk Assessment), the Protocol states:

> *8. To fulfil its objective, risk assessment entails, as appropriate, the following steps:*
>
> *(a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;*
>
> *(b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;*
>
> *(c) An evaluation of the consequences should these adverse effects be realized;*
>
> *(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;*

In other words, it is necessary to estimate the likelihood, and the consequences, of potential adverse effects of novel genotypic and phenotypic characteristics of GMOs that by themselves are novel and emergent biological constructions on the planet. Imagine an estimate of a likelihood of P = 0.000374 of the possible occurrence of ecologically harmful horizontal gene transfer from a given agricultural GMO. What is the standard deviation of this estimate? By which methods should it be calculated? Controlled laboratory experiments typically yield reproducible and reliable data, but their validity under other conditions may be unclear. Should one demand field trials, and in that case, in what surroundings, monitoring which other species? Is general ecological knowledge on, for instance, biological invasions and natural hybridisation relevant and to be included in the calculation of the estimates (Strand 2001)? The methodological uncertainties are so vast that technical uncertainties may appear irrelevant.

What about epistemological uncertainty in this case? What *can*, in principle, be known about the possible effects of novel and emergent artificial organisms? We cannot answer the latter question anymore than anybody else can. We can, however, show that the answer necessarily depends upon at least two crucial non-scientific factors: metaphysics and politics.

If the adverse effects to be studied are restricted to a small number of species and a short time-frame, it appears more likely that they could be monitored, or even perhaps some day predicted, than if one considers a large number of species and a long time-frame. The same applies if the problem is restricted to direct effects, and second- or higher order indirect effects and feedback cycles are not considered. In other words, how the problem definition determines what can be known and influences the uncertainty at all levels. This is not only a question of the overall number of effects to be taken into account, but also the specific choice of which effects to take into account. For instance, direct effects on production and profit are inherently more easily monitored than effects on, for instance, insect biodiversity.

Furthermore, there is a trade-off between the types of uncertainty. If one accepts a high level of technical uncertainty, allowing ‘fuzzy’, imprecise, qualitative, and anecdotal information, there is much more evidence available, which presumably would decrease the epistemological uncertainty (Marris et al. 2001). Often, however, such evidence is discarded as ‘unscientific’ because it is not cast in a precise quantitative form. In summary, there are a number of choices and decisions to be made on the framing of the problem affecting the research to be performed, which are not purely scientific (although the decisions often are made by scientists).
Metaphysics (or better, natural philosophy) also enters into the picture as the biosafety issue always requires an extrapolation from the known to the novel and emergent organism or novel

deployment and use. The philosophical question to be addressed is: 'What about potential surprises?' Some scientists, decision makers and citizens have a propensity for *complexity*, and tend to think that Nature has a large capacity for surprises. Others tend to think that science knows more or less all that is worth knowing about Nature's behaviour, and that surprises are unlikely or manageable. Both sides have some evidence to show in support of their beliefs. The latter refers to a large series of scientific successes, both in the theoretical and applied realms. The former similarly points to a large series of surprises and failures to control the surprises, as well as the development of chaos theory, complexity theory and other fields of research that show the limitations of linear models of Nature. We call this a metaphysical question because neither position is evidence-based today, and because we believe natural philosophy or worldviews play an important role in individuals' formation of beliefs (Strand 2002).

These philosophical subtleties about complexity are not irrelevant to the policy dimension, because from the perspective of complexity theory, uncertainty may be an essential and irreducible characteristic of systems and problems. In such cases, the rational option may be to increase efforts to *cope* with the residual uncertainty rather than wasting resources on uncertainty eradication.

3. The Evolving Relations between Science and Policy

What is the role of science in the governance of biosafety? And, more generally, what should be the relationship between science and policy?

First, we should clarify that there are two entirely different types of relationships between science and policy. The one hitherto discussed is that of science as *informing* policy. However, science is also the *object* of policy, in the sense that a number of policy decisions regulate scientific practice, above all in the life sciences and biotechnology. Likewise, it may be seen that the science that informs policy may successfully or unsuccessfully try to eliminate or reduce uncertainty, but at the same time scientific and technological practices are among the main world uncertainty *producers*, introducing novel and emergent technologies, organisms and forms of life. It is exactly this potential for innovation that currently enjoys the focus of attention in the research policies of many countries. With no more physical land on the planet to colonise, science (together with outer space) provides the 'endless frontier' to be conquered and capitalised upon (Bush 1945; Rees 2003).

On the other hand, the potential for unexpected surprising and possibly negative collateral effects is becoming increasingly acknowledged in the context of second modernity. The challenge, however, is that our societies have not developed the institutions required to handle the situation. Indeed, it appears that the main responses to production of uncertainty are those of 'ethical regulations' in the case of the medical life sciences and 'risk assessment/management' in the case of the science-based technologies, while the underlying assumption of the general desirability of accelerating research and innovation rates is left unchallenged.

In what follows, we will concentrate on the science that informs policy. However, the two distinct types of relationship between science and policy cannot be entirely separated. Sociologically, there may be connections or even overlap between the experts who inform and the scientists whose interests are affected by the policy decisions (De Marchi 2003). Epistemologically, there are definitely connections, in the sense that the practices to be regulated are based on a body of knowledge that also plays an important role in the policy advice. In more concrete words, in biosafety judgements, biotechnology expertise has often been given the central place, as opposed to, for instance ecology or sociology. We will return to this point later in this chapter.

As for the policy-informing function, we argued above that there are ample sources of uncertainty and complexity in biosafety issues. Alvin Weinberg (1972) coined the term 'trans-scientific' for 'questions which can be asked of science and yet *which cannot be answered by science*' (p. 209, original italics). Weinberg offered the example of the health risks of low-dose radiation, but he also discussed the general problem of weighing the benefits and risks of new technologies, decades before the debates on cloning, human embryonic stem cells, nanotechnology, and climate change arose.

It appears to us that Annex III of the Cartagena Protocol (as cited previously) is full of Weinberg-type of questions, and that biosafety issues on the whole might belong to the domain of trans-science. The problem is what to do about it. The *solutions* have been captured into five ideal types, or models, by Funtowicz (2006). We will present and briefly discuss them with regard to biosafety in the following.

3.1 The Modern Model of Legitimation

This model was already presented in the Introduction: science determines policy by producing objective, valid and reliable knowledge. Accordingly, to develop a policy is a matter of becoming informed by science and then, in a second step, sorting out values and preferences in order to formulate the correct and rational policy.

The idea of legitimation is central to this model. It is not a recipe for the articulation of policies; it is far too idealised for that. The key idea is that of a mutual legitimation. Governance and the foundation of the modern state are legitimised by the privileged status of scientific rationality. The modern European state also gradually adopted and supported the emerging scientific institutions to the extent that they achieved a hegemonic position as the official knowledge producers. The institutions of modern science and the modern state have co-evolved, justified and supported by the entire modern philosophical tradition since Descartes and Hobbes. Popper perhaps gave it its definitive form: science is the only guarantee of the open democratic society, and vice versa. According to Latour (1993), what happens is an ingenious mental division of labour. On the one hand, science is given the right to define (non-human) Nature and tell the truth about it, while staying clear of values and subjectivity. Politics, on the other hand, is given exclusive right to deal with values in society, but must leave questions of facts and truth to science. The achievement of making the citizens of modern societies think along these lines is the result of the philosophical endeavour of which the modern model is part, an effort that Latour calls the 'work of purification'. In Latour's view, the irony of modernity is that this mental work of purification is accompanied by a massive work of mediation between Nature and society through science: more and more connections among natural and human-made phenomena are established. Life technologies are changing the human condition and human activity is changing Nature (and perhaps has already irreversibly changed the climate). From the Latourian perspective, this irony is not accidental. It is exactly because modern societies have been led to think that nature and society/politics are completely separate realms, that they have accepted and endorsed the accelerating technological development.

This is not the place to discuss all the important features of the modern model. We hope to have shown, however, that a lot more has been at stake in defending this model than just the need to formulate an efficient policy-making strategy. The modern model has played a crucial part in the legitimation and consolidation of science, governance and political institutions in modern societies. It has also worked at a deeper cultural level in the modern state, securing the belief in the Enlightenment, progress and the superiority of the secular, Western scientific-economic rationality expressed quantitatively. On an anecdotal and biographical level, we have often

experienced that interlocutors will defend the modern model wholeheartedly and not just for pragmatic reasons. For some, it appears to be also a matter of identity and hope.

The problem arises then, (i) when complexities abound, (ii) when uncertainties cannot be reduced to probabilistic risks, and (iii) when experts disagree, are seen to be stakeholders themselves or simply do not know. The following three models can be seen as attempts to fix these anomalies (Kuhn 1962), to adjust and rescue the modern model from the challenges of uncertainty, indeterminacy and conflict of interest.

3.2 The Precautionary Model: Rescuing the Modern Model from Technical and Methodological Uncertainty

In real policy processes, it is quickly apparent that the scientific facts are neither fully certain in themselves, nor conclusive for policy. Progress cannot be assumed to be automatic. Attempts at control over social processes, economic systems, and the environment can fail, leading sometimes to pathological situations. During recent decades, the presence of uncertainty has become gradually acknowledged, in particular with regard to environmental issues. Because of the incompleteness in the science, an extra element in policy decisions is proposed, namely precaution, which otherwise both protects and legitimises decisions within the modern model. The second model to be presented here introduces the precautionary principle or approach into the modern model, in particular in the way it is being used in the European context. Precautionary 'principles' and 'approaches' have been introduced into a number of conventions, regulations and laws, notably the Rio Declaration on Environment and Development (UNEP 1992), the Cartagena Protocol on Biosafety and the 2001/18/EC Directive on the release of GMOs (see Chapters 29 and 30).

The exact description of the precautionary principles and approaches vary. However, the 'double negative' formulation of the Rio Declaration is illuminating and typical:

> *Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.* (Principle 15)

In the Communication of the European Commission (EU 2000: 1) on the precautionary principle, reference to scientific uncertainty is made, but it is emphasised that the precautionary principle is 'particularly relevant to the management of risk', and that

> *[t]he precautionary principle, which is essentially used by decision-makers in the management of risk, should not be confused with the element of caution that scientists apply in their assessment of scientific data.*

In the same communication, the Commission emphasises how arbitrary claims of precautionary measures cannot be supported by the precautionary principle. It is only to be invoked where a scientific evaluation concludes with evidence of risk, and only where precautionary measures are consistent with the principle of proportionality (between costs and benefits). This has prompted some critics to argue that the precautionary principle, in this and other similar formulations, is no more than an extended cost-benefit analysis.

Various episodes in the short history of biosafety illustrate the limitations of the precautionary model in the management of uncertainty. In the controversies surrounding Pusztai's studies on GM potatoes, and later, Quist & Chapela's (2001) studies on maize, much of the discussion centred on the scientific status of their claims. In the 1989–1999 controversy on the alleged harm to monarch larvae by transgenic pollen (Losey et al. 1999), the EU Scientific Committee on

Plants likewise maintained that there was 'no evidence to indicate that the [product] is likely to cause adverse effects' (see for instance Scientific Committee on Plants 1999).

The normative principle of precaution is accordingly framed and expressed in terms of quantitative science. One may ask about the difference in practice between the precautionary model and the modern model, given that scientific evidence is never 'certain'. The answer appears to be that there are situations where the scientific community largely believes in the existence of a certain harm or risk although the scientific evidence is not yet conclusive according to normal scientific standards. In other words, concrete and specific evidence of harm exists, but the technical and methodological uncertainty is slightly larger than what the standard conventions of scientific journals allow (usually 95% confidence in the case of statistical uncertainty[2]) (see also Gigerenzer 2004) (see also Chapter 17). Epistemological uncertainty, of the type 'we do not know what kind of surprises this technology could lead to', would be rendered unscientific and unsuitable by the precautionary model. This limitation is so severe that a complete reformulation of the principle is needed in order to accommodate epistemological uncertainty. In our view, it would have to be decoupled from science and from the future: a 'real' precautionary principle would not be contingent upon what will happen in the future, because this cannot be known. It would have to be framed by what is at stake today.

3.3. The Framing Model: Rescuing the Modern Model from Indeterminacy

We have discussed so far how a number of framing decisions may affect in a crucial way the outcome of scientific advice, as well as the resulting policy. With reference to biosafety, framing decisions include choice of types of effects, arrays of safety measures, species, scope of time and place, expert communities, and even scientific disciplines to consult. The virtually endless multitude of alternative framings is related to Wynne's (1992) concept of indeterminacy. There are no simple algorithms to resolve all these issues. Hence the framing of the relevant scientific problem to be investigated, even the choice of the scientific discipline to which it belongs becomes a prior policy decision. It can therefore become part of the debate among stakeholders. Different scientific disciplines themselves become competing stakeholders; whoever *owns* the research problem will make the greatest contribution and will enjoy the greatest benefits. Institutions are well aware of the problem of indeterminacy and of potential disagreement among expert communities. In an attempt to establish guidelines for the use of experts (COM 2002:713 p. 2), the European Commission states:

> *The Commission might be confronted by a panoply of conflicting expert opinions, coming variously from within the academic world, from those with practical knowledge, and from those with direct stakes in the policy issue. These opinions may be based on quite different starting assumptions, and quite different objectives. ... Increasingly, then, the interplay between policy-makers, experts, interested parties and the public at large is a crucial part of policy-making, and attention has to be focused not just on policy outcome but also on the process followed.*

The various attempts at accommodating the modern model to this challenge can be summarised in a framing model. The aforementioned guidelines primarily foresee an enlightened debate within the administration about how to frame the issue and choose the experts; other developments under the keyword of *governance* also envision participation by citizens and stakeholders in the framing process prior to scientific investigation – so-called upstream engagement.

[2]It should be kept in mind that the 95% threshold is due to convention and a result of history. Ronald A. Fisher, the leading statistician in the development of statistical tests and the concept of significance, wrote: 'It is open to the experimenter to be more or less exacting in respect of the smallness of the probability he would require before he would be willing to admit that his observations have demonstrated a positive result. ... It is usual and convenient for experimenters to take 5 per cent as a standard level of significance' (Fisher 1951: 13).

However, an incorrect framing of the problem (e.g. due to error, ignorance, poor judgement, and not necessarily wilful) amounts to a misuse of the tool of scientific investigation. Yet because there is no conclusive scientific basis for the choice of framework, it has to be admitted that, to some extent, the choice is arbitrary (or social), and certainly not a matter of 'objective science'. Acceptance of the principle of framing entails an acceptance of some degree of arbitrariness of choice (ambiguity), hence of the possible misuse of science in the policy context and, moreover, of the difficulty of deciding whether or not a misuse has occurred. Indeed, the judgement will itself be influenced by framing.

The framing model is interesting for several reasons. It can be seen as an attempt to acknowledge and somewhat redistribute the power balance between experts and lay people: the non-scientific framing exercise that scientists often implicitly (and unselfconsciously) perform, is taken away from them and democratised, at least at a superficial macro level. The framing constraints built into the methodological details of the scientific investigation, as well as the appropriation of knowledge by science, are not addressed. One could probably instruct experts to include harm to monarch larvae in their list of relevant biosafety issues, but the problem would still be under-specified. In order to know of and to specify all the crucially important criteria for quality of evidence to avoid any indeterminacy, non-experts would have to be experts and could just as well do the research themselves.

The framing model had precursors in the 20th century political culture: above all, certain Marxist and feminist intellectual traditions that had an ideological understanding of the framing issue and the existence of diverse perspectives. Their preferred solution was standpoint theory, that is, that political class, gender or other markers of political starting points should be the selection criteria. This is not without relevance in the biosafety issue; indeed, in many debates it is observed that experts or studies are discredited because they are identified with multinational corporations, countries or NGOs. Such framing claims are quite different to allegations of corruption or scientific fraud. Ideas of politically progressive, 'red' or 'green' counter-expertise belong to this intellectual tradition.

The aforementioned European Commission guidelines (COM 2002:713, p. 9) resolve the issue of indeterminacy in the framing by calling for a plurality of perspectives:

> *The final determinant of quality is pluralism. Wherever possible, a diversity of viewpoints should be assembled. This diversity may result from differences in scientific approach, different types of expertise, different institutional affiliations, or contrasting opinions over the fundamental assumptions underlying the issue.*
>
> *Depending on the issue and the stage in the policy cycle, pluralism also entails taking account of multi-disciplinary and multi-sectoral expertise, minority and non-conformist views. Other factors may also be important, such as geographical, cultural and gender perspectives.*

This might work only if the framing problem is one of bias and tunnel vision of each type of expertise: pluralism may then result in robustness, cancelling out the particular biases, hence approaching inter-subjective knowledge. Unfortunately, the framing problem cuts deeper – it is a matter of necessary choices, not of unnecessary biases. This cannot be accommodated by the framing model because it retains the ideal of certain scientific knowledge at its base.

3.4 The Demarcation Model: Rescuing the Modern Model from Conflict of Interest

The last adjustment of the modern model to be considered in this chapter is the demarcation model. This model resembles the framing model in the acknowledgement of expert disagreement and bias. However, both diagnosis and prescription are different. Where the framing model sees the need to specify better the values to be included in the experts system, the demarcation model is more concerned with supervising the values in action in the process of creating scientific advice:

> *The scientific information and advice used in the policy process is created by people working in institutions with their own agendas. Experience shows that this context can affect the contents of what is offered, through the selection and shaping of data and conclusions. Although they are expressed in scientific terms, the information and advice cannot be guaranteed to be objective and neutral. Moreover, science practitioners and their funders have their own interests and values. In this view, science can (and probably will) be abused when used as evidence in the policy process. As a response to this problem, a clear demarcation between the institutions (and individuals) who provide the science, and those where it is used, is advocated as a means of protecting science from the 'political interference' that would threaten its integrity. This demarcation is meant to ensure that political accountability rests with policy makers and is not shifted, inappropriately, to the scientists.* (Funtowicz 2006)

An example of the demarcation model is the desire for a clean division between risk assessment and risk management. Another is the attempt to establish 'independent' studies or research groups, and perhaps also the insistence on 'sound science', both of them keywords in the GMO controversies.

The main problem of the demarcation model is that it is no longer functional except in clear-cut cases of corruption. Post-empiricist philosophy of science showed that, in general, a total separation between facts and values is impossible, precisely because of emerging systems properties such as complexity and indeterminacy. Concretely, when the situation is highly polarised and conflict is apparent, it is extremely difficult to have a watertight separation between risk assessment and management. How do we decide (and who decides) in practice which is an input of fact and which is an input of value? Stakeholders may be experts (farmers and fishermen, for instance), and experts may be stakeholders (entrepreneurial science). This does not imply that expert are generally misled, corrupt or notoriously subjective, only that the ideal of isolated scientists having access to 'God's eye view' is unrealistic, and probably undesirable.

4. The Model of Extended Participation: Working Deliberatively within Imperfections

The alternative models described in this chapter can be considered as a progression from the initial modern model with its assumption of the perfect effectiveness of science in the policy process. Concerning the precautionary, framing and demarcation models, the imperfections can be seen to form a sequence of increasing severity, admitting incompleteness, misuse and abuse. There is still the desire, in each case, that the link between science and policy remain direct and unmediated. Respectively, the three models address the challenges of uncertainty and complexity by enabling precaution to modify policy, by including stakeholders in the framing of decision problems, and by protecting scientists from political interference. However, the core activity of the modern model, the experts' (*desire for*) truth speaking to the politicians' (*need for*) power is left unquestioned and unchanged. In what follows, we will question the legitimacy of this core activity, and sketch an alternative model of policy that arises from that questioning. We call this the *model of extended participation*.

The underlying ideas of the model are those previously developed by Funtowicz & Ravetz (1993) in their writings on post-normal science. When a policy issue is complex, decision stakes are high and facts are uncertain and/or in dispute, scientists may still endeavour to achieve the truth, but the many *truths* of the systems to be decided upon are simply unknown and, in any case, not available at the timescale of the decision. This does not imply that scientific knowledge is irrelevant; it does mean, though, that truth is never a substantial aspect of the issue:

> *To be sure, good scientific work has a product, which should be intended by its makers to correspond to Nature as closely as possible, and also to be public knowledge. But the working judgements on the product are of its quality, and not of its logical truth.* (Funtowicz & Ravetz 1990: 30)

To some extent, and in some cases, one might be justified to simplify the matters by dividing the task of quality assurance into an internal and an external component. The internal component would then correspond to the peer review system of academic science in which fellow scientists examine to what extent the scientific work has been conducted according to the methodological standards of the discipline. The external component would correspond to an assessment of the policy relevance of the advice. In sum, the issue of quality assurance would then have been divided into *facts* and *values* components. However, as discussed (when explaining the shortcomings of the framing and demarcation models), such a simplification would often be unjustified. Epistemologically, such a division renders invisible the relevance of political values for the myriad of methodological choices in the scientific work (the value-laden quality of facts), as well as the relevance of scientific information for the governance processes leading to the settling of relevance criteria. Sociologically, the simplification presupposes a clear division between disinterested and always self-critical scientists within a Mertonian academy and the lay public who by implicit contrast cannot be granted critical abilities.

We do not think that any of these assumptions holds in the general case. Curiosity-driven, economically-disinterested research is becoming the exception rather than the rule in ever more research fields. The mere expansion of the research world has led to worries about the quality of its own internal institutions for quality assurance, i.e. the peer review systems. On the other side, the knowledge and the critical capacities of the 'lay public' is becoming recognised as the ideology of scientism is giving way. Furthermore, with the development of Information and Communication Technologies (ICTs), access to technical information is increasingly hard to monopolise (in spite of the attempts of a corporate research world to close its open society into one of capitalising upon intellectual property).

The logical implication of this state of affairs is to extend the peer review community and let everybody contribute to the quality assurance process: allow the stakeholders to scrutinise methodologies and scientists to express their values. Hence, the vision drawn by the model of extended participation is one of democratisation, not just for reasons of democracy, but also with the aim of improving quality assurance. In this model, citizens are envisioned as both critics and creators in the knowledge production process. Their contribution is not to be patronised by using, in a pejorative way, labels such as local, practical, ethical, or spiritual knowledge. A plurality of co-ordinated legitimate perspectives (each with their own value-commitments and framings) is accepted. The strength and relevance of scientific evidence is amenable to assessment by citizens.

5. Conclusions

Quality assurance can thus be seen as a core commitment of post-normal science. Defined in terms of uncertainties and decision-stakes, quality assurance encompasses public interest, citizen, and vernacular sciences. In a period of domination by globalised corporate science, this effort to

make scientists accountable to interested groups presents a coherent conceptual alternative for the survival of the public knowledge tradition of science. Collegial peer review is thereby transformed into review by an 'extended peer community'.

There are now many initiatives for involving wider circles of people in decision making and implementation on policy (environmental, health, etc.) issues. For these new types of policy-relevant problems, the maintenance of scientific quality depends on open dialogue between all those affected. This we call an extended peer community, consisting not merely of persons with some form or other of institutional accreditation, but rather of all those with a desire to participate in the resolution of the issue. Since this context of science is one involving policy, we might see this extension of peer communities as analogous to earlier extensions of the franchise in other fields, such as women's suffrage and trade union rights.

Hence, extended peer communities are already being created, either when the authorities cannot see a way forward, or when they know that without a broad base of consensus, no policy can succeed. They are called citizens' juries, focus groups, consensus conferences, or any one of a great variety of other names; and their forms and powers are correspondingly varied (see Chapter 34 for models of participation). Their common feature, however, is that they assess the quality of policy proposals, including a scientific element, on the basis of the science they master combined with their knowledge of the ways of the world. Further, their verdicts all have some degree of moral force and are, as such, a contribution to governance.

These extended peer communities will not necessarily be passive recipients of the materials provided by experts. They will also possess, or create, their own extended facts. These may include craft wisdom and community knowledge of places and their histories, as well as anecdotal evidence, neighbourhood surveys, investigative journalism, and leaked documents. Such extended peer communities have achieved enormous new scope and power through the Internet. Activists in large cities or rainforests can use their weblogs to participate in mutual education and coordinated activity, providing themselves with the means of engagement with global vested interests that are on less unequal terms than previously.

The existence of extended peer communities and what is often called 'broader approaches to governance' is today uncontroversial in many parts of the world, while their justification still remains controversial. We will briefly address the practical and theoretical aspects of their justification. The practical aspect can be summarised as follows: if the function of extended peer communities is that of quality assurance, what will be the source and commitment to quality in order to replace the collegiate mutual trust of traditional research science?

The answer to this question could start with an analogy. There are many negotiations in the worlds of policy and business that work well enough to keep the system going. The operative ethical principle is called 'negotiation in good faith'. This concept is well established in many proceedings worldwide. It is sufficiently clear in practice that legal sanctions can be applied when one side fails to respect it. There is no reason to assume that technically trained experts are better equipped to practice this than are citizens. With such a regulative concept, there is no reason why dialogues in post-normal science situations should be lacking in the means to assure quality.
The theoretical aspect of justification is the question of legitimacy of the model of extended participation. By what argument do we claim that a de-differentiation of modern societies is legitimate, inviting citizens into the co-production of knowledge, and experts into the co-production of politics? As should be clear from the entire discussion of this chapter, the argument is based in a critique of modernity. Rather than beginning with the legitimacy of the extended peer community, we observe that the legitimacy of the modern model, with its strong

demarcations and dichotomies between facts and values, and science and politics, is *dependent upon the intellectual work of purification* (Latour 1993). The work of purification, however, can only be legitimised metaphysically or by recourse to its pragmatic successes. In a world in which there is no monopoly on worldviews and the problems of second modernity are ever more evident with respect to natural resources and the environment, the unconditioned legitimacy of the work of purification evaporates. What we are left with, is the world, inhabited and owned by everybody. Accordingly, the model of extended participation provides justification in the absence of forceful arguments in favour of exclusion. The type of justification is different, however, from that of the modern model. Leaving the modern model behind, legitimacy is no longer ensured by a technical argument proving the optimality of an algorithmic model of policy making.[3]
Finally, and returning to the issue of biosafety, it is not for us to specify the possible value of the model of extended participation. That extended participation takes place, is evident. In Northern Europe, this may take the form of consensus conferences and technology *fora* organised by the authorities, while in other countries NGOs and popular movements often play a more predominant role.

It is contrary to the idea of extended participation that we try to specify the legitimate domains of interest of such processes. In particular, we think that one ought not to abstain from what could be seen as a *politicisation* of the discourses and governance processes; indeed, the issue of biosafety is politicised as a matter of fact. Rather, it appears that the technical discourses of risks (and in some cases, the emerging technical discourse of bioethics) act so as to conceal the political nature of the issues. Indeed, one might foresee that broader governance with an extended participation might be able to increase the scope of vision of the issues related to biotechnology, asking not only 'Is it safe?', 'What are the known risks?' or 'Is it contrary to ethical principles?' within a capitalist logic of added value from innovation, but also 'Is it desirable?', 'What do we not know?' and 'What kind of future do we want?'

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[3]Obviously, there are circumstances in which there are valid arguments to support marked differentiation of expertise but to extrapolate and rely only on that knowledge uncritically is unwise (Wynne 1992; Lash et al. 1996).

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Chapter 17

The role of precautionary motivated science in addressing scientific uncertainties related to GMOs

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1. Introduction

Implementing a precautionary approach (as for instance specified in Article 1 of the Cartagena Protocol on Biosafety) might require a renewed look at the science underpinning risk assessment and management of GE and GMO release. Hence, in this chapter I will argue that the implementation of the Precautionary Principle may have implications on scientific practice. For instance, implementation of the Precautionary Principle requires that indications of adverse impacts are being documented in some way, and that risk-associated research is initiated. Such precautionary motivated research might initiate debates concerning the quality of risk-related scientific advice. Furthermore, it may identify areas where scientific understanding is lacking by investigating various models of risk and initiate basic research that concedes or rules out risks of ecological harm. Precautionary motivated science needs to be built on a basic research agenda; it involves broadening the scientific focus, reflexivity and allows for interdisciplinary approaches.

2. Scientific uncertainty with GE use and GMO release

Several reports have been written on the science-based concerns related to the use and release of GMOs (see for instance ESA 2004; NRC 2004; The Expert Panel of the Royal Society of Canada 2001, and in this book see especially Chapters 8–15). The uncertainties described in these reports can be placed in two categories: scientific uncertainty due to (a) lack of scientific understanding, and (b) scientific dissent.

2.1. Lack of scientific understanding with GE use and GMO release

Lack of scientific understanding with regard to the proposed benefits and the potential adverse effects of GE use and GMO release may be due to (see Chapters 4, 8 and 9):

- The new properties that are introduced by genetic modification of a plant, animal or micoorganism.
- Secondary effects of the introduction of the transgenes.

The lack of scientific understanding may be due to the novelty of GE and GMOs, in which case scientific uncertainty may be reduced by conducting more risk-associated research and by collecting more empirical data. In other cases, the lack of scientific understanding may be due to the variability or complexity inherent in the system under consideration. This form of lack of knowledge may be *irreducible* since it originates in the inherent randomness of ecological systems.

There is a need for more comprehensive studies of ecological effects of GMO utilisation, for instance with regard to potential secondary effects of GMO release on environmental processes and adverse effects on human and animal welfare. Experimental testing of carefully elaborated risk hypotheses may result in a solid basis for the avoidance of potentially harmful GMOs (see Chapters 8–15). A more holistic approach to GMO risk issues involves appreciation of uncertainty and implies assessment of time and complexity of ecological aspects. However, the

initiation of such risk-associated research involves some challenges to the scientific work since it questions the traditional conduct of science, i.e. with regard to reliance on methods, and the choice of hypothesis. These issues will be further elaborated in the remaining part of this chapter.

2.1.1 Reliance on models and methods

Models are often used in scientific research with the purpose of corroborating a hypothesis, by offering evidence to strengthen what may be already partly established through other means. Models can also be used to elucidate discrepancies in other models or for sensitivity analyses – for exploring 'what if' questions – thereby illuminating which aspects of the system are most in need of further study, and where more empirical data are most needed. Thus, the primary value of models is heuristic; models are representations, useful for guiding further study, but they are not subject to proof.

There is at present uncertainty with regard to the choice of methods and models to investigate the consequences of GMO use and release. This scientific uncertainty results from not fully understanding interactions among variables and the relevance of models used to predict the behaviour of multivariable systems. For instance, the potential for gene flow to the agricultural and natural environment is a new concern for regulators and scientists. This concern includes both a) economic and legal concern with regard to how to ensure coexistence, and b) environmental concern with regard to potential adverse effects on biodiversity.

When controls on field trials have included monitoring of horizontal gene transfer (HGT), the frequency has often been considered to have a low impact or to be insignificant. However, in two recent papers (Heinemann & Traavik 2004; Nielsen & Townsend 2004) it is argued that current techniques for sampling and monitoring of HGT from GM plants to soil microorganisms are too insensitive and that rigorous monitoring may be the only realistic way to detect HGT. Further, they highlight that the frequency of HGT is probably marginally important compared to the selective forces acting on the outcome. The two papers agree that new methods are needed to study HGT. However, while Heinemann and Traavik suggest a new method for studying HGT that is based on detecting iterative short-patch events, Nielsen and Townsend suggest a population-based approach. The fact that the two research groups suggest two different methods for solving the same problem is interesting. When conducting their research, scientists make assumptions and inferences based on the paradigms they are trained under, which in turn influence the scope and choices of methods and models to increase their scientific understanding. Furthermore, differences in training and other forms for socialisation may also have impacts on the choice of hypothesis and the threshold for significance of evidence.

2.1.2 Hypothesis testing: Type-I errors versus Type-II errors

In the practice of statistical testing, researchers often formulate a null hypothesis (H_0). The H_0 is usually stated in terms of 'no adverse effect'. If the outcome of a statistical test warrants the rejection of the H_0, the scientist will normally accept the alternative hypothesis H_1 – that there is an adverse effect. Hypothesis testing operates on the basis of limiting Type-I errors (which erroneously predict an adverse effect when there is in fact none), to ensure that the observed result supports the H_0. Hence, Type-I errors occur when one rejects a true H_0. In contrast, a Type-II error is made by not rejecting a false H_0, i.e. there is an ecologically adverse effect, and the H_0 is wrong (Table 17.1).

Table 17.1. Type-I errors and Type-II errors in ecological studies. Null hypothesis H_0 = There are no adverse effects.

Reality Test results	H_0 is true	H_0 is false
The investigation does not show adverse effects	Correct (1-a)	Type-II error False negative (ß)
The investigation shows adverse effects	Type-I error False positive (a)	Correct Statistical power (1-ß)

According to the traditional scientific norm, one ought to have complete and supportive information before claiming a cause-effect relationship. Consequently, the statistical significance of the result must be strong enough to allow only a small probability (p) that the result is due to chance or has been based on speculation. By convention, in a Type-I setting the probability of this error being made is determined by the significance level of α – often at 5%. Hence, if there is less than 95% confidence that there is an effect (1 in 20), the H_0 is not rejected. In such situations, scientists are prone to assume that the evidence is not strong enough to reject the H_0.
The conservative scientific demand of statistical significance before rejection of the H_0 is adequate if the statistical power is high. Statistical power, (Sp)=1-β (the risk of Type-II error), refers to the probability of correctly rejecting H_0, i.e. statistically detecting an effect if it exists. The risk of committing a Type-II error increases if the power of the data set decreases; i.e. there is limited scientific understanding and there is a scientific hypothesis of adverse effects. Minimising Type-I errors is necessary and adequate when doing laboratory research, as the parameters and variables are few, the results are in most cases reliably identifiable or quantifiable, and the purpose is to gain new understanding and avoid spurious results.

However, exploratory and monitoring research entails a practice that avoids making Type-I flaws (Lemons et al. 1997). Complex interactions in open systems cannot be adequately predicted; hence achieving complete and supportive information before claiming a cause-effect relationship may not be possible. This means that risks to society, health and the environment may remain obscured, because a bias towards avoiding Type-I errors discourages research into risk-associated aspects. In this context, the power of studies to detect relevant risk becomes important. In general, this is often overlooked, leading to a false sense of security from negative studies that fail to find a risk (Andow 2003). For instance, Lövei & Arpaia (2005) claim that power analysis is rarely considered in laboratory tests on the impact of GM plants on arthropod natural enemies. Hence, they argue that in future studies of non-target effects, power analysis needs to be employed since this may help research planning (for example, giving indications of sample size and duration of project) and contribute to clarifying the interpretation of the results.

2.1.3 Systematization of uncertainty may enhance quality and direct further research
The notion that uncertainty is only a statistical concept or represents insufficient data may leave out many important aspects of uncertainty when performing risk assessments (Giampietro 2003; Wynne, 1992). For instance, uncertainty with regard to GMO release and use can be presented at the level of uncertainty or that of ignorance.

Uncertainty refers to situations where we do not know or cannot estimate the probability of hazard, but the hazards to consider are known. This may be due to the novelty of the activity, or to the variability or complexity involved.
Ignorance represents situations where the kind of hazard to measure is unknown, i.e. completely unexpected hazards may emerge. This has historically been experienced with, for instance, BSE, dioxins and pesticides (EEA 2002). With regard to GMOs, unprecedented and unintended non-target effects may emerge. Non-target effects include the influence on and interactions with all organisms in the environment, and may be direct or indirect. Direct effects concern, for instance, ecotoxic effects on other organisms, while indirect effects concern, for instance, effects on health, contamination of wild gene pools or alterations in ecological relationships.

Employment of model-based decision support, such as the Walker & Harremöes (W&H) framework (Walker et al. 2003), may help to identify the types and levels of the uncertainty involved. The W&H framework has been developed by an international group of scientists with the purpose of providing a state-of-the-art conceptual basis for the systematic evaluation of uncertainty in environmental decision making. One of the main goals of the W&H framework is to stimulate better communication between the various actors in identification of areas for further research and in decision processes. In this framework, uncertainty is recognised at three dimensions:

1. Location (where the uncertainty manifests itself, (e.g. if it is contextual (ecological, technological, economic, social and political), if it is in the expert judgement, or in the models used (model structure, model implementation, data, outputs, etc.))
2. Nature (the degree of variability which can express whether uncertainty primarily stems from inherent system variability/complexity or from lack of knowledge and information)
3. Level (the severity of uncertainty that can be plotted on a gradual scale from ‘certain knowledge’ to ‘complete ignorance’).
4.

For instance, Krayer von Krauss et al. (2004) have demonstrated and tested the W&H framework with the purpose of identifying scientists’ and other stakeholders’ judgement of uncertainty in risk assessment of GM crops. In these studies the focus was on potential adverse effects on agriculture and cultivation processes by release of herbicide resistant oilseed crops. Krayer von Krauss et al. interviewed seven experts in Canada and Denmark. To identify the experts’ view on location uncertainty, the authors presented a diagram showing causal relationships and key parameters to the experts. To identify the level and nature of uncertainty, the experts had to quantify the level and describe the nature of uncertainty on the key parameters in the diagram. By asking the experts to identify the nature of uncertainty, it was possible to distinguish between uncertainty that may be reduced by doing more research and ignorance that stems from systems variability or complexity.

Approaches that define and systematise the uncertainty involved, such as the W&H framework, may help in using scientific knowledge more efficiently, in directing further research and in guiding risk assessment and management processes.

2.1.4 The unpredictability of complex systems

The study of complex systems is about understanding indirect effects and problems that are difficult to solve because the causes and effects are not clearly related (Chu et al. 2003; Gundersen & Holling 2002; Scheffer et al. 2002). Under such circumstances, the normal scientific approach of trying to produce a best estimate or final answers will not be useful, since it may not necessarily reduce uncertainty. This is because uncertainties regarding the behaviour of complex systems have nothing to do with a temporary insufficiency in our knowledge; it has everything to do with objective, structural properties of complex systems. Putting pressure on a

complex system at one place can often have effects in another place because the parts are interdependent. Hence, one needs to be aware that there will always be an inevitable gap between limited experimental conditions and reality, where the consequences of an activity can never be fully predicted. For instance, in observational studies of complex, poorly understood systems, errors in the independent variables, errors arising from choice of the wrong form of the model used to analyse and interpret the data, and biases from the way the study was conducted may arise (Kriebel et al. 2001).

With regard to GE use and GMO release, unanticipated effects may arise due to interaction between the introduced transgenes(s) and the recipient genome, or unanticipated interactions between the GMO and the ecological system. Designing adequate human and environmental models for determination of risks and identification of unpredictable effects are difficult tasks. Present approaches need to be supplemented with methods that study whole systems. This involves a perception that the dynamics of human and environmental systems cannot solely be described by the parts, as genes and proteins, but concern interaction with each part of the system. Some suggestions for how to study whole systems are presented in Chapter 10. In addition, it is crucial that methods for detection and monitoring are initiated with the purpose of following up the performed risk assessment, to map the actual health and environmental effects and to detect unanticipated effects. Long-term monitoring provides baselines against which to compare future changes and gives input data to improve regulation systems (Cranor 2003).

2.2 Scientific dissent with regard to impacts of GE use and GMO release

In a situation of lack of scientific understanding, analogies from well-known areas of research are often invoked. With GMOs the different scientific disciplines that are involved use competing analogies and models for basic assumptions to frame the scope for further research. For instance, agricultural biotechnologists refer to the practice of conventional plant breeding, while ecologists refer to the experiences based on the introduction of exotic species to make up for the lack of anticipatory knowledge. Since the principles and paradigms of the different scientific disciplines differ, they have no common ground to discuss means for gathering new scientific understanding. There are, for instance, divergent opinions among scientists about the relevance of various potential adverse effects, about the definition of potential 'adverse effects', and what action to take to prevent potential harm (Myhr & Traavik 1999; 2003). From this perspective, the demand for 'more research' is not sufficient to reduce scientific uncertainty, since the incapacity of science to provide a unified picture of the environment contributes to the uncertainty.

Sarewitz (2005) argues that scientific dissent in the case of highly complex and difficult to assess risk situations are due to different backgrounds/disciplines. The scientists' backgrounds may affect choice of hypothesis, methods and models, which gives conflicting data and causes disagreements among scientists. What Sarewitz denotes as 'excess of objectivity' refers to the observation that available scientific knowledge can legitimately be interpreted in different ways to yield competing views of the problem and how society should respond. Hence, the challenge is to manage the uncertainties that are characteristic of each field so that information of the highest possible quality can be obtained (Funtowicz & Ravetz 1990).

Reflecting on the role of scientific disunity in the interpretation of scientific uncertainty related to GE use and GMO release, the question arises as to how enhanced dialogue between competing disciplines can contribute to make explicit those values, interests and implicit assumptions that represent the frame for each discipline's approach to scientific uncertainty. For instance, an enhanced dialogue can be facilitated by involvement of a wide base of scientific disciplines as well as independent scientific institutions in the gathering of scientific understanding. Involvement on a wide basis of scientific disciplines will: (1) assist in the exploration of

alternative problem framing and alternative indicators that can be used in risk assessment; (2) function as a source of knowledge, data and information – including information on uncertainties – that may be of relevance for risk assessment; and (3) assist in the evaluation and critical review of assumptions used, method, process, and results. This will ensure diverse consideration of both mainstream and minority opinions, and cause avoidance of abuse of science by scientists biased to a specific agenda. Hence, the different methods and models representing the different disciplines may be seen as compatible providers of information and models for studying the problem or the system. With more diversity in the approach, more data will be generated and more responses will be available to understand complexity and changing conditions.

3. Conclusion

In this chapter I have argued that there is a need to achieve wise management of uncertainties with regard to potential adverse effects of GE use and GMO release. This challenge has to be met by scientific conduct and approaches that aim to manage risk and uncertainty, taking into account the complexity of the ecological systems that the GMOs are to be released into. Broad risk assessments of GMOs should include appreciation of uncertainty and complexity, and involve communication of early indications of harm. In this context, scientists and decision makers should become comfortable with making decisions based on the weight of evidence according to an approach that strives to reduce Type-II errors. A change to a more integrative risk assessment, where the Precautionary Principle has an important role may make science more accountable to public concern.

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Chapter 18

Indigenous Knowledge and Modern Science as Ways of Knowing and Living Nature: The Contexts and Limits of Biosafety Risk Assessment[1]

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> 'Attempts to draw a strict line between scientific and indigenous knowledge on the basis of method, epistemology, context-dependence or content, it is easy to show, are ultimately untenable'. (Agrawal 2002:293)

Introduction

In the context of the Cartagena Protocol on Biosafety to the UN Convention on Biological Diversity (CBD), the interactions between modern scientific cultures and indigenous cultures, and their ways of knowing nature, have become highly relevant. Indeed, as Hayden (2003a; 2003b; also Greene 2004) has noted, a particular strategic construction of indigenous (and scientific) ways of knowing and practising in relation to nature and biodiversity, is built into the CBD. This is the effective assertion, explained later, that 'in order to protect nature, first we have to *exploit* it'.[2] Western science has assumed a dual role in this global environmental, commercial, ethical, and political nexus – both as means of commercial exploitation of indigenous knowledge of biodiversity and (some of) its useful properties, and as medium of protection, through recording of loss, risk assessment, and related regulatory knowledge and action. This is the larger context within which the question of the adequate risk assessment of genetically modified (GM) crops, especially when globally exported into developing countries, has to be considered.

Global indigenous peoples have mobilised at the CBD over concerns about threats to their cultures, livelihoods and identities (see e.g. UNEP 2005; Oldham 2007). After a centuries-long history of direct and often violent exploitation, through land-grabs and many other forms of resource expropriation, more recently these threats have increasingly come from interventions performed in the name of *science* as modernisation – deforestation, disruption and chemical exposure in the name of 'modern' industrial agriculture, even expulsion from traditional cultural habitats in the name of environmental management (Leach & Mearns 1996).[3] These scientifically rationalised interventions have themselves been increasingly performed through genomics-related interventions, for example in the search for commodifiable plant-genetics properties from indigenous knowledge (Hayden, 2003a; 2003b), the analysis of indigenous human DNA for commercially exploitable insights into disease and disease-resistance (Oldham 2007), and the

[1]The singular terms are used for convenience. I do not intend to suggest that either scientific or indigenous knowledge-culture can be described as singular. The misplaced, if understandable, tendency to homogenise both these heterogeneous categories as if they were unitary 'systems' of knowledge or culture, has been critically addressed by, for example, Hobart (1993), though the implication is still suggested in recent works (e.g. Viveiros de Castro 2006), where some fundamental differences between Western modern culture and indigenous cultures are discussed, which inevitably implies an endogenous unity of each even while the author is also well aware of but is suspending other intra-category differences.

[2]As anthropologists have noted, 'nature' here has often included indigenous peoples and cultures, as exotic objects for instrumental study – and potential exploitation.

[3]The conflicting parochial cultural bases of modern scientific and indigenous ways of performing nature and society were well-exemplified in Verran's (2002) participatory study of environmental scientists' and aboriginal landowners' land firing practices in Northeast Arnhem Land, Australia.

attempted development of DNA taxonomies of biodiversity in 'the (global) DNA-barcoding of life'.[4]

The global export of GM agricultural science and technology to areas of the world where indigenous cultures exist has not been seen to impact primarily on these cultures themselves. However, in this chapter I highlight three dimensions of these intersections which are relevant to the question of whether prevailing institutionalised Western models of scientific risk assessment are adequate (Winickoff et al. 2005) for assessing the consequences and implications of modern agricultural biotechnologies in developing countries, where most of the world's biodiversity, and cultural diversity are located. These are:
The way in which scientific knowledge not only *informs* policy processes with relevant validated knowledge, but also frames the recognised *meaning* of the public issues. In other words, it presumptively plays a political role of defining what the salient questions are which need such information, and thus also what is to be ignored as a concern.
The ways in which institutionalised versions of scientific rationality, typically preached as 'sound science' in risk assessment arenas, omit and delete significant kinds of uncertainty and contingency, including ignorance. An intellectually rigorous science would attempt to identify, differentiate and logically address rather than confuse these, even if such response would rationally involve more than changes to scientific advice, but would also require institutional changes. This artificial reduction of uncertainties to only those for which (predictive) control can be claimed or at least promised, has the corresponding ethically provocative consequence of externalising unpredicted consequences onto unknown marginal others, in the future or present. Later, I suggest such institutional changes to risk assessment which are also salient to developed world contexts, and have been proposed to, for example, the European Union (EEA 2002; Wynne & Felt 2007).

This intellectually reductionist property of modern science, defined by its instrumental ethic, connects with a third form of intersection. This is that, contrary to prevailing beliefs,[5] modern scientific knowledge is not at all only observing and representing nature (Hacking 1983; Rheinberger 1997; 1999). It is also, as a function of its institutional and epistemological transformations over the 20th century, *intervening* in nature as it observes and represents it. Thus, scientific observation is always in some degree also manipulation of nature. This has increased as science and technology have industrialised and merged into techno-science, and scientific knowledge production has become the servant of – as imagined – endlessly accelerating global economic innovation, when recently it was seen as 'the independent republic of science' (Polanyi 1962), which supposedly 'speaks truth to power'.

Thus, a central point of this outline comparison of some key features of scientific and indigenous knowledge-cultures is not to romanticise the indigenous as the supposedly innocent counterpart to science's ethically-challenged, 'purpose-disoriented' instrumentalism. The point is to use these contrasts and comparisons to throw into perspective some of the aspects of GM agricultural techno-sciences including their 'sound-scientific' risk assessment which would otherwise go unnoticed and taken for granted, by default.

[4]For example: '[Indigenous Knowledge] is a short-cut to the discovery of new medically or industrially useful compounds' (Farnsworth 1990); 'the exploration of biodiversity [is under way] for commercially valuable genetic resources or materials' (Reid, cited in Moran et al. 2001).
[5]As articulated, for example, by former UK PM Tony Blair, in a speech to the London Royal Society (Blair 2002) 'Science Matters'.

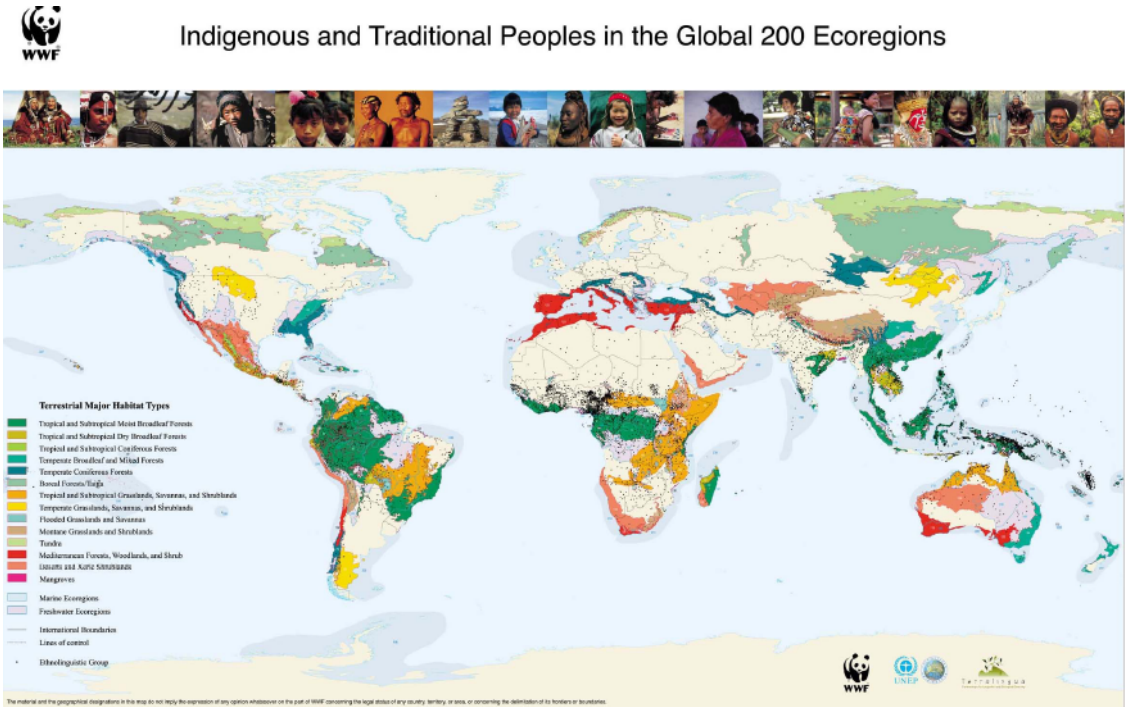

Figure 18.1
The map presented in Fig. 18.1, from WWF-Terralingua, shows the strong global correlation between loci of cultural diversity (measured by numbers of different languages) and biodiversity. To summarise, there are approximately 6000 different spoken languages worldwide, of which 4000–5000 are tribal or indigenous peoples' languages. An estimated 67% of such ethnolinguistic groups are in regions of outstanding international biodiversity and ecological importance (WWF-Terralingua 2000). UNESCO (2002) has stated that 3000 of these living community ethno-linguistic traditions are 'endangered, seriously endangered or dying'. Global biodiversity has also been described as being extinguished before we can even know what we are losing (Royal Society 2004).

Laird and Wynberg describe the global growth of GM or transgenic agricultural biotechnologies as 'escalating at a rate that surpasses that of any new technology ever embraced by the agricultural industry' (UNEP 2005: 19). The global market value of GM seeds and technology fees for 2004 was USD 4.70 billion, and cumulatively since commercialisation began in 1996, USD 24 billion. They have estimated the promised future market value of indigenous biodiversity knowledge for pharmaceuticals alone, leaving aside other imagined products, as USD 60 billion. Although this is promise, thus fiction not fact, it is this promise that drives such fervent global scientific and commercial investment in such knowledge. In this sense it is *material* imagination. Risk assessment alone, however good its scientific basis, cannot be expected to provide more than a part of the proper appraisal of these huge and sprawling global innovation fronts, driven by commercial ambition and founded on a determined prosecution of a particular and contestable vision of global social benefit.

In this chapter I use the comparison of indigenous and scientific cultures to hold up a mirror to scientific risk assessment as it has been institutionalised for GMOs in the EU, US and other developed countries, as well as in international bodies such as the Codex Alimentarius Commission and the World Trade Organization. This allows us to examine how it might need to

be developed – not only technically but also institutionally – from what are these free-market and free-trade priority settings, in order to navigate responsibly in fulfilment of the global, sustainability-founded agro-biodiversity requirements of the Biosafety Protocol and the CBD.

Some shifts in late 20th century science

Environmental risk assessment was introduced only in the 1970s in the US and Europe, and in quite limited ways. Later it began to be institutionalised for attempting to predict likely harm from new chemicals, radioactive or other non-ionising radiation exposures, and similar risky processes and materials. Indeed, the beginning of regulation of proposed deliberate environmental releases of GM plants for agricultural testing in experimental plots, with the 1990/220 Directive in the EU, was the first explicit reference to precautionary thinking (in the preamble, not in the legal articles), in that a step-wise risk assessment was established even though no environmental harm from such GM plants had yet been found.

Complexity and Reduction

This implied claim to a precautionary regulation just because no manifest harm had been seen before conducting risk assessment still begged serious questions about how well-known and under control or not were the biological processes involved as the basis of risk assessment knowledge as well as of the production of the GM constructs. Although the strong claim is that complexity has been embraced by modern science, including risk assessment, many examples show this to be untrue (Mattick & Gagen 2001; Grewal & Moazed 2003; Wynne 2005; Wilson et al. 2006). Here, without any claim to scientific literacy, ordinary people seem intuitively aware of issues which scientific regulatory authorities have neglected; of the limits of scientific control, the relentless generation of unpredicted consequences, and the breakneck speed of attempted translation from fragile cutting-edge genetic research-knowledge of possible technologies, to well-tested market products and profits (Marris et al. 2001). Here it is important to note that scientific claims for the extreme precision of GM technology were false, in that however precise might be the excision of genetic material from the original organism's genome, the insertion of such desired alien fragments into the new host plant's genome is extremely crude and unstable, requiring extensive monitoring, selection and discard. Moreover, the idea that such transgenes offer precise, reliable controlled traits was established before the human genome mapping indicated the falsity of the 'central dogma' of genetics, that one gene reliably creates one protein, which reliably codes for one specific trait. That the many fewer genes (*c.*20,000 found, against the 150,000 or so expected) do not operate so deterministically, because surprisingly there are too few genes for this mechanism to be valid, has major implications for the unstable behaviour, under varying conditions, of the crop and food genetic constructs so created, and thus for environmental and maybe also human or animal health risks. The EC (2005) recognised this problem implicitly in its evidence submitted to the WTO in the dispute with the US, when it affirmed that:

> It is not scientifically reasonable to simply translate and extrapolate the limited risk assessment results on the toxicity of Bt maize to human and non-target organisms from USA, Australia or some other non-European countries because the
>
> regional growing environments;
>
> scales of farm fields;
>
> crop management practices;
>
> local/regional target and non-target species considered most important
>
> in the agri-ecosystem;

> interactions between cultivated crops; and surrounding biodiversity;
>
> could each differ from published non-European studies, and could differ substantially between regions and countries within the EC.

Yet paradoxically, in EU regulatory practice itself, these EU-generated observations are ignored, and standard EU-wide risk assessment is accepted as sound science. The unstated reason is the greater priority given de facto to the EU single market, which to be viable requires a single regulatory risk assessment system. The WTO's Agreement on Sanitary and Phytosanitary Measures (SPS Agreement) risk assessment rules operate in a similar fashion, thus undermining the need in rigorous science for all situation-specific local variations in salient conditions to be recognised (Winickoff et al. 2005).

As indicated, a first basic shift in science since the 1950s is its central and pervasive role as policy authority, not only in answering the questions posed by policy, but also now in defining what those questions should be. The more this role has intensified, the less 'science' has been identifiable with scientific *research*, the more industrialised and instrumental it has become, and as a result, the more intellectually reductionist. Hence, the inability of institutionalised risk assessment to address complexity has been exacerbated, just as complexity and the condition of lack of control (that is, ignorance) which always stalks science have become more important.

Representation as Performance: Technoscience, Innovation and Social Benefits
As science has become more intensely and systematically commercialised, imaginations of its social purpose have increasingly closed around commercial exploitation in existing systems of value. These are rich Western consumer markets, and demand in such systems does not at all correspond with priority human needs in the global systems of investment, techno-scientific research, innovation, production, and exchange which prevail. In a special issue of *Nature Biotechnology* published in October 2004, on systems biology, an article asking 'Can Complexity Be Commercialised?' explained how:

> With a top-down approach which characterises much of today's systems biology, researchers start at the phenotypic or event level of a disease and *drill down* through functional pathways to only what is important in a specific disorder, because that disease phenotype is what they want to change … to speed up drug discovery and development and to make it much more efficient … and to use information from disparate data-sets to create computational models that can describe and predict phenotype at the cell, tissue or organismal level [so as to assist commercial drug-development for] … systems biologists to come up with tangible results to show investors. (Mack 2004)

This is the same systems biology which the UK's basic biology science research council (the BBSRC), describes as 'the science base' – pure science which is supposed to be free of social imaginations as to what might be the applications of science. The protein scientist Hans-Jorg Rheinberger (1997; 1999) has described in the same light how the molecular biological approach is no longer to try to observe and represent what is happening in sub-cellular processes. It is to use these processes within the cell as a technological experimental micro-laboratory, to see what can be made to happen.[6] The same has been true of plant science research, where the social imaginations of GM technological global agriculture have shaped innovation trajectories well before questions of risk arose for assessment.

[6]Of course, these are not mutually exclusive projects. The technological project still generates valid knowledge as a by-product, but the selectivity of this knowledge is systematic, limited by the technological ends which are imagined and invested in it. Thus, it excludes potential knowledge too, and these blindnesses may be just the origins of future unpredicted consequences externalised and deleted from responsibility by the risk culture that pretends to encompass all possible uncertainties with risk scientific knowledge.

The logic of recognising this unnoticed embodiment of unaccountable innovation commitments into science *before* risks and consequences become a question, and the endemic inadequacy of risk assessment to identify all possible consequences is not to shut down all innovation. It is to ask the extra questions that *rigorous* risk assessment requires – is the innovation worth it? What imagined social benefits is it intended to bring? To whom? Are these the most important purposes, and the most effective means?

Here it is worth thinking of the Access and Benefits-Sharing (ABS) issues being negotiated under the CBD. The arrangement is that indigenous original knowledge-holders have their knowledge recognised by giving them a right to a share of the benefits (e.g. profits from a pharmaceutical product sold in rich Western markets), if and whenever these might materialise, perhaps in twenty years. These financial benefits, it is envisaged, will prevent those peoples from destroying their local biodiversity 'goose' which laid such 'golden eggs'. However, as Mack's account makes clear in passing, there is no debate or reflection as to what might constitute an imaginable benefit worth respecting. It is pharmaceuticals, or equivalent high-value goods which will be available to rich Western consumers. Any possible alternatives are simply not entertained as a question, let alone a serious possibility worth assessing. Similarly, this is how risk assessment has arisen in Western regulation, in that if any technological innovation, such as a drug, is promoted by anyone for licensing and thus regulatory risk assessment, it and the profits it may bring to someone are automatically defined as a public good, and thus no debate about benefits to society is even imagined. Under the pressure of indigenous reactions to bioprospecting, and the CBD ABS arrangements, these questions, about what kinds of benefit should be defined and accepted, are now being posed for consideration, by the indigenous networks represented (Oldham 2006). Likewise, interestingly, equivalent benefits questions are now being entertained by European regulatory authorities responsible for risk assessment, as a possible fourth-hurdle regulatory set of questions in addition to risk questions themselves. As explained in the EEA (2002) Precautionary Principle book (see also Wynne & Felt 2007), a rigorously sound scientific approach to scientific uncertainties in risk assessment would address the predicament of unknown as well as known possible risks, and one logical response to this (among several) is to address the question of whether the promised benefits are (a) realistic, (b) socially accessible to all, in principle, and (c) important, for whose social needs? Thus, a fourth-hurdle regulatory question with regard to social benefit and the need to weigh these in with the risk questions, is a logical consequence of rigorous scientific risk assessment.

Given the certainty of such unknowns and thus ignorance in developing countries' risk assessments for GMOs under the Biosafety Protocol, and given the different conditions salient to social benefits appraisal, such a social benefits question is rational for developing countries too.

Risk Assessment and Falsehoods of Control: Risk as a Relational Issue

Returning to indigenous knowledge, anthropologists (e.g. Richards 1993; van der Ploeg 1994; Graeber 2001; Ingold 2003) have shown how social-relational concerns and commitments are built silently into reasoning and valuation processes in such cultures. They are not so exposed to deliberate instrumental forces as they are in scientific cultures, and knowledge is embedded more into such social practices and relations. With Western scientific risk assessment, these relational dimensions are simply buried by the scientific framing. Once one recognises that risk as *known* possible consequences *always* carries further questions about unpredicted and *unknown* consequences, the relational issues stand out starkly. If there will be unpredicted as well as predicted consequences, it is necessary to ask who will be in charge of the social responses to such surprises – and can we trust them to react responsibly, in the public interest? Publics understand that they depend on such institutions, unavoidably; thus, this relational trust question

follows unerringly from appreciation of the predicament of ignorance which unavoidably attends scientific knowledge – and which becomes more significant the more ambitious science's interventions, and claims.

This relational question is intrinsic to risk; it is not an optional extra. Yet it is buried by the way science has been institutionalised in risk assessment and regulation and policy, and how it has thus been shaped intellectually. It has been recognised only belatedly that past interventions since colonial times into indigenous peoples' ways of life and environments, have been founded on the false premise that their culture was irrational and intellectually vacuous – similar in key respects to the same kinds of false patronisation of developed-world publics (Irwin & Wynne 1996; Wynne 2006). That both indigenous peoples and Western publics in their different ways seem to recognise complexities beyond the imagination of instrumental science (which is characterized by its commitment to the control, reduction and externalisation of unknowns) is a deep cultural and ethical difference which science has yet to acknowledge. The lack of expectation of control practised by both indigenous cultures and typical Western publics allows for these complexities to be sensed, recognised and adapted to through ad hoc improvisations, even in the absence of highly-elaborated instrumental knowledge.

In indigenous cultures anthropologists have described the various skills for handling these kinds of unknowns and insecurities, in belief systems which are more rooted in living relationships and dwelling practices than typical mainstream Western culture, and where public knowledge is required to be impersonal and objective. One important perspective on this is given by Ingold (2003), who distinguishes between engagement and living in the world, and detachment and alienation from it, as in Western notions of 'the global environment':

> To the extent that it has been used to legitimate the disempowerment of local people in the management of their environments, this ['global environment' discourse] – the privileging of the global ontology of detachment over the local ontology of engagement idea has had serious practical consequences for those amongst whom anthropologists have conducted their studies. To adopt a distinction from Niklas Luhmann, it might be argued that the dominance of the global perspective marks the triumph of technology over cosmology. Traditional cosmology places the person at the centre of an ordered universe of meaningful relations .. and enjoins an understanding of these relations as a foundation for proper conduct towards the environment. Modern technology, by contrast, places human society and its interests outside what is residually construed as 'the physical world', and furnishes the means for the former's control over the latter. Cosmology provides the guiding principles for human action *within* the world, technology provides the principles for human action *upon* it. … It is a movement from revelation to control, and from partial knowledge to the calculated risk. (Ingold 2003: 216)

We should also notice how this shift to 'the calculated risk' also involves an implicit projection of an exaggerated degree of control, and a tacit externalising of any lack of control onto others, thus a denial of responsibility for unknown consequences, even ones which may have been engendered by the same modern practices. The extant unresolved big question is whether we can find ways out of a treatment of these distinctions as monolithically either-or. Can we, as Verran (2002) asks, work them together, so that our instrumental techno-scientific powers might be organically regulated, and inspired, by cultures of negotiated human-relational, societal ends and priorities, rather than become their own instrumental, self-justifying ends?

An example of what Ingold's distinction means in practice is given in the work of fellow-anthropologist Paul Richards (1993) on African indigenous agriculture. He describes a complex system of intercropping of different crops and cycles, which is described by Western scientists as a combinatorial logic of a quite sophisticated, pre-planned design. From close observation over

long periods of living with the farmers, he notes instead that there is a continually adaptive practical culture, in which the eventual outcome is not previously imagined and planned. Instead, it is achieved as a contingent outcome, through a succession of sequential improvised adjustments to unpredictable changes. As Richards says, a scientific frame of thought imposes a 'fallacy of misplaced abstraction', which replaces what are situated practices with no prior design, only adaptive skills and resources, with a false notion of 'indigenous knowledge-system' akin to the conventional image of science.

In other words, indigenous knowledge-culture is bringing tacit skills learnt from practice and historical experience to bear on a particular matter in a particular situation. Science also relies on tacit situated practice-knowledge (Polanyi 1958; Collins 1983), but its 'situations' are much more highly-orchestrated, limited and controlled. This exposes profound cultural differences, not just 'knowledge-gaps', between indigenous and scientific cultures. Hobart (1993) and Vitebsky (1993) describe these knowledge-practices in similar terms to Ingold and Richards, as situated, continually adaptive and learning in an experimental practical form, but within an ethical and epistemic idiom which does not expect nor seek control (and thus deny and externalise uncontrolled effects) in the way that scientific culture does.

Scientific Reductions: Indigenous Complexities

Van der Ploeg (1993) has described similar deep cultural dislocations in the interventions of Western scientific potato breeding into indigenous Andean potato-farming cultures. The approach of Western science is first to develop in a research laboratory one (standard) 'optimal' seed/plant (genotype), then to manage conditions – soil, inputs, environment, farmers' practices, etc. – to optimise production according to the laboratory object's standard conditions. According to van der Ploeg (1993; 217), 'One of the consequences of this … is that the new genotype will only prove to be an effective and rational innovation insofar as these required conditions can be effectively reproduced in the fields'. This also makes the crops dependent on a single optimised harmonisation of genotype and conditions, thus making them more vulnerable to change and variation, i.e. less resilient.

The potato farmers interactively cultivate different plots, using and exchanging multiple cultivars whose history and performance under different conditions they know, and share in their communities. Each farmer deals with a huge variety of ecological conditions: soil, temperature, water, drainage, wind, past cultivars, height, shelter, sun, rain, pests, etc. One factor may alter another. They thus begin from variable phenotypical qualities and environmental conditions, and select multiple – up to one hundred – seeds/plants (and their genotypes) to suit these. In this more complex and experimental optimisation process they use 'folk taxonomies' economically to describe their cultivars. For example, one potato variety (*ccompi*) is sometimes called another (*calhuay*) not because of error, but because *in certain conditions*, it shows properties of the other. Likewise, environmental and other inputs variables interact in complex dynamic ways, and these are reflected in economical forms of tacitly combined reference. Thus, 'high-low' altitude interacts with 'hot-cold' temperatures, depending on wind, shelter, soil richness, etc., so that a plot higher in scientific altitude terms, may be 'lower' in indigenous farmer terms, because the soil was previously more tilled, or because of lower wind exposure. Unaware of the indigenous meanings, scientists deem this ignorant. Echoing Vitebsky's aforementioned observations, 'when one separates these concepts from the people who use them, or from their context, they do indeed become 'inaccurate'' (van der Ploeg 1993: 212)

These indigenous descriptive terms do not refer to a supposed universal and abstracted reality, as is the assumption of scientific culture, but they are locally specific, flexible and practice-related in meaning. Moreover, these more informal theoretical terms are open to change, according to

experience and need. The farmers are collectively practising a form of experimental knowledge which is being continually developed according to empirical experience and social-cultural needs, including normative cultural commitments to long-term sustainability and 'pay-off', not just short-term. Thus, van der Ploeg (1993) notes, the forms of farmers' technical reference and practice are consistent with, and reflect and help to sustain existing community relations and social practices. They have not been isolated into individualistic or short-term notions of optimisation, productivity, efficiency, and 'validity', whereas the scientific culture assumes implicitly that if its own system's yields begin to drop after a few years – as, indeed, was found to happen in van der Ploeg's situation – new laboratory genotypes and/or artificial inputs such as chemicals will remedy this.

Once the Western scientific system entrenches itself therefore, the dependency of the indigenous culture on the techno-scientific inputs and corresponding modes of life relentlessly increases, and the independent, experimental collective knowledge-capacities of its members is relentlessly diminished. This social-technical knowledge-capacity may be a very substantial positive value whose systematic destruction has yet to be adequately recognised in existing forms of regulatory appraisal and risk assessment, and their narrow and parochial definitions of (physical) 'harm'.

Conclusions

In this chapter I have used what are now common anthropological insights into indigenous cultures (including into 'indigenous' citizen cultures in developed-world societies) to provide a clear profile against which to see some of the unseen cultural dimensions of scientific risk assessment as the defining modern approach to decision making about such issues such as the commercial use and international trading of GM crops and foods between developed and developing social and agricultural settings. The point is emphatically not to ask which of these is better. It is more to help develop a more mature, indeed more scientifically rigorous and self-reflective, practical culture in the established methods and processes of risk assessment which define these policy decision-making commitments. This would also bring (a) a much-needed modesty to such regulatory claims about the scientific knowledge which is so used to claim public authority for the innovation trajectories as well as the risk assessment judgements themselves, and (b) a recognition that rigorous approaches to risk assessment take us beyond existing reductionist scientific framings of 'risk', and require institutional developments such as social benefits appraisals to prevailing regulatory 'risk assessment' processes.

Such a comparative outline, albeit far too brief, shows how the defining institutional scientific methods and assumptions of global and national risk assessment are already framed in various ways which prejudice outcomes in particular directions. For example, the universally institutionalised presumption that only risks need to be assessed and not claimed or imagined benefits, because historically – until the European controversy over GM crops and foods emphasised public refusal to believe promotional claims about benefits – it was just taken for granted that if anyone wanted to promote a particular innovation, by definition it was a social benefit. For GMOs this has now been challenged openly, but its more general logic has not been at all adequately assimilated into an institutional and policy change which, as the 1995 Norwegian Gene Technology Act has been almost unique in doing, would ask the fourth-hurdle, social benefit questions and weigh these in with the risk questions. For the very different conditions, needs, and priorities of developing countries this may be an even more important extra regulatory question than it is for developed societies; and it would almost certainly lead to different outcomes, in whichever particular direction. Of course, the collective answers to such benefit questions would not be precise 'revelations' from nature by technically validated analysis, as risk assessment is supposed to be. Yet as many authors have shown, this is not fully true for

risk assessment anyway (Wynne 2001; Stirling 2003; Jasanoff 2005; Chapter 3 in Wynne & Felt 2007).

When, as these analysts have done, we examine risk assessment as knowledge-culture more closely, it is clear that important social-cultural premises have always framed the scientific risk knowledge process – about what count as salient, defining questions, including what counts as harm, to what socially valued entities. Moreover, the precision that is always taken to define sound science itself tacitly embodies and imposes normative values commitments which are thus protected from accountable debate. This epistemic criterion inevitably reduces recognised risk possibilities to immediate, measurable ones, and from immediately recognisable elements of the technological trajectory which is being socially appraised for decision. The larger, longer term more extensive commitments realistically rendered more likely by the first (more precisely definable) step, are framed out of risk assessment defined by the precision criterion, even though realism and wholism as other legitimate criteria for valid knowledge ought to include such larger questions, even if they are less precise.

The point here is that a proper debate about social benefits and purposes of innovations, as in agricultural change, would automatically include debate about the social purposes and values of prevailing knowledge, including the knowledge invested in the innovations in question, its degree of social centralisation, private ownership and control, and the extent to which it encourages or prohibits distributed knowledge skills of groups such as farmers in van der Ploeg's example of the previous section. This kind of institutional and intellectual framing would then also render scientific risk knowledge more socially responsive and grounded in realistic reflection of such societal debates, values and needs. This would not at all reject science, just frame it more openly, constructively and effectively, as proposed, for example, in developing country GMO cases in Chapter 10, as well as by earlier science policy reports which have been ignored (USNRC 1996; UK RCEP 1998).

Resonating with the aforementioned conclusions are also the logical outcomes of taking the uncertainties within and underlying scientific risk knowledge more rigorously than institutional risk assessment does. The differentiation between risk, uncertainty, ignorance, indeterminacy, and ambiguity exposes the point that while risk assessment may include uncertainty in the form of known possible consequences whose probability we cannot estimate, it never addresses ignorance, because this is strictly impossible. However, this does not make it negligible. Nor does it justify denial, as presently happens to the public discredit of risk assessment and scientific institutions. Addressing benefits questions, as explained, is one such logical response; but so too is building in debate and assessment of alternative trajectories, and not only in technical risk terms but in social terms too, including the distributed knowledge-capacity issues outlined earlier in this chapter. Diversity of portfolios may also be a rational approach, especially when exaggerated speed of commercialisation and immature scientific knowledge underlie the technology and its regulatory risk appraisal.

The key differences between scientific and indigenous may be more in their different, perhaps incompatible ethical, cultural and social substance, than in any more systematic logical aspects. To use a common philosophical parlance, it may be more about forms of life, than about abstract logical or reason-based, intellectual criteria.

I have tried to summarise the implications of insights into scientific and indigenous cultures for biosafety assessment of GMOs, bearing in mind that 'biosafety' questions cannot as a matter of reasoned principle be divorced from wider questions about social benefits, and purposes, thus about what kinds of institutional structures, of ownership, control, accountability, and direction

are shaping innovation scientific research and not only biosafety research in a domain of this kind. It cannot make sense to attempt to do rigorous assessment of the potential impacts of a science-driven technology such as GMOs without also asking about the quality and (im)maturity of the scientific knowledge that has given rise to them in the first place, what aims and expectations were driving it, and what technical and social alternatives are, or if we invested in the research, could be, available.

Examining the relations between scientific and indigenous knowledge-cultures thus provides some helpful perspectives out of which to construct more robust, more just and sustainable forms of innovation and more rigorous, more publicly legitimate forms of risk assessment and appraisal, than have so far been established in developed or developing countries, for innovating and shaping agricultural development globally.

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Chapter 19

Genetic Engineering, Biosafety and Indigenous Peoples

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1. Introduction

There are diverse views on the developments and decisions in science and technology because there are varying perceptions of the world and values that are deeply embedded in particular cultural and socio-political contexts. Indigenous peoples have their own worldviews and values which guide them in their relations with the natural world and with other living things and these influence the positions they take in issues being addressed by the Convention on Biological Diversity (CBD). This chapter will discuss some of these views as they relate to biodiversity, biotechnology, particularly genetic engineering, biosafety, the patenting of life, and collection of human genetic materials. Most of it will be basically be a critique of genetic engineering and the collection of human and other genetic materials from indigenous peoples and their territories. This will be seen within the nexus of biodiversity, cultural diversity, and indigenous peoples' rights and development. It will establish the relationship of our advocacy for the protection and sustainable use of biodiversity, the implementation of the Cartagena Protocol on Biosafety and our struggle for the respect and protection of our basic collective and individual rights as indigenous peoples.

On 29 June 2006, the newly established UN Human Rights Council adopted the UN Declaration on the Rights of Indigenous Peoples. For us, indigenous activists, who have been actively involved in the negotiations of this in the last 24 years, this was a major victory which we hope will make our task in asserting and claiming these rights easier. It is a historic decision, which will have direct implications for biosafety capacity building related to implementation of the Biosafety Protocol of the CBD.

One of the sticking points during the negotiations of the Biosafety Protocol was the issue of socio-economic considerations over genetically-modified organisms (GMOs). This is a key concern not only for developing countries but also for us, indigenous peoples, whether we come from developed or developing countries. Any new technology, including GMOs, should be assessed and considered not only on technical and scientific grounds, but also on the basis of actual and potential social, economic and cultural impacts.

These impacts are directly related to our rights to own and control our lands, territories and resources, our right to practice and live our cultures and to use and maintain our indigenous knowledge systems. Most of the world's remaining biological diversity is found in our territories, because our ancestors and the present generations consciously protected and used these sustainably. If GMOs invade our territories and obliterate the traditional varieties we use for food, medicines and forage, then the sustainable use and the traditional knowledge around these will be forever lost. If our traditional knowledge on the use and sustenance of these biological resources are stolen through patenting, our indigenous ways of sharing this knowledge for the common good will be destroyed. It is our right and obligation to Mother Earth and to future generations to continue to protect and nurture this biodiversity.

The first part of this chapter will trace the history and rationale of our involvement with the discussions on biodiversity, genetic engineering, biosafety and intellectual property rights. The CBD is only concerned with the biodiversity found among plants, animals, microorganisms and ecosystems. For indigenous peoples, however, it is hard to talk about biodiversity without relating these to the issue of human and cultural diversity. Thus, the second part of this chapter addresses the issue of the collection of human genetic materials of indigenous peoples under the Human Genome Diversity Project, which we thought was stopped only to discover that it has been reincarnated recently into the Genographics Project of the National Geographic Society. The last part will deal with our critique of the patenting of life and the actions and responses of indigenous peoples related to this.

2. Asserting Our Worldviews and Our Rights

In 1993, during the first meeting of the United Nations Commission on Sustainable Development (CSD), a small group of indigenous activists met with Rafe Pomerance, the then head of the US Government delegation. He patiently answered our questions about biosafety, and his country's refusal to sign the Convention on Biodiversity (CBD). However, when he said, 'everything within the Convention is negotiable except for one issue, which is intellectual property rights' I became concerned. I explained that our views diverge from his, from that of transnational corporations, and from Western thinking in general. We simply do not believe that the Western intellectual property rights (IPRs) regime should be imposed on us, nor on the rest of world for that matter. 'That is why you need to be part of the global market: to protect your intellectual property rights', he responded. However, this is one of the problems: we do not have any control over the global market economy. How can we protect our rights in an arena where we do not have any say over the rules of the game and we are not even acknowledged as key players? It is precisely the market economy which marginalized our indigenous economic systems.

A year later, I was on a panel with Andre Langanay, a former committee member of the Human Genome Diversity Project (HGDP), at a conference held in Berne, Switzerland, in October l994. This project aimed to collect genetic materials, which the HGDP calls 'isolates of historic interest', from indigenous peoples all over the world to be stored, studied and manipulated. He discussed the objectives and promises of the HGDP and I presented my critique of this, which included our call for a moratorium on this. In response, he said he could not understand what indigenous peoples have against the extraction of their genetic materials if this means that they can contribute to the discovery of new cures for diseases. If he were asked to do this in order to help others to get well, he would have no second thoughts about it, he argued.

Langanay's statement shows how different our worlds are. He has not gone through the experience of being colonized and having his community militarized because the government or a corporation wants to appropriate his people's lands and resources. Most indigenous peoples have gone through this experience and therefore are wary in giving out easily what remains from their territories and from their bodies.[1]

[1]While the official documents on the HGDP did not mention that what they are doing is for profit, I am positive that eventually some scientist or corporation will apply for patents on some of the collections. The example of a US corporation called Incyte, which in April 1994 applied for a patent on 40,000 human genes and DNA fragments, provides a strong basis for my suspicion. The patent application of the T-cell line infected with HTLV-11 virus of the Guaymi woman in Panama by the US Department of Commerce and another application over the T-cell line infected with HTLV-1 virus of a Hagahai man in Papua New Guinea by the same agency, to me indicates that commercialization of these viruses will be a logical next step. While these were not directly linked to the Human Genome Diversity Project, these precedents already indicate the future of the genetic collections of the HGDP.

Our participation in the Convention on Biological Diversity is to ensure that the state parties to the Convention understand and integrate our concerns in the implementation of the programme of the Convention. We want to be effectively involved in the decision-making processes of the CBD so that we can protect the biodiversity in our territories and our traditional knowledge in the use and development of this. One way of protecting our territories from being contaminated by GMOs is to work for the proper implementation of the Biosafety Protocol in the countries where we are found. Another more strategic and effective way is to ensure that the CBD is implemented from a human-rights based approach. This means that it does not just deal with technical and scientific issues but links these up with the protection of human rights and fundamental freedoms recognized in international human rights law. There is no way that indigenous peoples address issues of biodiversity protection without linking with the need for recognition of our rights to our lands, territories and resources.

Unfortunately, it is not just the biological diversity in our territories which is being appropriated but also our human genetic materials. Hence, we consider that in fighting for our rights, especially our right to own and control our territories, resources, knowledge, and cultures, we should not forget to include our rights over our own bodies. These rights are threatened by projects such as the HGDP and extraction of the biodiversity found in our territories. Our struggle for these rights began with our ancestors fighting against foreign colonizers more than five hundred years ago and continues against nation states which continued the colonization process.

3. Gains Achieved By Indigenous Peoples

In our struggles for the recognition and protection of our collective and individual human rights, we have achieved some gains over the years. In some countries, such as the Philippines, we successfully lobbied the government to enact a law which recognizes and protects our rights. This is called the Philippines Indigenous Peoples' Rights Act of l997. At the global level we have actively participated in the formulation and negotiations of the Draft Declaration on the Rights of Indigenous Peoples which started in the early l980s until it was adopted after more than twenty years by the UN Human Rights Council. This happened on 29 June 2006 at the first Session of the Human Rights Council. So, it is no longer a draft; we now have a United Nations Declaration on the Rights of Indigenous Peoples.[2]

The specific reference to issues under discussion in this chapter is Article 31 of the UN Declaration (UN Document A/HRC/1/L.10 2006):

> Article 31
>
> Indigenous peoples have the right to maintain, control, protect and develop their cultural heritage, traditional knowledge and traditional cultural expressions, as well as the manifestations of their sciences, technologies and cultures, including human and genetic resources, seeds, medicines, knowledge of the properties of fauna and flora, oral traditions, literatures, designs, sports and traditional games and visual and performing arts. They also have the right to maintain, control, protect and develop their intellectual property over such cultural heritage, traditional knowledge, and traditional cultural expressions.

[2]See the UN Document A/HRC/1/L.10, 30 June 2006, Report to the General Assembly on the First Session of the Human Rights Council, Geneva, United Nations. This document contains the adopted UN Declaration on the Rights of Indigenous Peoples. It was a decision taken through a vote: 30 voted 'yes', 2 'no', and 12 abstained.

> In conjunction with indigenous peoples, States shall take effective measures to recognize and protect the exercise of these rights.

We worked very hard for the establishment of policies, spaces and mechanisms in the United Nations which address indigenous peoples' rights and development. These include the UN Working Group on Indigenous Populations, the UN Permanent Forum on Indigenous Issues, and the UN Special Rapporteur on the situation of Human Rights and Fundamental Freedoms of Indigenous People. In these bodies we denounced projects such as the HGDP and we secured recommendations calling for a cancellation or a moratorium on the HGDP and the Genographic project. At the national level, we made our communities aware of the existence of this project and alerted them to be watchful over activities undertaken to collect their genetic materials.
In the Convention on Biological Diversity, indigenous peoples also engaged actively with its various working groups, such as the Working Group on Article 8j, which looks at the protection and sustainable use of traditional knowledge of indigenous peoples and local communities. We are participating in the negotiations of the international regime on access and benefit sharing of genetic resources. Further, a few of us took part, although not in a very sustained manner, in the negotiations for the Biosafety Protocol.

4. Indigenous Biotechnologies

Because of our concern over the adverse environmental, socio-cultural and economic impacts of genetic engineering and on how intellectual property rights are being used to undermine our rights, we raised our views on these at various forums. We do not have a homogenous view on these issues. However, there are basic elements which we agree upon, which will be reflected in this chapter. Most of what I will share are my own views and experiences in dealing with these issues.

Biotechnology can be defined as 'any technique that utilizes living organisms (or parts of organisms) to make or modify products, to improve plants and animals or to develop microorganisms for specific purposes' (Hobbelink 1991). By this definition, biotechnology is as old as humankind. Ancient farmers, women, and indigenous peoples, have been domesticating and cross-pollinating plants since time immemorial.

There are a host of cross-breeding efforts involving animals and plants which have been undertaken by indigenous peoples. Potatoes have been domesticated and bred by Huancapi Indians of the Peruvian Andes. The Igorots (in the Philippines) have been cultivating and breeding a wide variety of *camote* (sweet potato), which was a staple for them before rice was introduced. When rice was introduced, different varieties were developed by our people to suit the environmental conditions in our territories. In one village alone, there are more than ten varieties of rice seeds planted for different weather and soil conditions. Many varieties of other root crops, such as cassava and taro, were also developed. Such human interventions have led to the further development of biodiversity, complementing the acts of nature.

Indigenous biotechnologies include fermentation technology to brew beer, wines and other food preparations, and also the domestication of wild plants and animals. We, the Igorot people in the Cordillera region of the Philippines, have been fermenting our own *tapey* (rice wine) and *basi* (sugar cane wine) since time immemorial. *Tapey* is made with a native yeast called *bubod*, which is made by the women. *Basi* is prepared with seeds called *gamu*, which come from the forest. Indigenous peoples have also discovered a vast array of medicinal plants, and have continued to use many of these through the generations. Thus, to say that indigenous peoples have contributed significantly to the present body of knowledge possessed by scientists, such as ethnobotanists,

ethnopharmacologists, as well as by agriculturists, foresters and food technologists, is an understatement. The development of these indigenous biotechnologies is still continuing. However, the recent moves of biotechnology and agribusiness corporations towards appropriating what we have and know may have serious impacts on the continuing development of these indigenous knowledge and technologies.

5. Difference with Genetic Engineering

Of course, these innovations are fundamentally different from the biotechnology we now have, which rests on a host of applications and techniques – from manipulation of life to detection and unobtrusive marker-assisted breeding. Today, biotechnology is more often associated with the most modern technologies, particularly genetic engineering, new cellular procedures based on the old technology of tissue culture, and embryo transfer. This kind of biotechnology poses a major threat to our indigenous values and belief systems, lifestyles, biological diversity, and the last remaining indigenous sustainable resource management systems, and socio-politico-economic formations. The philosophical, social, economic, ecological, and cultural implications of these are serious, not only for us indigenous peoples but also for the whole world. In this chapter, biotechnology will refer to these new biotechnologies, particularly genetic engineering.
The big difference between the new biotechnologies, specifically genetic engineering, and indigenous biotechnology is that with the traditional cross-breeding of plants and animals the reproductive process ran its natural course. Genetic engineering not only short circuits the reproductive process, but it creates new life forms and new rates of evolution never before seen on the face of the earth. Genetic materials of humans or animals are put into plants. Microorganisms, plants, animals, and human beings are the main raw materials for the biotechnology industry, just as inanimate, non-renewable matter (mineral ores, oil, petroleum, etc.) were the main raw materials for the industrial revolution. The history of colonization and exploitation of many indigenous peoples in various parts of the world is the story of how the colonizers and corporations got their hands on the rich deposits of minerals and the abundance of forests and forest products found in indigenous peoples' territories. Many of our territories are not only rich in minerals but are also biodiversity-rich. Now, with the promise of profits in the genetic resources of our bodies, and in plants, animals, and microorganisms found in our territories, we are faced with a more insidious and dangerous threat.

6. Diverse Views on Technology

As indigenous peoples are diverse, they also have varying views on how to regard this kind of biotechnology. There are those who believe that the march of science is inevitable, so if gene hunters and collectors come into our communities, the only option for us is to negotiate the best possible contracts. The aim is to ensure that we can have a share from the benefits derived from these genetic resources which come from our bodies, lands and territories. This may be the pragmatic approach, one which follows the advice given by the US delegate (Rafe Pomerance) mentioned earlier.

A related view is that there is nothing inherently wrong with genetic engineering. The problem does not lie with the science and technology, per se, but with who has control over it. If we can have control, then we will be able to use it to our own benefit and to the benefit of humankind. Thus, the strategy should be focused on how to ensure the transfer of this technology to the Third World and to indigenous peoples. We can lobby governments to exert greater control over the technology to make sure it benefits the people. Alternatively, we secure the power ourselves, and run the government so that we will be able exert our own control over this technology.

A third view is that technology, in this case genetic engineering, has its own inherent logic, dynamics and dangers, which will define not only the directions development will take but also the dominant worldview and individual consciousness. Its inherent logic will define how one will interpret and organize one's systems. With this view, there are various strategies to take. The first is to make sure that the precautionary principle is strictly applied in this case. The science, or the worldview which underpins this science, and its environmental, economic, political, and socio-cultural impacts should be analysed and critiqued. Appropriate policy frameworks, regulatory and corrective systems should be put into place to control the technology and its adverse impacts. The Biosafety Protocol is the key regulatory instrument which can be used by indigenous peoples to pursue their concerns regarding the adverse impacts of genetically-modified organisms. The second strategy is to ensure that the collection of genetic materials from indigenous peoples' bodies and territories cannot be done without their free, prior and informed consent. A third strategy involves fighting against the patenting of life forms. Most indigenous peoples support this third view. They ask that they should have the option to choose technologies which are socially, culturally, ethically, and environmentally appropriate for them. This is part of their right to self-determination and their right to lands, territories and resources.

Many indigenous peoples have held protests and conferences which have issued declarations and positions against life patents, calling for a ban on the Human Genome Diversity Project, and a moratorium on biopiracy in indigenous peoples' territories.[3] Unfortunately, we know that in spite of these protests, biopiracy is still taking place, collections of human genetic materials are continuing, and various life forms are still being patented.

Those of us who have resisted colonization, and whose economies have not been thoroughly eroded by the capitalist market economy, have managed to retain aspects of our pre-colonial cultures. Our cosmologies still revolve around the need to live and relate harmoniously with nature. Our technologies are still rudimentary and not as powerful as those developed in industrialized countries which are capable of redirecting nature and channelling its forces elsewhere.

Indigenous peoples who are in this state of development still maintain an intimate union with nature. Indigenous religion, which is usually a form of animism, reflects a reverential attitude towards creation, in general. Even those who have converted to any of the dominant world religions maintain a folk religiosity which combines the dominant religion with indigenous beliefs and practices. This is not to say that our own traditions are unchanging in spite of all the developments around us and those brought into our communities. Our cultures are not static. The alienation between humanity and nature which is characteristic of highly industrialized societies is rarely experienced by indigenous peoples, who still largely rely on nature for their basic survival. Even those who have been introduced to the sophisticated mechanical technology developed since the industrial revolution have somehow consciously kept aspects of their ancestors' belief systems and cultures. This can be seen among the indigenous peoples of industrialized countries. The hunters and fisher folk among the Inuits in Alaska, Canada and Greenland, for example, do not relate to their prey in the same manner as those who own and manage commercial fish trawlers. They are aware of the need to harvest sustainably to allow for the regeneration of species. They strive to maintain their communities even amidst the strong pressures from the dominant society to assimilate and integrate with the ways of the white people.

[3]Examples of the declarations issued and congresses held are: Mataatua Declaration on Cultural and Intellectual Property Rights of Indigenous Peoples of June l993 issued in Aotearoa; National Congress of American Indians (3 December 1993); Guaymi General Congress (Panama, 1994); Latin and South American Consultation on Indigenous Peoples' Knowledge (Bolivia, l994), Beijing Declaration of Indigenous Women (Beijing, l995).

The ways of life and spirituality of the Igorots, many of who are still small holders/tillers, are very much attuned to the agricultural cycle.[4] Community rites and rituals are not practised only during births, weddings and deaths but also during the agricultural seasons of planting, harvesting and weeding. There are also rituals to call for the rains to come. The agricultural seasons are determined by the seed varieties we plant and by the climate. For many generations we have used indigenous seeds. The introduction of the high-yielding, hybrid seeds of the Green Revolution, however, disrupted the usual periods for community rituals. This is one reason, along with the required chemical inputs, which made many of our farmers revert to the use of indigenous varieties.

7. Implications of Genetic Engineering

7.1 The Philosophical Plane

Genetic engineering carries with it a worldview or philosophy which is reductionist and determinist. A living organism is reduced into its smallest biologically relevant component, the genome. The explanation of the way the organism behaves is sought in its genes. This worldview also regards nature as something which should be controlled, dominated, and engineered or re-engineered. With the invention of technologies which control and re-engineer nature, human beings have succeeded in setting themselves apart from nature. This is what happened after the industrial revolution and is now happening with the biotechnology revolution. Plants, animals and humans are reduced to their genetic components and their integral wholeness is not important anymore. These separate components can be manipulated and engineered at will and for commercial purposes. Hence, the way biotechnology further promotes and reinforces the mechanistic, materialistic, reductionist, and dualist worldview is a major concern for indigenous peoples.

For indigenous peoples, biodiversity and indigenous knowledge or indigenous science cannot be separated from culture and territoriality. Thus, the genetic determinism of biotechnology conflicts with the holistic worldview of indigenous peoples and general ecological knowledge. The cosmology of most indigenous peoples regards nature as divine and a coherent whole, and human beings as a part of nature. Thus, it is imperative that humans live in meaningful solidarity and harmony with nature. This is the 'web of life' concept or what is now referred to as the ecosystem approach which appreciates the relationship and bonds of all of creation with each other. Human beings have to work and live with nature and not seek to control and dominate it. Whether we recognize it or not, we humans are totally dependent on water, air, soil, and all life forms, and the destruction or pollution of these will also mean our destruction. The integrity or intrinsic worth of a human being, plant or animal is measured in relation to how it affects and relates to the others. The engineering mindset is becoming the norm. Efficiency, not only of machines and human beings but of all living things, is the goal. Because profits and economic growth are the most important parameters used to measure development and progress, the adverse environmental, economic, cultural, and social impacts of biotechnology are viewed as insignificant. Reductionist science is given preference over wholistic science to explain how the world and the human body work and how to diagnose and cure human diseases as well as to eradicate poverty.
Indigenous peoples who have not totally surrendered the cosmological vision inherited from their ancestors, and have, indeed, developed it further, are in a better moral and ethical position. If indigenous peoples keep asserting their own philosophy and their right to believe and practice it, we might someday evolve a different philosophy or perspective which provides a balance between the two extremes.

[4]See Victoria Tauli-Corpuz (1996), Reclaiming Earth-Based Spirituality, Indigenous Women in the Cordillera, p. 101.

7.2 Ecological and Economic Implications

The ecological risks of genetic engineering have been amply elaborated by NGOs and scientists (see Chapters 10-12). Since indigenous peoples' territories are the last remaining biodiversity-rich centres, the erosion of this biodiversity could be facilitated by the invasion of more evolutionary advantaged transgenic plants, animals and microorganisms (see Chapter 11). The role that the Biosafety Protocol will play in controlling the release, transport and sale of these GMOs, especially their contamination of traditional varieties and wild relatives of their most important food, medicinal and forage crops is crucial.

The appropriation of indigenous knowledge on plants and plant uses, along with the destruction of indigenous sustainable resource management and agro-forestry practices is also facilitated by biotechnology. Patent applications by scientists, corporations, and even governments, for medicinal plants used by indigenous peoples since time immemorial are increasing each day. In India, the neem tree and the plant from which turmeric is derived are very much used by the tribals. Similarly, Ayahuasca and quinoa in Latin America, kava in the Pacific, the bitter gourd in the Philippines and Thailand are all plants which are widely used by indigenous peoples. Quinoa (Chenopodium quinoa), for instance, is used to make a high protein cereal which has been a staple in the diet of millions of indigenous peoples in the Andean countries of Latin America. It has been cultivated and developed since pre-Incan times. Two researchers from the University of Colorado received US Patent Number 5,304,718 in 1994 which gives them exclusive monopoly control over the male sterile plants of the traditional Bolivian Apelawa quinoa variety. This crop is exported to the US and European market and the value of Bolivia's export market on this is currently USD 1 million per year. The most logical development is that the patent will be taken over by corporations. The hybrid varieties will be used for wide-scale commercial production in the US or Europe, and the Bolivian exports will be prevented from entering the US and European markets.

This will lead to the displacement of thousands of small farmers, most of which are indigenous. The other possibility is that lands will fall into the monopoly control of corporations who own the patents or their subsidiaries in Bolivia who will produce quinoa using the hybrid commercial varieties. Thereby, the genetic erosion of the diverse quinoa varieties developed by indigenous farmers over centuries will take place.[5]

This process is the most probable course of events for many indigenous peoples in different parts of the world. This is made possible because of developments in biotechnology and the legal systems that grant intellectual property rights to those who are able to innovate in high technology laboratories. The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) of the World Trade Organization has become the standard through which IPR laws are being harmonized worldwide. This is further perpetuated by regional and bilateral free trade agreements, whereby developed countries such as the US demand high standards of IPRs, even beyond what is mandatory under the TRIPS Agreement. The contributions of indigenous peoples to preserving, sustaining and developing biodiversity and resource management systems are not recognized and valued by this prevailing system.

8. The Human Genome Diversity Project and Genographic Project

The ambitious Human Genome Project, is a 20-year project funded by the National Institutes of Health (NIH) and the Department of Energy in the United States of America amounting to USD

[5]GRAIN (Genetic Resources Action International) c, Patenting, Piracy and Perverted Promises: Patenting Life, the Last Assault on the Commons, GRAIN, Barcelona, l997, p. 5.

20 billion. Scientists working on this project realized early on that even if they were able to produce an entire DNA sequence, they still would not have information on the variation of DNA among humans. Their aim is to know the genetic basis of the biodiversity among humans. Hence, in l991, they established a committee to develop the Human Genome Diversity Project (HGDP): 'The objectives of the HGDP are to collect, analyze, and preserve genetic samples from a host of vanishing human populations'.[6] This involves a massive survey of human genetic diversity. By discovering the specific DNA differences between populations, they may be able to reconstruct the origins and historical relationships among groups of peoples. They also hope to be able to establish the hereditary basis for differences in human susceptibility to disease. Researchers have already identified 722 human communities for DNA sampling, and have drafted plans to collect and analyse 10–15,000 samples at a cost of USD 23–35 million. They will collect DNA by extracting blood, scraping the inner cheek and collecting hair roots. The collections will be termed 'isolates of historic interest' (IHI). Preservation techniques will be used upon collection, and the researchers will then induce the white blood cells to grow permanently in culture or in vitro.

In a paper titled '*Call for a Worldwide Survey of Human Genetic Diversity: A Vanishing Opportunity for the Human Genome Project*', the HGDP researchers stated:

> The populations that can tell us the most about our evolutionary past are those that have been isolated for some time, are likely to be linguistically and culturally distinct, and are often surrounded by geographic barriers … [i]solated human populations contain much more informative genetic records than more recent urban ones [7]

The scientists involved are aware that their target populations are vanishing fast, so for them, time is of the essence. Cavalli-Sforza (1991) believes that humans are an endangered species in terms of genetic diversity. He describes the HGDP as an 'urgent last ditch effort' to collect DNA from vanishing peoples, and is determined to finish the mapping within five to ten years. Due to the massive protests of indigenous peoples which reached the UN Working Group on Indigenous Populations and the UN Commission on Human Rights, the funding for this project has been reduced and then there was nothing much heard about it. After the demise of the HGDP it reappeared as the Genographic Project of the National Geographic Society. Cavalli-Sforza, the man behind the HGDP is also leading the Genographic Project.

8.1 Critique of the HGDP and the Genographic Project

What do indigenous peoples have against the Genographic Project? The aims of the project appear to be noble and we can grant that the scientists who are involved in it are sincere in pursuing such aims.

It is good that scientists acknowledge that most of the world's human genetic diversity lies with indigenous peoples and that they are endangered; this underscores the urgent need to save this genetic diversity. Indigenous peoples themselves are saying the very same things. Yet, there is a lack of decisive moves on the part of governments and international bodies to address the genocide and ethnocide of indigenous peoples.

In a statement I read before the High Level Meeting of the United Nations Commission on Sustainable Development (UN-CSD) in April l993, I said 'After being subjected to genocide and ethnocide for 500 years, the alternative is for our DNA to be collected and stored. This is just a

[6]Report of the Second Human Genome Diversity Workshop. Penn State University, 29–31 October l992.
[7]See Kidd, et al. 1993.

sophisticated version of how the remains of our ancestors were collected and stored in museums and scientific institutions.'[8]

There are many serious concerns to be raised surrounding the Genographic Project. These revolve around ethical and moral questions. Indigenous peoples' cultural and religious values and rights are likely to be violated by this project. How are the genetic materials and the information going to be used? Who are going to use them and who will benefit from such use? Some of the problems foreseen with the Human Genome Project (HGP), the Human Genome Diversity Project (HGDP) and now the Genographic Project are as follows:

l. Methods of collecting DNA

Many of the methods employed by corporations to collect genetic materials from indigenous peoples are unethical. One example is the attempt of Hoffman-La Roche to collect the genes of the Aeta people in the Philippines. After the Aetas became the victims of the eruption of the Mt. Pinatubo volcano in l991, medical missions visited them once in a while. In 1993, Hoffman-La Roche approached the Hawaii-based Aloha Medical Mission, which often visits the Aetas.[9] They tried to link up with this group to collect the genetic materials they needed. For people facing calamity, any group that offers charity will be warmly welcomed.

How thoroughly will processes of informed consent be followed, considering the time constraints imposed by the proponents on themselves? Will the collectors be thoroughly briefed? It is easy for people from the Department of Health to visit indigenous peoples' communities and gather blood, cheek tissues and hair roots under the guise of medical missions. The proponents are thinking of making use of such government agencies to facilitate the collection phase. Health departments do not have a good record of providing health education and services to indigenous peoples, however. In fact, indigenous women have been subjected to forced sterilization without their consent. For such a controversial project there is a strong possibility that informed consent will not be applied as it should be.

The need for sophisticated laboratory equipment to study and preserve the genetic collections means that these collections will be kept in the developed countries. While the HGDP has proposed to leave duplicate samples of the DNA with the national governments or in regional institutions, the problem of financing such laboratories still remains. While the proponents acknowledge that storage laboratories can be in indigenous communities, they still have a caveat which says 'A condition for establishing such labs … would have to be that they cooperate on an open basis with investigators interested in the region'.[10]

2. Potential uses of the genetic materials

A new eugenics?

Based on the current uses of genetic materials collected for the Human Genome Project (HGP), there is much to worry about. With the discovery of genetic 'defects' and 'superior' genes, doctors can already proceed with screening 'effective' or 'superior' embryos and foetuses. The

[8]Victoria Tauli-Corpuz, Statement presented during the 2nd Session of the UN-CSD on behalf of the Cordillera Peoples' Alliance, New York, l994.

[9]The information on the collection of genetic materials from the Aetas was relayed to me by my NGO friends in the Philippines. I was sent copies of the exchange of letters between Dr Philip Camara of the Makati Medical Centre in the Philippines and Elizabeth Trachtenberg of Roche Molecular Systems. The exchange of letters took place between March 1993 to July l994. A fuller account of this exchange can be read in the book The Life Industry: Biodiversity, People, and Profits (1996).

[10]Cavalli-Sforza et al. (1991)

next foreseen step is to abort 'defective' foetuses and to clone 'superior' ones. Who will determine what genes are bad and what genes are good?

While the proponents claim that the results of the study will erase the basis for discriminating against indigenous peoples, they are not in any position to assert this. The information can be used against indigenous peoples for political purposes. When it falls into the hands of those who want to perpetuate their power over the world, political motives could overrule the original intent of the research.

Patenting and commercial production of genetic materials
With the additional information and materials which will be gathered from the HGDP, what other possible programmes will be developed? If their aim is to determine the susceptibilities and resistance to diseases, how will such discoveries be used? Will they clone the proteins conferring disease resistances and develop and sell these for profit? The fact that biotechnology corporations are already competing for the control of such materials, and investing in their commercial production and sale, says more than enough.

Patenting is the first step toward the industrial production of inventions or discoveries. Industrial production means the reproduction of millions of identical goods, such as cars, machines, clothes, etc. The patenting of life forms will naturally encourage the reproduction of isolated or modified genetic materials, plants, animals, and human beings.

Craig Venter, a former US National Institutes of Health (NIH) researcher doing gene mapping and sequencing, has applied for patents on more than two thousand human brain genes. If approved, this will give him and NIH ownership of over five per cent of the total number of human genes. Andrew Kimbrell, in his book *The Human Body Shop* (1993: 46), says:

> [should] any one of the genes prove to be extremely valuable, perhaps a key gene for brain cancer research or future therapies to increase I.Q., the researcher and NIH could then form lucrative licensing agreements with biotechnology companies for exclusive commercial exploitation of the genes ... The entire human genome, the tens of thousands of genes that are our most intimate common heritage would be owned by a handful of companies.

The patent application of the US Department of Commerce for the T-cell line infected with human T-cell lymphotrophic viruses (HTLV) Type 1 of a 26 year old Guaymi woman from Panama was the first attempt to patent genetic materials from indigenous peoples. This application was submitted as early as l993. International NGOs led by the Rural Advancement Foundation International (RAFI) (1997) discovered this application. An international campaign was launched and Isidro Acosta Galindo, the President of the General Congress of the Ngobe-Bugle (Guaymi) wrote to the US Secretary of Commerce demanding that he withdraw the application. The patent claim was denounced by indigenous peoples and NGOs at meetings of the Convention on Biological Diversity and other international gatherings. Because of this international outcry, the patent application was eventually withdrawn, citing the high cost of pursuing a patent claim.[11]

An indigenous man of the Hagahai people of the highlands of Papua New Guinea had his DNA patented by the NIH on 14 March l995. This patent covered a cell line containing an unmodified Hagahai DNA, and was also withdrawn under international pressure.
How will genetic materials and genetic information be used?

[11]Baumann et al. 1996: 137.

Indigenous peoples are well known for resisting 'development' or mal-development projects which will destroy their traditional territories. Many indigenous communities are also presently waging armed resistance against the states which are oppressing them. Will genes increasing susceptibility to diseases be used to get rid of belligerent indigenous peoples who are against 'development' or 'progress'?

If genetic information shows that a certain indigenous group is descended from people from other countries, for instance that the ancestors of the Igorots come from Southern China, will this be used to deny them their rights to their ancestral lands? What if a group is found to have a genetically high risk of contracting a certain disease? The history of colonization of indigenous peoples would show that biological warfare was often used on them. Smallpox viruses were spread among the resisting Native Americans in North America. Diseases carried by colonial missionaries and soldiers decimated a significant number of Hawaiian natives.
Indigenous peoples have always been discriminated against, and have been portrayed by colonizers as primitive and barbaric. In a world where Western standards and culture are being propagated by media and corporations, the intolerance for diversity is increasing. Will the collection and immortalization of the cell lines of indigenous peoples, be a justification for actions which will lead to their final disappearance?

3. Genetic determinism
It is worrisome to see how DNA or genes are being regarded by scientists. How can one explain one's sexual orientation and behaviour, for example, by saying that there is a homosexuality gene or a violence gene? Genes are part of a whole system and an individual is part of a family and society which are major factors in configuring who that individual is. There is an overestimation of the role played by genes in determining the behaviour and personalities of peoples.
What could be the possible implications of such conclusions? If the propensity to be a criminal lies in a violence gene, can the person be cured through gene therapy? The line of thinking promoted by the HGDP and the Genographic Project is fraught with dangers. The value of analysing society and better understanding the dynamics between the individual and society will be diminished significantly if we believe that social problems such as criminality can be solved by gene therapy, genetic engineering, or by aborting foetuses that are shown to have the 'criminality genes'.

The Human Genome Project, the Human Genome Diversity Project and the Genographic Project have facilitated the invasion and colonization of the human body by the market economy. Genes are said to be the building blocks of life; thus, if life is to be considered sacred, so too should the genes. The effort to map and sequence genes will not just help us learn more about humanity's genetic diversity, but it is leading directly toward the commercial exploitation of genes. The patenting of these genetic materials will pass the control over life from nature or God, to the patent holders.

8.2 Responses to the HGDP, Genographic Project and Patenting of Life
The World Council of Churches made a statement in 1989 calling for a 'ban on experiments involving the genetic engineering of the human germline'. The outcry of indigenous peoples' groups against the HGDP is another response. Obviously, there is a great need to speak out against this sacrilegious treatment of human life.

Indigenous peoples have sustained their protests against the HGDP. In June 1993 a conference was held in Aotearoa, New Zealand, and from this emerged the Mataatua Declaration on the Cultural and Intellectual Property Rights of Indigenous Peoples. This called for a moratorium on the HGDP until such time that its impact has been fully discussed. As early as 1994, I presented a

statement at the UN Commission on Sustainable Development asking for a ban on the HGDP. In February 1995, Asian indigenous peoples presented a statement at the European Parliament also calling for a halt to this project. During the Fourth World Conference on Women in Beijing, through the leadership of the Asian Indigenous Women's Network, participants agreed on the Beijing Declaration of Indigenous Women which again condemned the HGDP and called for it to be banned.

In 1995, seventeen organizations in the Americas signed the Declaration of Indigenous Peoples of the Western Hemisphere Regarding the Human Genome Diversity Project. It called on international organizations to protect all life forms from genetic manipulation and destruction. This statement criticized the efforts of Western science 'to negate the complexity of any life form by isolating and reducing it to its minute parts ... and [thereby] alter its relationship to the natural order.[12]

The whole discussion of biotechnology and biopiracy cannot be tackled without discussing intellectual property rights and the role of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) of the World Trade Organization (WTO).
This recognition had pushed us in the Tebtebba Foundation[13] to organize a workshop of indigenous peoples on Article 27.3.b of the TRIPS Agreement. This was held in Geneva on 24–25 July 1999, just before the 16th Session of the UN Working Group on Indigenous Populations. This workshop developed a statement titled No to Patenting of Life: Indigenous Peoples' Statement on Article 27.3.b of the TRIPS Agreement. This has been sent all over the world via the Internet and at present there are already more than 200 signatories. Almost all of the major indigenous peoples' organizations and networks from all the continents of the world have signed up.

In Seattle, during the 3rd Ministerial Meeting of the WTO there was a group of indigenous peoples who held their own caucus meeting, from which emerged the Indigenous Peoples' Seattle Declaration. Again, this included the protest against the patenting of life.

The Africa Group in the WTO has consistently maintained a position against the patenting of life forms and their parts. A group of indigenous persons met in 2003 to revisit their positions on the ongoing negotiations in the WTO and in the World Intellectual Property Organization (WIPO). This meeting supported the Africa group position, which called for a revision of Article 27.3 (b) of the TRIPS Agreement to prohibit patents on plants, animals, microorganisms, and essentially biological processes for the production of plants and animals.

In addition, a few of us have participated in the negotiations leading to the adoption of a Biosafety Protocol in the Convention on Biological Diversity. The Biosafety Protocol, which was adopted in 2000, primarily regulates the transboundary movement of genetically modified organisms (GMOs). The Tebtebba Foundation has worked closely with the Third World Network (an international NGO based in Penang, Malaysia) on this issue. Many indigenous peoples in different parts of the world are also taking part in the campaigns against GMOs and products containing GMOs. The campaign launched by indigenous peoples' organizations and NGOs

[12]UN Document E/CN.4/Sub.2/AC.4/1998/4, Standard Setting Activities: Evolution of Standards Concerning the Rights of Indigenous Peoples, Human Genome Diversity Research and Indigenous Peoples, Commission on Human Rights, Geneva, p. 4.

[13]Tebtebba Foundation (Indigenous Peoples' International Centre for Policy Research and Education) is an indigenous peoples' NGO whose objective is to help build the capacity of indigenous peoples to fight for their own issues. It carries out research work, lobbying and advocacy in the national and international arenas, holds training workshops and produces publications.

against the GMO contamination of corn in Mexico is another response which highlighted how the contamination happened and what remedial measures can be taken by the Mexican Government and the international community. We are part of an international network against genetic engineering and food security which is composed of various community-based organizations which are working for sustainable agriculture in Asia, Latin America and Africa.

At the national levels there are various efforts of indigenous peoples' organizations to monitor the state of biopiracy taking place and also to lobby for laws that will regulate bioprospecting. In the Philippines, for instance, there is Executive Order (EO) 247, which is expected to regulate research and bioprospecting in the local communities. The Executive Order requires prior informed consent before the researchers can even set foot in the communities. There are still a lot of weaknesses in terms of how this is being implemented but it has served as a deterrent against the rush of biopirates. This has since been superseded by the 1997 Indigenous Peoples' Rights Act which has specific provisions that require corporations and researchers to obtain the free, prior and informed consent of indigenous peoples in communities where research or bioprospecting is being done.

Today we do not hear about the Human Genome Diversity Project. However, as mentioned earlier, in 2005 the National Geographic Society unveiled the Genographics Project, which is a reincarnation of the HGDP. This project aims to collect 100,000 DNA samples from indigenous peoples all over the world to show how they are interconnected. Now, they are undertaking collections through different channels. The UN Permanent Forum on Indigenous Issues (of which I am the current Chairperson) in its latest session held in May 2006 made the following recommendation:

> 88. The Permanent Forum recommends that WHO and the Human Rights Council conduct an investigation of the objectives of the Genographic Project which proposes to collect 100,000 DNA samples from the indigenous peoples of the world in order to formulate theories on historic human migrations, that the Genographic Project should be immediately suspended and that they report to indigenous peoples on the free, prior and informed consent of indigenous peoples in all communities where activities are conducted and planned.[14]

Conclusions

The position of indigenous peoples vis-à-vis genetic engineering is still evolving. The common thread in the various positions is the view that life forms should not be patented. If the ownership of patents on life forms is the main incentive for scientists and corporations to invest in biotechnology, it might be a good idea not to allow this. The benevolent motives avowed by scientists who want to contribute to sustainable development should not be tainted by the commercialization or commodification of life.

There is a common concern among indigenous peoples about the dangers of releasing GMOs and commercializing them as it is already proven that these can contaminate wild and traditional varieties of food crops, medicinal plants, wild foods, and forage. Much more work needs to be done to increase the engagement of indigenous peoples in the implementation and monitoring of the Biosafety Protocol.

It is also generally agreed that the harmonization of intellectual property rights regimes to fit the mould of Western IPRs, particularly TRIPS, is morally and legally indefensible. This is being

[14]See UN Doc. E/2006/43/ and E/C.19/2006/11. Report on the Fifth Session of the UN Permanent Forum on Indigenous Issues (15–16 May 2006) p. 15.

done to further legitimize the desire of industrialized countries and their transnational corporations to have monopoly control over biotechnology. Those who have contributed their centuries-old knowledge to develop and protect the rich biodiversity in their communities will now be accused of biopiracy because the right to this knowledge is passing into the hands of the corporations through IPRs.

It should be recognized that indigenous peoples have a right to their intellectual and cultural heritage; this is clearly articulated in the UN Declaration on the Rights of Indigenous Peoples and other UN standards. This right is being blatantly violated by developments in biotechnology. Even the collection of genetic materials from indigenous peoples' bodies through the HGDP and the Genographic Project and other similar projects is a violation of the rights and dignity of indigenous peoples.

We indigenous peoples all agree that the protection of biodiversity and cultural diversity cannot be effectively guaranteed if our rights to our ancestral territories are not recognized and respected. Therefore, protests against biotechnology cannot be separated from the call for the recognition and respect of indigenous peoples' rights to territories, right to own their lands and resources, including genetic resources, and the right to their intellectual and cultural heritage.

The UN Declaration on the Rights of Indigenous Peoples contains the minimum standards which should guide states, corporations and society in general on how they should respect and protect indigenous peoples' rights. It was the result of more than two decades of intensive dialogues between indigenous peoples, experts and government delegations. It is the articulation of the collective values and aspirations of indigenous peoples from all parts of the world. The recent adoption of this is a historic milestone which will have strategic implications for our fight to sustain biodiversity in our lands and keep these safe from being polluted with GMOs.
The march of science and technology will likely proceed in spite of protests from indigenous peoples and NGOs. In the face of the aggressive recolonization of indigenous peoples' territories, bodies and minds, which is facilitated by the new science and technologies, it is imperative to support the struggles of indigenous peoples and to ensure that the UN Declaration on the Rights of Indigenous peoples is respected by governments, corporations and the broader society. Whatever gains indigenous peoples will make will also be gains for the whole of humanity and nature.

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Chapter 20

Potential Socio-Economic, Cultural and Ethical Impacts of GMOs: Prospects for Socio-Economic Impact Assessment

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1. Introduction

Socio-economic and cultural considerations related to the use and release of genetically modified organisms (GMOs) have received less attention than the natural scientific and technological aspects. This trend sends a signal that the debate about the adequacy of GMO use and release is essentially technical-scientific and is only open for scientists and experts to engage in. The small body of literature on socio-economic considerations related to GMOs could be explained by a number of reasons. Socio-economic impacts of any technology take years to become evident, as the world has experienced with other new technologies as for instance the Green Revolution. By the time the impact is evident, it has already become widespread and in most cases, become deeply institutionalized. For instance, the introduction of the Green Revolution created a new class of agricultural laborers, and changed gender relations by increasing the burden of women in farming (Paris 1998). By the time social scientists began looking at these phenomena, they had already been well entrenched in social institutions and dramatically changed social relations. GMOs may cause both ecological and socially irreversible changes. While this may be the case for most technological innovations introduced in any society, GMOs have unique characteristics that make their ecological and social impacts even more serious and far-reaching. The fundamental ethical and social debates emanating from the fact that GMOs involve manipulation of life forms and processes, as well as the socio-economic and ecological impacts of GMO contamination, are among the many aspects that are unique to this particular technology. Even when the technology is withdrawn or people totally discontinue adopting the technology, its socio-economic impacts may persist and leave a permanent imprint in society, its history and its people. This is even more serious in GMOs which may introgress with wild populations or contaminate conventional crops long after farmers decide to stop planting GM crops. This stark reality underlines the critical importance of assessing the potential socio-economic impacts of GMOs before and during their introduction in any societal context.

2. Technology and Society

Technology cannot be separated from the social context where it is introduced. No technology in the world's history – from the discovery of fire to the domestication of plants and animals, traditional biotechnology, the Industrial Revolution and the Green Revolution – has ever happened in a social vacuum. Accordingly, the different spheres of society – be they economic, political, social, cultural, or ethical – are all affected by the introduction and adoption of a technology, though different in manner and pace. Throughout humankind's history, technological and scientific innovations have greatly impacted socio-economic relations and political life, some in subtle ways while others are highly visible. In a subtle way, the introduction of mechanized farming during the Green Revolution increased the inequity between small-scale and large-scale farm communities (Conway 2003) and reduced the availability for agricultural jobs performed by women (Paris 1998). As a result of the intensive rice production promoted under the Green Revolution, rural societies have been restructured by the birth of a new economic class of merchants that specializes in rice trading, as well as a new breed of agricultural laborers who do seasonal work in rice farms.

In the same way, the different components of society also have some influence on the way a technology is adopted and disseminated in society. Culture, ethics and religion have perhaps the most powerful influence in defining the way technologies are introduced and disseminated in any given society (Figure 20.1). In the case of GMOs, ethical and religious dimensions are the most dominant aspects of the controversies in many countries where religion remains a strong societal force. For instance, whether GMOs can be considered *halal* or *haram* sets the tone of the debate on their acceptability in Muslim societies (Safian & Hanani 2005).

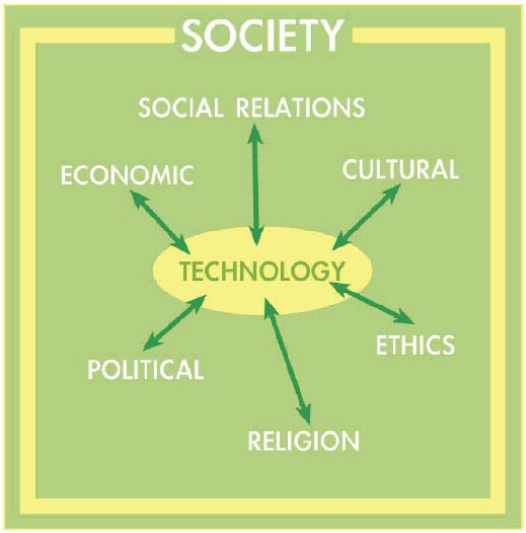

Figure 20.1. GMOs and Society (Garforth 2004; La Vina & Fransen 2004).

3. Socio-Economic Considerations 'Defined'

There have been several attempts to define what socio-economic considerations in the context of GMOs actually mean. The attempt has so far not been successful, and some have argued that socio-economic considerations must be dismissed since they are 'too vague' and 'totally outside the domain of biosafety'. To some, socio-economic considerations are simply 'uncontrollable', even 'unwieldy' and the best way to deal with these is to defer the discussions, or worse, ignore them.

Like other evolving concepts that defy concrete or precise definition, 'socio-economic considerations' have been loosely described as:

> taking into account a broad spectrum of concerns about the actual and potential consequences of biotechnology, such as impacts on farmers' incomes and welfare, cultural practices, community well-being, traditional crops and varieties, domestic science and technology, rural employment, trade and competition, the role of transnational corporations, indigenous peoples, food security, ethics and religion, consumer benefits, and ideas about agriculture, technology and society. (Garforth 2004; La Vina & Fransen 2004)

The elements in this definition are not exhaustive or static. Some of the socio-economic considerations, which are not covered in this definition, will be further expounded in this chapter. The intention is to provide a better understanding of the width and breadth of the issues involved, to promote more concrete definitions of terms, and to evolve assessment tools that could be used by regulators and civil society to minimize or avoid the potential adverse social consequences of GMOs.

4. Importance of Assessing Socio-Economic Impacts of GMOs

The need for assessing the potential socio-economic consequences of GMOs is hinged on several important reasons/values:

1) Social Responsibility. Scientists who develop and introduce technology into any society need to bear the moral and ethical responsibility for the impacts that their innovation may have on society. This involves also potential socio-economic impacts of technologies beyond the controlled confines of laboratories and greenhouses. Recent history in technology introduction stresses that the role of scientists and technologists should not end once a technology leaves the laboratory, but becomes even more important as it is introduced into society.

2) Inter-Generational Responsibility. A technology's aim should be to contribute to sustainable development and is therefore hinged on the inter-generational responsibility of developers of the technology and government regulators. Assessing the socio-economic impacts of GMOs would not only ensure that adverse effects are avoided, or at least minimized, but may also protect the interests and needs of the present as well as those of future generations since socio-economic impacts of technologies are felt throughout generations.

3) Social Acceptance. By giving serious consideration to the potential socio-economic impacts of GMOs, developers and regulators would have a better sense of society's acceptance of the technology and/or its product. As will be explained in later parts of this chapter, effective assessment of the potential socio-economic consequences of GMOs would require the active and broad involvement of various social actors.

4) Reducing Long-Term Costs. A primary concern in socio-economic assessment of GMOs is the costs related to the processes of broad participation of various stakeholders and actors and the period of time it takes to go through these processes. While this may be a valid concern in the short-term, it ignores the possible long-term costs of the technology on society arising from its potential adverse impacts. Hence, by taking socio-economic considerations into account in decision making on GMOs, irreversible social, economic and cultural costs may be avoided or minimized.

Developers and regulators cannot escape the ethical dimension of introducing GMOs without carefully assessing their potential socio-economic impacts. Unlike laboratories and greenhouses where the factors and conditions are all within the control of the scientists conducting the experiment, social and economic forces are beyond anyone's control. Thus, a strong sense of ethical responsibility underpins the need for thorough assessment of socio-economic considerations before GMOs are introduced in any given societal context.

4.1 Socio-Economic Considerations in Relation to GMOs: Legal Recognition

Owing largely to the strong lobby by civil society organizations and several developing countries, particularly the Africa Group, socio-economic considerations have officially been taken on board in the Cartagena Protocol on Biosafety (see Chapter 25).

Article 26 of the Protocol on Socio-Economic Considerations states:

> 1. The Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities; 2. The Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts of living modified organisms, especially on indigenous and local communities.

While the Protocol has recognized that there are socio-economic considerations arising from GMOs, and that these may be taken into account in the decision-making process, research on socio-economic considerations is not a requirement for decision making. Nonetheless, the international community has thus acknowledged that socio-economic considerations are important components of the biosafety decision making process.

4.2 Socio-Economic Impact Assessment (SEIA)

In order to give meaning to the provision of the Biosafety Protocol on socio-economic considerations, tools have to be developed and applied to guide decisions on research, development, movement and introduction of GMOs. One such potentially powerful tool is the socio-economic impact assessment (SEIA), which is adapted from the existing mature tools adopted in environmental impact assessment.

SEIA can help in assessing the potential consequences on the various aspects of the society in which a particular technology is being introduced. It is basically a participatory assessment tool which maps local knowledge in a particular societal context where new technology will be introduced. By being participatory and interdisciplinary, e.g. focusing on economic, social, cultural, political, and ethical aspects, a SEIA entails involvement of different actors/stakeholders and a plurality of aspects in the assessment.

Overall, SEIA can help regulators and civil society groups to weigh the potential benefits of GMOs side by side with their potential risks and adverse impacts on the different socio-economic spheres. There are evolving frameworks on socio-economic impact assessment that are being developed in different contexts. The Philippines, for example, had initially set forth the importance of SEIA in the drafting of its national biosafety framework, although the final regulatory framework did not make it a mandatory requirement in applications for GMO releases. As the Philippine experience has shown, despite the presence of a mature environmental impact assessment framework from which lessons can be learned, the development of tools for socio-economic impact assessment remains a challenge to policy makers, regulators and civil society organizations.

5. Socio-Economic Considerations: What to Assess?

The breadth and depth of what is involved in socio-economic considerations are quite overwhelming, especially to those who want to make the commercialization of such a complex technology as GMOs as least complicated as possible. However, society is a complex organism that has evolved in specific contexts where economic, political, social, cultural, and ethical spheres constantly interrelate with each other in an intricate manner.

This section will attempt to identify some of the components of socio-economic considerations by using general headings representing the key spheres of society and the specific areas in each sphere that GMOs may have potential impacts on. Examples, mostly from experiences in and observations from developing countries, will be used to illustrate key points and critical concerns.

5.1 Economic Considerations

Control over Tools of and Relations to Production. Assessment of the potential socio-economic consequences of GMOs should take into account the issue of control over agricultural production and relations to production in the particular context where the technology is introduced. The potential impacts of introducing GMOs in a rural context have to be studied carefully, bearing in mind the lessons from technologies such as the Green Revolution which reinforced income inequality and wealth distribution in the rural areas, despite the increase in rice and corn production (Conway, 2003). The high costs of agricultural inputs introduced by the Green Revolution made them inaccessible for the rural poor who became heavily indebted to the rural elite who already had better control over the tools of production even before the new technology was introduced.

In the context of GM crops, the control over seeds and the accompanying inputs that complete the technology needs to be the core consideration in socio-economic assessment. The question of control over seeds is relevant at different levels, from the corporate interest in the development and distribution of GM seeds, to the local channels for technology dissemination. Key issues that need to be assessed are: will the dissemination of GM seeds provide opportunities for poor farmers to have some control over the tools of production, or will it further entrench control of particular segments of the community over farm inputs, processing and marketing? These questions may be difficult to answer, but lessons from recent experiences with the introduction of agricultural technologies as well as simulation exercises with the participation of representatives from key sectors can provide meaningful inputs.

Income and Wealth Distribution. Companies that develop GMO products intend to recoup their investments on research and development, through the intellectual property rights (IPR) system and marketing schemes, as well as by profits from the sale of these products. Since price segmentation is an unsound business practice, GMO seeds, for example, are generally sold at a standard price in a country where they are commercialized, which means that the same price applies to all farmers, whether rich or poor.

> For instance in the Philippines, Monsanto's MON 810 (Bt corn with cry1ab transformation event from the soil bacterium Bacillus thuringiensis) is sold at more than twice the price of the counterpart non-GM hybrid corn seed varieties. In a country where at least 60 per cent of corn farmers do not own the land that they till, this price is too high. Given this market reality, Monsanto adopts a targeted marketing scheme that primarily offers its Bt corn products to rich and middle-income farmers who can afford the higher cost of seeds as insurance against corn borer damage. Granting that the company's claims are true with regard to the benefits of Bt corn, those who will benefit from this promise are obviously those farmers who can afford the cost of seeds and who already have relatively high income to start with. This situation will expectedly aggravate the problem of income inequality and wealth distribution in the rural areas. While some may argue that the increase in the income of rich farmers will contribute to higher investment and employment creation in rural areas, this scenario highly depends on whether the promises of better yield and higher income from planting GM crops become a reality. The assertion is also hinged on the expected 'trickle down' of the benefits from those who are supposed to gain from planting GM crops to those who cannot afford the technology.

Income Security. The impact of GMOs on farmers' net income is another important economic consideration that needs to be seriously looked into. Economic cost-benefit analyses would be useful in this regard, taking into account the specific farming practices and conditions of farmers who have adopted the technology. Basic questions about the costs of GM seeds and other required inputs and their share in the total cost of production should be posed, along with the potential net income (or losses) that farmers can expect from using the seeds. Hidden costs, such as environmental and health effects, should ideally be considered too.

Rural Labor. Rural labor is one economic concern that is especially relevant to many developing countries where widespread rural unemployment is a perennial problem. Most GM seeds available in the market today are developed by biotechnology companies based on the needs and conditions of farmers in developed countries where agriculture is predominantly industrial in scale. The situation in industrial agriculture, where the cost and availability of labor is a major production cost, is vastly different from the situation in household-based farming that characterizes agriculture in many developing countries where labor is readily available, abundant and often cheap.

For instance, the introduction of herbicide-resistant GM crops that eliminates the need for weeding or tilling of the soil during land preparation will potentially have grave long-term impacts on rural labor. Less labor requirement on farms using herbicide-resistant GM crops would mean less employment opportunity for poor agricultural workers, especially in areas where there is high rate of rural unemployment. Some may argue that the use of GM seeds that cost more than conventional seeds but that require less labor would make more economic sense than hiring farm labor, which does not only involve paying legal wages but also complying with core agricultural labor standards as well. Such an argument reinforces the potential adverse impacts of GM crops on socio-economic relations in rural areas as well as in overall income distribution. It is argued further that the use of labor-saving GM seeds could theoretically create higher economic surplus that could contribute to increased investments and job generation. The global trends in decreasing investments in the rural areas and the declining contribution of agriculture to overall national income, however, point to the reality that whatever economic surplus is generated in agriculture is not substantially reinvested in the sector to benefit the rural poor.

Markets. Since the price of agricultural commodities is highly sensitive to and dictated by supply and demand, GMOs that promise yield improvements may affect market behavior. Particularly vulnerable are developing countries whose economies are highly dependent on the production and export of specific agricultural products. Spikes in the production of or expansion of areas devoted for the production of Bt cotton in the US or India, for example, could affect the potential market for cotton produced in poor western African nations where millions of farmers depend on cotton cultivation for their livelihood. Since GM commodities such as Bt cotton are produced largely for processing into textile materials and animal feed, they are not segregated from conventional cotton, and hence they will compete with each other in the market.

Even in cases where GMOs are segregated from their conventional counterparts, as in Europe and Japan, which do not accept GM commodities unless they are properly labeled, this could have potential impacts on the market. Segregation, while beneficial for consumer awareness, meaningful labeling and precaution, may ultimately result in price segmentation where the non-GM products could bear a higher price and would be primarily intended for markets that can afford them. On the other hand, GMOs could be channeled to markets with less capacity to pay or where such segregation is not legally required. While this may make sense from a purely market perspective, it would pass on to consumers the price of segregation, which should have been part of their inherent right to information in the first place.

Trade. One of the issues in trade that needs to be considered is the ability of developing countries to compete in the international market if they decide to venture into commercial production of GM crops. In order to compete with the commodities of bigger and wealthier countries in the export market, developing countries bear the burden of meeting high international standards, such as sanitary and phytosanitary standards. While GM crops promise to address specific problems related to particular pests and diseases, the quality of the product largely depends on the conditions in which they are produced and the management practices under which they are

grown. In the case of corn from the Philippines, for example, the most serious problems that affect the crop are fungal diseases, which affect the quality of the harvest and could diminish the chance of meeting international export standards. While promising to increase corn yield as a result of less corn borer attacks, none of the varieties of Bt corn commercially available in the local market address fungus infestation which negatively affects the quality and overall production of corn locally and hence the prospects of exporting surplus corn production to other countries. With stringent sanitary and phytosanitary measures imposed internationally on imported corn and the strict risk assessment processes required in key industrialized markets resulting from strong consumer rejection of GMOs, the prospects of Philippine GM corn competing in the export market do not look very promising.

Coexistence and GMO contamination. The risk of transfer of pollen is particularly high for cross-pollinating crops, such as corn and canola (see Chapter 12). Producers of organic crops risk having their crops contaminated by nearby GM crops whose pollen can travel long distances by wind or with the aid of insects. Coexistence as a policy is extremely challenging, with evidence pointing to the reality of GMO contamination of conventional crops, and even involving GMO crops grown experimentally on a limited scale and those that have not been approved for commercial planting. This situation is expected to be much more complicated in most developing countries where landholdings are much smaller and distances between farms are much shorter. GMO contamination of conventional crops, and of wild and weedy relatives, poses serious threats to biodiversity and the genetic base for long-term food security. Also at risk are the economic prospects that countries and farmers hope to gain from organic cultivation of agricultural products.

Organic Agriculture. In countries where GMOs are already legally commercialized, the prospects for farmers to venture into organic agriculture may be limited by the widespread cultivation of GM crops. There is a consensus in the available literature that the most obvious and potentially devastating impact of GMOs is their direct effect on organic agriculture through contamination. Already, this has become a controversial issue in the US and Canada, where organic farms have been contaminated by GMOs and some farmers have filed ongoing legal suits demanding damages (Nature Biotechnology 2002; SOS Food 2002).

Considered as the fastest growing sector in agriculture worldwide, organic agricultural products have increasingly become important to the economy of many developing countries in recent years (Patton 2006). Organic certification standards generally do not allow GMO contents, and agricultural products containing even small traces of GMOs do not merit the organic label. Should contamination of organic crops occur, farmers would lose the organic certification status for those crops and the premium prices they command.

Food Security. For developing countries where agriculture is a primary activity to ensure family subsistence and provide food supply to the domestic market, a key economic concern that needs to be considered is the potential impact of GMOs on long-term food security. The majority of the GMOs commercialized worldwide are not considered as food crops in developing countries where food security is at the core of agricultural development. Most of the GM corn, soybeans and cotton cultivated and traded worldwide are intended as animal feed. With cultivation of GM crops in the developing world, household food security faces the threat of conversion of land areas traditionally planted with food crops for the production of commodity crops for industrial use and export. Already, many poor and even medium-income countries have high incidences of malnutrition despite increased agricultural production, mainly due to crop uniformity and the erosion of traditional food bases that used to supply balanced and readily available nutrients to family members. A sound socio-economic impact assessment should seriously look into the

impacts of widespread promotion of GM crops for industrial use on the overall food security of communities in view of land limitation and the declining productivity of agricultural land due to intensive production.

Food Aid. While ensuring long-term food security remains a great challenge for the developing world, many poor countries are confronted by emergency situations that inhibit farmers from producing their own food, particularly in areas affected by war, widespread conflicts, natural calamities, drought, and famine. In such emergency situations when countries have to depend on international assistance for the survival of their people, economic sovereignty is often compromised. For instance, the issues involving GMOs in food aid were dramatized some years ago when some countries in Africa affected by drought and famine, namely Zambia, Mozambique and Zimbabwe, formally rejected the food aid brought in by the United Nation's World Food Program (WFP) on the grounds that the corn shipped from the US contained GMOs. Zambia, especially, held its ground by declaring that its decision was based on its responsibility to protect the health of its people and the integrity of its environment (Manda 2003). The WFP had to respect the stand of Zambia, and the controversy led it to formulate its position in procuring food aid from sources that could assure GMO-free food supply, whenever possible and available.

Intellectual Property Rights (IPRs). The issue of IPRs has received extensive attention and is the subject of intense debates at the international level. GMOs and GM products that are commercially available, even those that are still being developed, are protected by IPRs owned by the companies and institutions that developed them. The proprietary stake of companies over these products is at the heart of the discussion on who controls the technology and the resulting concentration in corporate hands that directly mould the relations to production and control over production in a given society.

Concerns about the implications of IPRs for GMOs extend beyond the economic sphere. The impacts of IPRs on public access to knowledge and technological innovations are far-reaching. IPRs have arguably hampered the free flow of information, knowledge and genetic materials that have served as the foundation of research and development efforts in public institutions. Proprietary control over useful technologies severely limit the potential of public institutions to pursue research that serves the interests of the poor, which is not considered a lucrative market for corporate products.

5.2 Social Considerations

Impacts on Farmers' Rights to Save Seeds. The potential consequences of GMOs on the traditional practice of farmers in saving, reusing, sharing, exchanging, and selling farm-saved seeds is a very important consideration in the assessment of socio-economic impacts of the technology. This is especially relevant in developing countries where farmers widely practice traditional seed saving and free exchange of planting materials, which may not be the case in developed countries where industrial agriculture is the dominant farming system. The traditional seed saving practices of farmers are widely regarded as the foundation of the immense genetic diversity in agriculture today. Thus, developments that may limit this practice, such as the stringent application of the IPR system on seeds, are seen as potential threats to the long-term food security of rural communities in particular and countries in general.

The inherent right of farmers to seed saving and exchange is legally protected by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) under the Food and Agriculture Organization (FAO). Governments are entrusted to protect farmers' rights through national legislation, a task which has not been easy for many countries that have also committed to protect IPRs of seed companies under international trade agreements, such as the Agreement

on Trade-Related Aspects of Intellectual Property Rights (TRIPS) of the WTO. Despite the flexibilities in the TRIPS Agreement, only a few countries have exercised the political will to protect farmers' rights to seeds while giving recognition to the proprietary rights of companies over innovations.

Part of farmers' rights to save and exchange seeds is their right to make decisions on their farms. The potential of GMOs to further entrench rural inequalities and marginalize poor farmers could also have long-term impacts on their capacity to decide on what, when and how to plant on their own farms. Experiences under the Green Revolution have shown how capital-intensive technologies could foster dependence on input providers among poor farmers who do not have the necessary capital required for adopting a new technology.

Impacts on Women. The impact of new technologies on women and gender roles in general is an area that should be looked into. The recent history of introduction of modern agricultural technologies has shown how rural women have been further marginalized and their roles made even more invisible by innovations which are generally designed for men (Paris 1998). In the case of herbicide-resistant corn that aims to eliminate the laborious task of weeding, women would be significantly marginalized since weeding is one of their primary tasks in corn cultivation, as for example in the Philippines. While this could decrease the burden of women in corn farming, their role will become further invisible, with men taking the primary decision making role on what varieties to plant.

Consumer Concerns. While GM seeds mean higher input costs on the part of producers, the technology promises to provide cheaper products to consumers resulting from higher and more efficient production. While price matters for most consumers, especially in developing countries, it is not the only factor that determines consumer responses to new products introduced in the market. Consumer acceptance is highly influenced by cultural and ethical values, and perceptions on health and environmental safety of the product – which are most relevant in the case of GMOs, as shown by a number of examples from developed and developing countries in recent years. Japan, Thailand and South Korea, following the trend set in Europe, now require labels on GMOs. While consumers in industrialized countries are generally less accepting of GMOs, their counterparts in developing countries can assert their right to choose.

6. Institutionalizing the SEIA

SEIA as a tool for decision making on approval and releases of GMOs needs to be institutionalized in the biosafety processes of countries. The specific government institutions responsible for implementing the SEIA processes need to be identified and their mandates have to be clearly formulated. Governments may decide to tap existing biosafety bodies or specialized agencies, independent institutions such as the academe, or create a special body for this purpose. In the case of the Philippines, for example, where socio-economic impact assessment is not obligatory, existing institutions responsible for biosafety decision making are tapped.
In order to be an effective tool for decision making, SEIA needs to be integrated in the biosafety decision-making policy and processes, such as the national biosafety framework, biosafety regulation or biosafety law of a country. SEIA should not be a stand-alone process, but should be an integral component of biosafety decision making. SEIA should neither be limited to an assessment after decisions on GMOs have already been taken, but should be integrated in different stages of the biosafety process – from the contained experiment, to the limited field trials up to the time prior to the commercial release of GMOs. Regulators should bear in mind that most of the socio-economic consequences of GMOs are likely to be irreversible and beyond anyone's control once the products have been disseminated to and adopted by society.

7. Socio-Economic Impact Assessment: Guiding Principles

To be effective in guiding decision making concerning GMOs, SEIA needs to involve the following key principles:

'Bottom-up' Approach. SEIA is essentially a bottom-up approach, involving the actors who may be affected by the potential impacts of GMOs. As a bottom-up approach, SEIA involves broad participation of the different actors of society who would most likely be affected by GMOs, which could differ according to the nature of the product involved. For instance, in the case of GM seeds, farmers are most likely to bear the costs or reap the benefits, and thus should play the biggest role in SEIA.

Based on public awareness. Active participation can only be expected from an informed public, which underlines the role of governments and civil society in providing balanced information and explaining the issues to the public.

Transparency and public access to information. Participation in decision making is largely determined by the trust and confidence of people in the government that initiates such processes. Public trust and confidence, in turn, are gained by governments that conduct their business in transparent and accountable manners, hence appropriate mechanisms need to be established so that the public has access to information on the status of approvals and on the basis of decisions made by regulators.

Provide alternative technologies and options. Awareness-raising efforts should also extend to broadening the public's perspective on other technologies and practices available to attain the same objectives aimed by a specific GMO. Information provided to the public should not be merely limited to a yes-or-no scenario but should provide inputs on technological alternatives to GMOs.

Multi-disciplinary assessment. SEIA clearly involves a multidisciplinary assessment and the role of social scientists in SEIA is largely limited to facilitating the process and providing necessary inputs that provide the appropriate context to the discussions with the various actors involved. Integrate in biosafety decision making and technology assessment framework. SEIA has to be considered as an integral part of the entire biosafety decision-making package in any given context, not as a stand-alone process. It should be explicitly recognized as such in national biosafety frameworks, regulations and laws.

Develop context-specific assessment tools. Regulators need to develop context specific socio-economic impact assessment tools with inputs from the different actors. In general, the processes involved in the SEIA and how they are actually implemented in reality would determine the credibility of the exercise as a basis for decision making on GMOs.

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Chapter 21

Putting Farmers First In Sustainable Agriculture Practices

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Long before development agencies and banks, Western-educated technocrats and consultants introduced irrigation to increase rice production in Asia and elsewhere, the Balinese communities had developed and practiced their own community-managed irrigation system called *subak*,[1] which is now known worldwide and is described in the literature on irrigation systems. Rather than a purely technical and hydrological process like the modern irrigation scheme, *subak* is a holistic socio-religious system with technical know-how on agricultural water management. Similarly, traditional sustainable agricultural practices, as will be described further in this chapter, are holistic approaches to food production and community welfare, as opposed to the narrow technological approach of conventional agriculture systems.

To return to the aforementioned irrigation example, during the Indonesian Green Revolution era beginning in the early 1970s, the community irrigation system, particularly in Java and Bali, was taken over by the government and the entire system was reduced to merely an issue of technical management of water for agriculture. Farmers were reduced from being water managers to water users. While practically all community-managed irrigation systems have disappeared in Indonesia, the Balinese *subak* system still exists, albeit under severe constraints.[2] In the same manner, community seeds and cultivation practices were taken over by single high yielding varieties and monoculture practices.

In general, Western-educated engineers, governments and international agencies unfortunately had, and still have, the mindset that communities 'have no technical know-how; they have to be given technology to improve their lives'. They tend to think community-based technologies do not exist, or are not viable. The *subak* case is just one example in terms of holistic water resources management for agriculture. This and other similar practices have proven otherwise. The technical know-how, and the management skills in agriculture exist; it is just that they are ignored, or sometimes considered non-marketable.

Thus, in discussing alternative agriculture, in the context of (conventional agriculture and) genetically modified (GM) crops that are being developed currently, it is important to note that alternatives exist; in fact 'alternative' agriculture systems are still, to a certain extent, mainstream practices in many parts of the world. There is increasing recognition that 'alternative' systems such as *subak* can constitute viable sustainable agricultural practices. *Subak* will be used frequently in this chapter to provide an example of holistic practice in agricultural resource management, because it illustrates the complex interlinkages between ecology, culture and

[1]The earliest historical mention of subak is found in Balinese ancient records from 1071. However, the system could have been in place before that, as the wet land rice cultivation was mentioned in the Sukawana AI record in the year 882 and the word 'water channel digger' (undagi pengarung) was mentioned in the Bebetin AI record in 896 (Purwita 1997). For an interesting account and understanding of subak as a socio-cultural religious system, see Lansing (1991).

[2]Sutawan (2004) in a personal communication said that the socio-religious aspect in Bali is so strong, that when the irrigation system was taken over by the government, the tertiary water channel was still managed through the subak system. However, the current threats to subak are posed by tourism development and the decreasing profitability of the agricultural sector.

technology, it has existed in a community for hundreds of years, and it has proven to be resilient, despite being ignored and underestimated.

This chapter describes key principles and approaches of sustainable agriculture, particularly at local community level, as a key alternative to GM crops and industrial agriculture systems, and is followed by an account of the successes of sustainable agriculture practices in some parts of developing countries, illustrated by three case studies. It concludes by arguing for the need for a paradigm shift in agriculture and outlines what changes such a paradigm shift would entail.

1. Principles and Approaches of Sustainable Agriculture

Sustainable agriculture is a practice of various techniques and principles ranging from IPM (Integrated Pest Management) to permaculture and agroecological systems. The key issue in sustainable agriculture is that there is *no single approach* that can be applied all over the world in a uniform manner; different techniques and systems are applied, *and* adapted, in different ecological and socio-cultural systems.

Sustainable agriculture follows the definition of sustainable development, i.e. meeting fundamental human needs while preserving the life-support systems of the planet. This is a concept that is easy to discuss but hard to implement because it requires a holistic approach within which science and technology are integrated with the social and political aspects of society, as well as with local and national economic development. However, there has been a de-coupling of science and technology from the social and political processes that shape the sustainable development agenda (Kates et al., 2001 in Buchori 2006). This is precisely what is happening with the development of GM crops, where scientists and technocrats develop new crop varieties and agricultural policies away from the reality of problems faced by farmers.
The holistic nature of sustainable agriculture is shown through the principles of IPM and agroecological approaches. Table 21.1 highlights the differences between IPM and non-IPM approaches in agriculture.

Table 21.1. Differences between non-IPM, conventional IPM and ecological IPM agriculture approaches.

Aspect	Non-IPM	Conventional IPM	Ecological IPM
Decision/target based on	Pest	Pest and natural enemies	Flora and fauna in the agro-ecosystem
Basis of control	Calendar or based on damages	One-dimension control threshold*	Multiple dimension control threshold**
Intervention method	Pesticides	Multiple intervention	Design of agro-ecosystem to minimize intervention
Diversity	Low	Low-Medium	Medium–high
Spatial scale	Plot	Plantation area	Landscape
Time scale	Immediate	One planting season	Long-term
Strategy	Chemically preventing	Responsive	Pre-emptive and responsive

Source: Buchori (2006)
* One dimension control threshold means that pest control will be conducted when the threshold of only one dimension is crossed. For instance, farmers will practice pest control when the population of a pest organism exceeds a certain level.

** Multiple dimension control means that pest control is based on the threshold of several dimensions. For instance, farmers will conduct pest control after getting information on various dimensions: i.e. the population threshold of a pest organism, the population of natural predators, the environmental conditions, the price of pest control, safety, etc.

IPM evolved particularly in Southeast Asia as a response to pest attacks on High Yielding Varieties (HYV) of rice in the 1980s, about ten years after the Green Revolution was adopted. As Table 21.1 shows, it evolved from a simplified response to pest attack into an ecological IPM approach, which is both pre-emptive and responsive. This shows that sustainable agriculture is a dynamic process in which knowledge management plays an important role. The principles of IPM are mainly: (1) to grow a healthy crop; (2) to enhance the role of natural pest predators in order to keep pest populations under control; (3) to understand the functional roles of different species, and therefore farmers conduct weekly observation of their fields (taking on the role of scientists); and (4) to have farmers as experts taking a central role in agriculture (Buchori 2006). Decision making is in the hands of farmers through observations and learning. In contrast, GM crop development is largely decided by scientists, companies and government officials without involving farmers. Under a GM crop regime, farmers are de-coupled from their crops and work; they will be less competent as they have a limited understanding of the molecular techniques used. Also, the ability and even legal right to act will be reduced due to patented genes.

A more comprehensive set of principles for sustainability is provided in the agroecosystem approach. According to Altieri (2002), agroecology goes beyond the perspectives of genetics, agronomy, hydrology, and so forth, to devise an understanding of *co-evolution* at ecological and social levels of agricultural systems' structure and functioning. Agroecosystems are communities of plants and animals interacting also with their physical and chemical environments, which have been modified by people to produce food, fiber, fuel and other products for human consumption. Thus, sustainable agriculture is not merely to produce food but provides other needs as well. As Uphoff (2002a) says, 'better human nutrition is a more important goal than food production alone, and will not be achieved only through greater grain output'.

By understanding the ecological relationships and processes in nature, agroecosystems can be enhanced to improve the production of food, fiber, fuel, and medicinal herbs as well as other commodities so that they become more sustainable, with other and more sound ecological and social impacts (Altieri 2002). To this effect, ecological processes can be optimized by applying the following ecological principles (Rejntjes et al., 1992 in Altieri 2002):

- Enhance recycling of biomass to optimize nutrient availability and to balance nutrient flows over time
- Provide the most favorable soil conditions for plant growth
- Minimize loss of energy and other growth factors, among others through microclimate management, water harvesting and better soil management
- Diversify species and genetic resources
- Enhance beneficial biological interactions and synergies.

In terms of economic components, agroecological approaches optimize the use of locally available resources. Socially, agroecological approaches build up and take full advantage of local knowledge and practices. Thus, the strategy is to encourage development methodology that supports farmer participation, use of traditional knowledge, and adaptation of farm enterprises to fit local needs and match up with socio-economic as well as biophysical conditions (Altieri 2002). In this respect, agroecological practices are often enhanced and strengthened by local institutions

and policies as opposed to uniform techniques at the national and global level. A key principle of sustainable agriculture is therefore the development, enhancement and protection of local biodiversity and local social capital, including local institutions, cultural practices, etc.
The *subak* situation described at the beginning of this chapter is a case in point. *Subak* is cultural capital for the Balinese in terms of managing water resources for agriculture (Pitana, personal communication 2004). The implementation of *subak* involves natural resources (water), human resources (experts on water systems) and cultural resources (Hindu- and Balinese-based institutional arrangements). The technical know-how is developed and governed by these three aspects. When modern irrigation systems were brought in, a complex interactive system was replaced by an alien single-unit system based on a single aspect: the technicality of bringing water to the fields. This system reduced farmers from managers to mere water users, with their competence overruled and their fate decided by government water 'experts'.
Thus, sustainability has to do with embracing the fundamental character of interactions between nature and society (Kates et al. 2001 in Buchori 2006). The following diagram (Figure 21.1) shows the elements and interactions of sustainable agriculture.

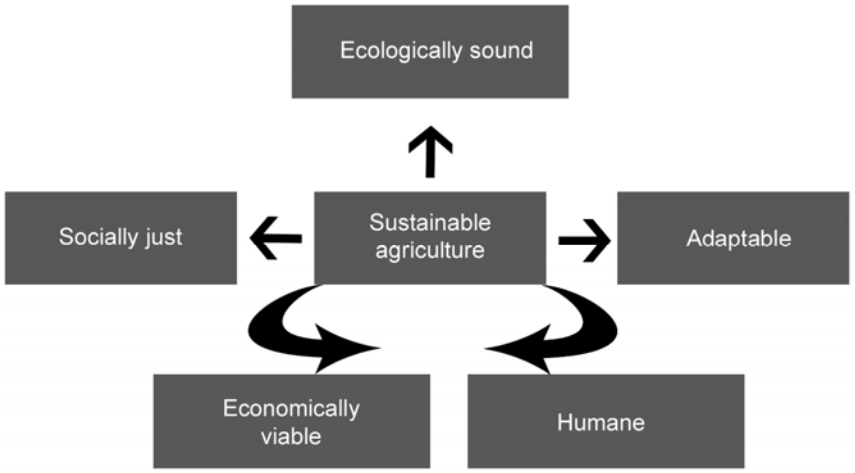

Figure 21.1. Elements of sustainable agriculture (Buchori 2006).

Two further elements can be added to the diagram: spirituality and culture. Again, the *subak* system serves as an example of a system that is rooted in religious (i.e. spiritual) and cultural elements. Another example of a spiritual element is the practice of providing a goddess status to staple plants, such as rice and corn. In Java and Bali, farmers traditionally treated rice as the Goddess Sri (*Dewi Sri*). The entire act of farming from seed selection, to sowing, to reaping the harvest was traditionally centered on the treatment of rice as a living being. Just before harvest, for instance, the Javanese farmers conducted the *wiwitan* ceremony. This is a ritual where farmers offered part of their harvest and various kinds of food to the Goddess Sri and asked her to bless their harvest. In effect, this is a seed selection process because farmers took the best rice stalks from the middle parts of their fields to be offered to the Goddess. These stalks were then saved and planted in the next season.[3] Such ritrituals were gradually abandoned with the adoption of the Green Revolution in Java, but still exist in Bali, albeit in a reduced form.

[3]Information from an interview with farmers in Central Java as part of an on-going process of documentation of sustainable agriculture practices by Third World Network (TWN).

From a scientific and technological point of view, such rituals may be seen as a 'waste of time and effort'. However, in sustainable agriculture, such rituals constitute a communion with nature, and involve cultural identification, as well as being part of the development of knowledge about local agroecological systems. Further, this is what sustainable agriculture is all about – providing nutritious food, medicine and fiber without taking cultural identities and power away from communities.

2. Sustainable Agriculture in Practice

There is a growing body of evidence that sustainable agriculture practices have been able to increase productivity with minimum damage to the environment compared to monoculture, i.e. industrial-scale agriculture. Notable among this is the study conducted by the University of Essex in the early 1990s. The study involved projects on more than four million farms in 52 countries to explore how the world's poor can feed themselves using cheap, locally available technologies that will not damage the environment. The findings showed that switching to environmentally and socially responsible farming improves harvests by an average of 73% (Greenpeace 2001). More recently, an international study team, led by Jules Pretty from the University of Essex, strengthened the previous finding. The team found that farmers in 286 projects in 57 countries have improved crop productivity by an average of 79% since the early to mid-1990s, while simultaneously increasing water use efficiency and carbon sequestration, and reducing pesticide use. Farmers used a variety of resource-conserving technologies and practices ranging from IPM and agroforestry to water harvesting and livestock integration (Lim 2006).

Alternative or sustainable agriculture practices are often not new but draw on traditional knowledge and practices, some of which have now been positively evaluated by scientific methods. With appropriate development and applications, they offer opportunities to increase food production (Uphoff 2002b). Case studies presented by Uphoff (2002c) show that new and better combinations of plant, soil, water, and nutrient management practices, combined with livestock and/or fish and IPM, can increase production by 50% to 100%, sometimes even to 200% or 300%. The crops reported in the case studies included rice, corn, beans, and potatoes. The experiences presented were not of particular technologies for selected crops (as is the case with GM crops) but rather the application of *principles* (italicized as in Uphoff) that can capitalize on existing genetic potentials. For instance, even a simple principle of intercropping two rice varieties can reduce crop losses and raise yields, as demonstrated in Yunnan province, China.[4] This simple technique stems from the knowledge about local agroecology, rather than a single technical idea.

While there are many reports showing the success of transitions to sustainable agriculture, these are mainly local- or community-based initiatives or studies at research centers located in different areas. In most cases, there are no national policies or institutionalization of these efforts and national governments also rarely design programs for sustainable agriculture. The exception is perhaps IPM, which was adopted as a national policy in many Southeast Asian countries during the 1980s. For example, Indonesia adopted IPM through a Presidential Decree in 1984. Since then, farmer field schools were established and within a few years there was a substantial reduction in pests as well as in foreign exchange spending for importing pesticides. Another

[4]Zhut et al (2000, cited in Uphoff 2002c) reported that planting rice varieties that are susceptible to blast with non-susceptible varieties reduces blast disease by 94% compared to rice grown in monoculture. The yield from susceptible rice varieties was increased by 89%. This was first practiced in 1998. Disease reduction was so successful that by 2000 farmers no longer used fungicidal sprays and the method was used over 40,000 ha.

example is the adoption of SRI (System of Rice Intensification), as described in one of the following cases.

The following cases illustrate further some of the benefits of sustainable agriculture practices.

2.1 Pesticide-free village

Punukula, a small, predominantly tribal, village in the state of Andhra Pradesh (AP), India – declared itself pesticide-free in 2003, even for crops which are notorious for their high pesticide consumption. Farmers in this village claim that their ecological approach to pest management is saving them Rs 3 million (approximately USD 64,000) a year, as reported by Kuruganti (2005). Farmers in Punukula began to use pesticides about 15 years ago when migrant farmers introduced cotton. Initially, the pesticides worked well and farmers bought them on credit from the shops in a nearby town. Gradually, however, pests became resistant to the pesticides and farmers had to spend more money to buy greater quantities of pesticides. In addition to selling pesticides, fertilizers and seeds on credit, the agrochemical dealers also began lending money to farmers at high interest rates. When the debt trap closed in, farmers who could not repay their debts began to commit suicide.

In 1999, a local NGO, the SocioEconomic and Cultural Upliftment in Rural Environment (SECURE), introduced ecological methods of farming. Five self-help groups run by village women provided the determination and support to help make this shift possible. Instead of chemical sprays, the farmers began preparing sprays made with inexpensive local materials such as neem seed powder and green chilli-garlic extract. The sprays were supplemented by hormone traps to attract the moths and destroy them before they started mating. Some farmers also used 'crop traps': planting marigolds and castor, which the pests preferred, alongside cotton.
One season was enough to demonstrate the difference: spiders, wasps and beetles – which feed on cotton pests – returned to the fields once the chemical spraying stopped. In the next season, many other farmers tried out this new approach. While men still found it more practical to buy pesticides, women took on the work of preparing the ecological anti-pest sprays, and ensuring that no one brought pesticides into their village.

By 2003, most farmers in this 200 household village had stopped using pesticides. The new methods were used not only in cotton fields, but also for chilli and paddy as well. In August 2004, the women groups, with support from SECURE, bought a machine to crush the neem seeds into the powder used for the sprays. Punukula farmers now have money to invest in house repairs, buy land, invest in livestock, and repay their debts. They believe that the way to get rid of pests is to rid their fields of pesticides. Neighboring villages are beginning to show an interest in the approach because of the successes.

2.2 Adoption of SRI in Cambodia

SRI (System of Rice Intensification) is a method of rice cultivation that uses less input, especially water, among other efforts.[5] The Government of Cambodia has integrated SRI promotion into its national development plan for 2006–2010, given the results demonstrated by these methods. As reported on the SRI Group Website[6] at Cornell University (January 2006), SRI was introduced by the director of the Center for Studies and Development of Cambodian Agriculture (CEDAC), Dr Koma Sang Yaing, who first tried SRI methods in 1999. In 2000, CEDAC was able to persuade

[5]SRI is a method of rice cultivation that combines using less water, fewer seeds and more organic fertilizer. In SRI, the field does not have to be flooded, rather excess water has to be drained. Seedlings are transplanted when they are only two weeks old and planted farther apart, with one seedling in one hole instead of several seedlings. The harvest is often more than double the conventional method. For more detailed information on SRI see http://ciifad.cornell.edu/sri

[6]http://ciifad.cornell.edu/sri/

28 farmers to try out the methods for themselves. The good results encouraged 400 farmers to use SRI in 2001, and 3000 farmers used it in 2002. The spread of SRI has been driven particularly by farmers' own initiatives.

CEDAC conducted an evaluation of the SRI experience of 120 farmers who had used SRI methods for three years (2001, 2002 and 2003). Even though not all the farmers were using all of the SRI methods as recommended, the evaluation showed that even partial use of SRI enabled them to harvest 2.75 ton per hectare on average, compared to 1.34 t/ha using conventional means. Fertilizer use has reduced from 116 kg/ha to 67 kg/ha on average, and chemical pesticide use has declined from 35 kg/ha to 7 kg/ha. Costs of production have been reduced by half, and household incomes, even with use of SRI on only part of the land used for growing rice, have almost doubled. Of the farmers who were surveyed, 55% said SRI reduced their labor requirements, while only 18% said it increased labor requirements, and 27% said it made no difference. Another evaluation of SRI was conducted by GTZ**,** the German development agency, in February–April 2004. Data were gathered from 500 farmers, randomly selected in five provinces, 400 of them being 'SRI users' and 100 'non-SRI' for comparison. Not all of the SRI users were using all the recommended practices, or using all as recommended, but even so, a 40% increase in yields was documented, along with a 75% increase in net income per hectare, due in part to substantial reductions in farmers' costs of production. Most significantly, the study found that there was no real increase in labor requirements for using SRI. Labor savings made during transplanting (a time of peak labor demand, when 10 person/days per ha were required) offset the increased labor needed for weeding (which could be done with flexible timing). Also, reducing the need for cash expenditure at the start of the planting season, when household cash reserves are lowest, was beneficial for farmers. One farmer, who received an award for highest SRI yield, attained an average level of 14.6 t/ha, with one crop-cut of 2 kg/m2 (20 t/ha).

The Cambodian Ministers of Agriculture and Environment have promoted SRI because it fits with the national strategy for the agricultural sector: intensification (including SRI), diversification (facilitated by SRI gains in land productivity), compost use to improve soil fertility, and fish culture (SRI makes it possible to free up land area for fish ponds). Farmers are now making many modifications in their farming systems, based on SRI, to diversify production for both better income and nutrition.

2.3 Experience of an organic rice farmer in Java, Indonesia

Giyanto, a farmer from Delanggu, Central Java, Indonesia, switched to organic farming in 1999.[7] This was a period of economic crisis, when the price of inputs soared due to the declining value of the Indonesian currency (Rupiah) to the US Dollar. As agrochemicals and their component materials had to be imported, their prices increased drastically. Farmers could not afford the use of agrochemicals.

Giyanto began organic rice farming by adopting the local, almost-extinct variety of *menthik wangi*. He found that the production costs were reduced, partly by using the traditional method of *singgang*. In this method, during the first rice harvest, farmers leave 10 cm of the stalk, measured from the ground. The plant will flower again and produce another harvest of rice. The first batch of rice is harvested after 120 days of planting, and the second (after the *singgang* treatment) can be harvested after 80 days. This reduces the cost twofold. However, the *singgang* method is not easy and requires patience. It can only be done twice to ensure quality; but this practice is also a way to maintain pure lines of a certain variety. This method does not work for conventional rice farming.

[7]Notes from ongoing documentation of sustainable agriculture practices in Indonesia by Third World Network (TWN).

Giyanto also rears chickens and uses their waste as manure. He can produce 1 ton of manure a month from his 1000 chickens, enough to fertilize 2000–3000 m^2 out of his 8000 m^2 of farmland. In addition, he does not plant paddy throughout the year like many Javanese farmers. Giyanto plants onions during the dry period of June–November when there is less water, for two reasons. First, this reduces the risk of rice harvest failure due to lack of water. Secondly, it breaks the cycle of rice pests. When rice is planted all the year through, the pests have plenty of food to eat and they become prolific. This can lead to a disproportionately high pest population. Planting a non-rice crop, even for one season, breaks the food supply of pests and therefore can reduce pest incidence in the next rice planting season.

Giyanto sells his rice harvest to the SAHANI (SAHAbat PetaNI, or friend of farmer), an organic fair trade shop in Yogyakarta whose management is farmer-driven. This shop collects the harvests from farmers, thus reducing the costs incurred by farmers. The shop buys the rice at a fixed price, so farmers do not have to face fluctuating market prices. Giyanto said SAHANI gives a better price for the organic produce compared to the market price for non-organic rice. For instance, organic *menthik* rice is Rp 5000/kg while the non-organic variety is Rp 4700–4800 per kg.

In Indonesia, Java is the centre of agrochemical agriculture, particularly for rice. The organic movement has grown over the past ten years but faces many constraints. First, most farmers either own only 0.3 ha of land (Giyanto is a rare exception) or no land, i.e. they are farm laborers with no decision-making power over what to plant. Second, farmers have been so used to agrochemicals over the last 35 years that it is difficult for them to change their mindset; they have also lost much of their cultural wisdom. Third, farmers want better and fixed market prices for their organic produce as the initial costs are higher as a consequence of the soils having been degraded for so long. However, there are small groups of farmers who have realized the value of organic farming and have gradually made the transition that Giyanto has made.

Several lessons can be learnt from the aforementioned cases. None of them involve a single technological innovation per se. Rather, they involve policy, institutional and marketing issues. First, in India, it was the women's group that made the shift to sustainable agriculture practices. In fact, the role of women has been sidelined in the Green Revolution. Projects to promote the Green Revolution in the villages mostly involved men; this took away many of the decision-making powers (crop selection, food storage, etc.) and jobs (weeding and harvesting) that used to be the domain of women. The shift from sustenance to a market economy was made by men. At the same time, women suffered serious health impacts due to the excessive use and misuse of agrochemicals, particularly pesticides. The introduction of GM crops is likely to repeat the situation as these crops are targeted towards the market rather than for local food security. Thus, sustainable agriculture is a way to restore the domains of women in food production as well as to improve peoples' health, local competence, and economy and incomes.

In the Cambodian case, it was the research institution that became the agent of change together with farmers, leading to adoption of alternative methods by the government. The research institution took the initiative to try the new method, but farmers were involved in the trials and had the decision-making power as to whether or not to adopt SRI. Indeed, farmers adopted this method partly because of the increased yield, but also because they were free to modify and adapt the method, unlike a single technological fix that cannot be easily locally adapted.
In Java, farmers tried to revive an old practice that can cut costs, while cooperation with a farmer-friendly shop under the fair trade regime ensured the income of farmers reverting to sustainable practices.

Such complex issues as have been described cannot be solved through a single technological approach such as GM technology. Instead, what is needed is a complete paradigm shift to a more holistic (but diverse) approach that takes these complex issues and the various sustainable agricultural principles into account. This new holistic paradigm would integrate diverse socio-economic, cultural and ecological aspects with adaptive technology development based on local knowledge and innovation, and local resources.

3. The Need for a Paradigm Shift

A paradigm shift, especially in knowledge systems, is needed because the current conventional agriculture system, and its extension to GM agriculture, is based on a dichotomy between a single technical knowledge system and diverse local knowledge systems. In fact, diverse local knowledge systems have either been ignored or marginalized. The following example illustrates this point. Approximately 50 years ago, Mukibat, an Indonesian farmer, devised a technique that can increase the yield of cassava by five times or more. He merely grafted cassava tubers on to the root of a wild rubber tree from the same genus as cassava (*Manihot*); this gives the growing tubers more access to sunlight and nutrients (Forest et al., 1994 in Fernandes et al. 2002). Since then this has been called the Mukibat Technique. Yet this technology has aroused little scientific attention and was only reported in the literature more than 20 years after it was introduced. This could reflect the indifference or ignorance among researchers about farmer innovation, or of cassava simply being regarded as a low-status staple crop despite the fact that hundreds of millions of people depend on it for sustenance (Fernandes et al. 2002).

The Mukibat case is a clear indication that the current scientific system does not accommodate local knowledge systems. Yet, any new technological innovation that comes only from the scientific and technocratic communities will not solve the food and agriculture problems facing the world today. Instead, a multi-stakeholder process, diverse knowledge systems and consideration of the interlinkages between all aspects of agriculture are needed to solve these problems.

The basic paradigm shift needed is the recognition that governments, scientists and corporations cannot feed the world in the absence of policies and practices that allow communities to feed themselves. Thus, the solution lies not in feeding the world, but in allowing the world to feed itself (Greenpeace 2001). This is a complex problem that requires a holistic paradigm, policies and practices, not a single, quick technological fix.

To bring about the paradigm shift in agriculture, the following five elements are necessary. First, we need to recognize that *alternatives to conventional and GM agriculture exist.* As stated before, many of these ‘alternatives’ are actually mainstream practices in many parts of the world. They exist as local innovations, and are dynamic in the sense that they can be modified to adapt to current situations. What is urgently needed is the right institutional, economic and policy support to ensure that these alternatives are scaled up.

Second, *farmers are innovators and applied scientists at the micro level*. They have the appropriate knowledge about their work and local socio-ecological conditions. The Mukibat technique is a case in point. Ignoring such innovative practices is technocratic arrogance that hinders efforts to achieve food security. The lack of recognition and acceptance of indigenous knowledge have regrettably led to many (although not all) mainstream scientists ignoring traditional farmers’ rationales and imposing conditions and technologies that have disrupted the integrity and sustainability of native agriculture (as argued by Altieri).

Third, *diversity and interlinkages of agroecology and socio-culture must be recognized and taken into account.* Conventional chemical- and technological-based farming systems have converted agri-'culture' from a socio-cultural and ecological process by delinking the technical aspects from other socio-cultural and ecological processes. Whereas, in traditional systems, social relationships and cultural patterns govern technical know-how, the modern practices reduce such social systems into 'monoculture technical know-how devoid of (local) culture'. As an example: the world cannot plant a single Bt cotton variety all over the earth from the US to Australia through Africa and Asia, where ecosystems and socio-cultural systems differ. A holistic food production system must be put back in place.

Fourth, *we need to get the policies and institutions and incentives right.* The current banking system, for instance, favors chemical- and technological-based agriculture for credit loans. Governments (often centralized), institutions and policies wipe out diverse local and indigenous institutions that govern agroecological systems, as is shown in the *subak* case. *Subak* used to be a *self-organized irrigation institution* until the government took over irrigation management. Such local institutions, if they still exist, need to be supported rather than demolished. Similarly, appropriate government policies are required to protect, nurture and develop local agroecological systems.

Fifth, *any agricultural innovation must guarantee equity for the farmers*. Many technological innovations widen the gap between rich and poor farmers as they are not governed through local institutions. GM technology, for instance, can only be adopted by rich landowners who can take higher risks in agricultural practices. The Green Revolution process has shown how farmers become impoverished when they enter a debt trap, usually through credit to buy agrochemicals, as shown by the case cited from India.

Finally, it cannot be overemphasized that the world will feed itself better and in a more sound and ecological manner through *farmer-driven, locally-adaptive and diverse systems*.

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Chapter 22

A general introduction to the regulation of GMOs and gene technology

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1. Introduction

In this chapter some of the historical background of regulations connected to GMOs and gene technology will be elaborated. Most of the gene technology regulations developed throughout the world have many similarities: they are based on the same regulatory and management mechanisms and principles, and have a common historical background.

During the 1970s and 1980s national research institutions, scientific societies and authorities (e.g. National Institutes of Health (NIH) and the National Academy of Sciences in the USA), international organisations (e.g. Organization for Economic Cooperation and Development) and regional unions (European Union) were heavily involved in debating safety issues linked to recombinant DNA technologies (rDNA). The different molecular methodologies used in the technology development are often combined in the terminology: *gene technology* or even the wider term of *modern biotechnology*, as defined in the Cartagena Protocol on Biosafety (see Chapter 23 on terminology and definitions). In this chapter, the term *rDNA organisms*, which stands for recombinant DNA organisms, will be used mainly synonymously with genetically modified organisms (GMOs) if not otherwise stated. 'rDNA organisms' was a terminology that was more commonly used in the earliest phase of the development of the technology, but at a later stage and at present most people use the term 'GMO'.
The majority of the OECD countries developed and enacted their regulations during the late 1980s and the beginning of the 1990s, while most developing countries are currently in the process of developing their GMO policies and regulations, or have recently finalised them. This is in accordance with the obligations prescribed in the Cartagena Protocol on Biosafety, which entered into force in September 2003 (Cartagena Protocol on Biosafety 2000). One important observation is that the OECD countries had their regulations in place when the first GMO entered the market in 1995, while most developing countries are struggling with developing their policies and regulations as an increasing number of GMOs are entering the world market today.

In this chapter I will also elaborate on some of the main systems, terminology and principles used in regulations and guidelines, and explain both the political and scientific rationale behind their development and usage in the regulatory context (e.g. case-by-case handling and the step-by-step procedure). I will describe the most common elements encompassed in regulatory approaches linked to contained use, deliberate release, and ethical, social and socio-economic considerations, including public participation, using examples from existing legislation (see Table 22.1 for definitions and use of some central terms).

Table 22.1. Explanations regarding some of the most used terminology and principles that connect regulations of GMOs to their development, application/notification and use.

Topic/ Subject	Regulatory use	Rationale behind the use
Contained use	Term used for production and research with GMOs, including general usage of gene technologies, in specific contained facilities. Usually found in most countries' GMO regulations.	Prevent the spread of GMOs and transgenic molecules outside the contained facilities. Protect the environment, animals, workers, and the public from possible known and unknown risks and hazards that might arise (e.g. when developing, doing research, production, etc.) with GMOs in laboratories or other contained facilities.
Deliberate release	Intentional release of GMOs in any way, through experimental or commercial releases into the environment or to the market. Term used in most countries' regulations.	Term used in application procedures for releases of GMOs. Separate actions conducted with GMOs from those in contained use and accidental releases. Often used in connection with risk assessments and risk management procedures and requirements for both experimental and commercial releases.
Case-by-case principle	Regulatory principle in order to separate management of specific GMO applications from other GMO applications that authorities receive.	Connected to risk assessment procedures. The rationale is that each GMO transformation event may differ, and therefore should have a separate peculiar evaluation by the authorities (and the applicant), in order to evaluate all possible hazards and risks of that specific GMO.
Step-by-step procedure	Used as a part of the scientific research in development of GMOs in order to prevent possible hazards from being realised. Knowledge gained through this stepwise procedure is an important basis for collecting information needed in risk assessments and application of specific GMOs.	The step-by-step procedure is used during research and development stages, and includes that a GMO should be characterised and carefully observed, whereby safety and performance data are collected at each research stage from e.g. laboratory, microcosms, glasshouses, before small and larger field testing is conducted. If a hazard or negative potential is identified, the organism can be brought back to a higher confinement level for safety reasons, or the experiment can be terminated.
Risk assessment	A very important part of the GMO regulation, evaluation and management system. Found in most countries' GMO regulations connected to the application and decision procedures.	A thorough systematic evaluation to identify all possible risks and hazards connected to a specific GMO and its possible usage. Risk assessments can be executed in many different ways, but should always be based on the best updated and relevant scientific data and information regarding the GMO in question, in order to be conducted appropriately. Risk assessment is a cross-cutting issue procedure with many scientific fields involved.
Risk management	Measures and strategies to regulate, manage, control and prevent risks from being realised. Different regulatory approaches to risk management are found in most countries' regulations and handling of GMOs.	The rationale is to introduce e.g. appropriate mechanisms or measures to prevent harm or hazards from GMOs that might have been identified in the GMO risk assessment or might happen unexpectedly. In many cases a risk assessment will not give a definite answer to possible risks; risk management measures may therefore be essential to prevent unexpected damage.
Traceability	Traceability is used, e.g. in EU regulations, to facilitate tracing and withdrawal of products where unforeseen effects occur. It also facilitates	Traceability can be implemented in order to facilitate control of GMOs in the market, due to lack of knowledge of possible unforeseen adverse effects from GMOs on the environment, biodiversity, human health, and society. Segregation, labelling and monitoring of GMOs after approval for marketing, are therefore a central part of

	risk management measures and labelling requirements of GMOs.	traceability regimes in order to reveal possible adverse effects (includes product information preservation).
Co-existence	Co-existence refers (especially in the EU) to the ability of farmers to make practical choices between conventional, organic and GMO production, in compliance with legal obligations for labelling and/or purity standards within the EU.	Cultivation of GMOs is likely to have implications for organisation of agricultural production. The possibility of unintended presence of GM crops in non-GM crops raises the question of how a producer's choice of different production types can be ensured. Co-existence regimes are therefore important in monitoring, labelling and segregation of GM crops from conventional and organic crops. Further, co-existence regimes, together with registers for cultivation and monitoring regimes, will simplify tracing of adverse effects from GMOs, if such effects occur.

2. Historical background of biosafety regulations and regulatory policy development

2.1 The first initiatives for regulations were taken in the USA

One of the first occasions where worries were clearly pronounced and debated in connection to gene technologies took place at the Gordon research conference on nucleic acids in the USA in 1973. At that time recent advances in DNA methodologies and related research activities made scientists concerned regarding the newly developed methodology of replicating bacterial plasmids with e.g. introduced virus genes. At the conference, scientists raised concerns about possible adverse effects of the ongoing recombinant DNA (rDNA) research activities. They identified, to some degree, the need for adequate methods to prevent the spread of rDNA molecules due to lack of knowledge and uncertainties in predicting possible negative effects. This led the US National Academy of Sciences to ask Dr Paul Berg to head a committee on recombinant DNA molecules.

In 1974, the 'Berg Committee' published their well-known letter in *Science* (Berg et al. 1974). The Berg committee requested the National Institutes of Health in the USA to consider the establishment of an advisory committee. They also requested scientists working in this field not to conduct certain experiments on bacterial plasmids and rDNA molecules involving antibiotic resistance, bacterial toxins, and cancer and tumour development.

The Berg committee wanted an advisory committee to be in charge of: i) overseeing an experimental programme to evaluate the potential biological and ecological hazards of certain types of rDNA molecules; ii) developing procedures which would minimise the spread of such molecules within human and other populations; and iii) devising guidelines to be followed by investigators working with potentially hazardous rDNA molecules.

As a result of the recommendations from the Berg committee and the concerns raised by scientists working in this field, the International Congress on Recombinant DNA Molecules was organised in February 1975 at the Asilomar Conference Centre in California (Berg et a1. 1975). Many of the conference participants were among the leading molecular biologists in the world, but journalists were also represented. The Asilomar Conference made a statement that was approved by its Executive Committee and the Governing Board of the National Research Council acting on behalf of the United States National Academy of Sciences. The following quotation is from the summary statement:

> *The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the Conference agreed that most of the work on construction of recombinant DNA molecules should proceed provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, are employed. Moreover, the standards of protection should be greater at the beginning and modified as improvements in the methodology occur and assessments of the risks change. Furthermore, it was agreed that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. In the longer term serious problems may arise in the large scale application of this methodology in industry, medicine and agriculture. But it was also recognized that future research and experience may show that many of the potential biohazards are less serious and/or less probable than we now suspect.* (Berg et a1. 1975)

The conference identified some experimental designs and conditions that should be followed when conducting research with rDNA molecules. These included containment levels for minimal, low-, moderate- and high-risk experiments, and matching types of containment with types of experiments. They also identified certain experiments that should be deferred, such as cloning of recombinant DNA derived from highly pathogenic organisms, DNA containing toxin genes and large-scale experiments using rDNA that are able to make products potentially harmful to humans, animals or plants. Due to the recommendations and discussions from the Gordon research conference, the Berg committee and the Asilomar conference, the first NIH guideline on rDNA was developed and entered into force in 1976. The intended application of the NIH guideline was for scientific research on bacteria and rDNA molecules in containment. The NIH guidelines were effective only for research conducted within the USA and funded by the US Government. The guideline was voluntary for privately funded research institutions and industry. Many national authorities and research communities in other countries followed the discussions in the USA closely and took steps to introduce similar management strategies in their countries. In the years to come, the NIH guidelines were revised many times.

Already in 1975 the first basic outline of what we can call the GMO regulatory approach was drawn up. This includes an advisory committee, something that is common in many countries today, and ‘containment guidelines’ or regulations to minimise unintended release and possible negative effects. This first NIH ‘containment guideline’ was mainly linked to safe handling and possible spread of rDNA molecules and recombinant microorganisms from laboratory research and development facilities. Later, due to scientific developments, the safety focus shifted from contained research and production systems to deliberate release of GMOs for different types of usage in the release environment, or as marketed products.

During the next ten years the development of methodologies improved and research progressed greatly, including experiments with both recombinant plants and animals. At the same time, the potential of the methodologies within many different biological research fields and production systems was clearly recognised, and was also regarded as having a very optimistic future by both the private sector and governments. Modern biotechnology therefore became a fast, hot growth area for future research development and economic investment.

2.2 OECD (Organisation for Economic Cooperation and Development)

In 1983, OECD member countries established an ad hoc group of governmental experts on safety and regulations in biotechnology. This was due to the ongoing discussions regarding safety issues, rDNA guidelines and different regulatory processes, where the wish for future harmonisation of guidelines and regulations between the member countries was also an issue. The group’s mandate was to:

> *i) Review country positions as to the safety in use of genetically engineered organisms at the industrial, agricultural and environmental levels, against the background of existing or planned legislation and regulations for the handling of microorganisms*

> *ii) Identify what criteria have been or may be adopted for the monitoring or authorisation for production and use of genetically engineered organisms in: industry, agriculture and the environment. Explore possible ways and means for monitoring future production and use of rDNA organisms in: industry, agriculture and the environment.*

In 1986 the OECD published the report from the Ad Hoc Committee, titled *Recombinant DNA Safety Considerations*, the so-called 'Blue Book' (OECD 1986). Although the committee stated that they '*recognised that there is no scientific basis for specific legislation to regulate the use of recombinant DNA organisms*', paradoxically the work of the Ad Hoc Committee, and the introduction of safety considerations and risk assessment procedures, which to some degree were outlined in the Blue Book, in many respects became the basis for regulations of GMOs and gene technology in the Western world.
The first chapter of the OECD book lists examples of successful ongoing research activities, and gives a particularly optimistic perspective for the future application of rDNA techniques within many areas. Most of these optimistically predicted applications have never been successfully realised, but in some areas, especially in contained production with rDNA microorganisms, the 'dream came true' to some extent. Today, there are many products on the market developed from contained production with microorganisms, e.g. enzymes for pharmaceutical and industrial usage. In other areas, especially rDNA-plants for crop production, experimental release trials increased dramatically during the beginning of the 1990s, and marketing of GMOs for production as food and feed, after 1995. This contributed to bringing forward the scientific and regulatory political controversies linked to possible negative effects from rDNA plants on the human health, the environment including biodiversity.

In the second chapter of the OECD book, safety considerations are outlined, and we are given a first introduction to risk assessment methods and considerations linked to rDNA organisms. Linked to application of rDNA micro-organisms, much of the methods described were adopted from a report by the US Office of Technology Assessment (OTA 1981). However, the Ad Hoc Committee had intended for the methods described to be also, in principle, applicable to plants and animals.

With special references to agriculture and environmental applications, the OECD Ad Hoc Committee stated that an independent review of potential risks, on a case-by-case basis, of rDNA organisms was recommended. This is still the main requirement in governmental regulations connected to handling of GMO applications and risk assessment procedures, but there are options for fast track procedures in some countries' regulations, and also in the EU directive on deliberate release of GMOs.

The OECD's Blue Book describes the step-by-step procedure, a process of progressively decreasing physical containment, and recommends that the procedure should be used as a part of the scientific research and development of GMOs in order to prevent possible hazards from being realised. The knowledge gained through these stepwise procedures would therefore be important in the risk assessment of a specific GMO. The step-by-step process conducted during research and development stages means that a GMO should be characterised and carefully observed, whereby safety and performance data are collected at each research stage from laboratories, in microcosms or other contained environments, before small and larger field testing is conducted. In this way, predictions can be made of the organism's behaviour in subsequent less confined stages of development. If a hazard or negative potential is identified, the organism can be brought back to a higher containment level for safety reasons, or the experiment can be terminated.

The OECD's Group of National Experts (GNE) on safety in biotechnology continued the discussions throughout meetings and workshops for many years. Since 1995, the OECD's working group on Harmonisation of Regulatory Oversight in Biotechnology has been active (complemented by the OECD's Task Force for the Safety of Novel Food and Feed), although probably not as important in setting the international agenda for discussion today as during the

1980s. The different OECD groups and workshops that have been arranged have made a considerable contribution to risk assessment guidelines and biosafety regulations that are documented through a huge number of OECD publications (for further information see the OECD's database BioTrack at: http://www.oecd.org).

2.3 Some examples of national regulatory approaches

Although most OECD countries in the earliest years of GMO discussions did not have separate regulations, some of the aspects of modern biotechnology were regulated through already existing regulations, such as regulations on industrial production, pollution control, product certification, etc. Some countries had introduced recombinant advisory committees, that gave advice both to authorities and researchers, and in many cases the committees also initiated and arranged conferences, workshops and informed the public about modern biotechnology.

Due to scientific progress, especially with genetically modified (GM) plants, scientists and the emerging biotechnology industry wanted to conduct field trials. There was therefore an increased focus on environmental safety in connection with GM plants and field releases. Some countries (e.g. USA and England) developed guidelines for safe field experiments with GM plants. Later, during the first half of the 1990s, some countries also developed experimental guidelines for aquatic animals (fish), microorganisms and viruses.

Denmark was one of the first OECD countries that developed a separate Act regulating gene technology in connection with the environment. The purpose of the Danish Act, enacted in 1986, was '*to protect the environment, nature and health, including considerations of nutrition in connection with the application of gene technology*'. At the end of the 1980s many European countries considered following the Danish example and developing specific regulations on modern biotechnology (e.g. Norway), while many other countries in the world preferred voluntary guidelines (e.g. Australia and USA). During this period, the discussion regarding the need for new EU regulations on biosafety started, and at the beginning of the 1990s all contained use of GM microorganisms, and experimental and commercial releases of GMOs, were regulated with the implementation of the new GMO directives (Directive 90/220/EEC and Directive 90/219/EEC).

How to manage the regulations by national authorities was also intensely debated in many countries. Some countries chose to divide the management of the regulations among those authorities with jurisdiction over similar problem areas related to conventional organisms or production systems, while others invented new solutions. In most cases, the ministries of environment, agriculture, fisheries, and health, and their underlying institutions or authorities, are involved in the management of biosafety regulations, GMO applications and risk assessments in some way or another. It is also common that different types of national committees are more or less involved in the regulatory processes, give guidance to authorities, and in some countries they are also the appointed authority connected to GMO applications. During this period, public debate started to increase, especially in Europe. In many countries the debate had political influence on the development of new regulations, including e.g. requirements for labelling.

2.4 The European Union regulatory approach

The EU regulatory system linked to GMOs and gene technology has developed into one of the most comprehensive and advanced regulations in the world. I will therefore, to a large extent, use the regulations and management system in EU as an example and basis for explaining regulatory approaches, problem areas and the reasoning behind regulations. This will later be linked to the definitions of GMOs and what is usually not covered in existing regulations (Chapter 23), which is also a challenge linked to the Cartagena Protocol on Biosafety and for all countries' authorities. First, I will briefly explain the general regulatory system in the EU and some of the history behind the revision of the directives.

The two EU Directives, 90/220/EEC on deliberate release of GMOs and 90/219/EEC on contained use of genetically modified microorganisms (GMMs), were adopted in 1990 and

entered into force in 1991. Directive 90/220 regulated both experimental and marketing releases of GMOs. Directive 90/220 did not give the member states the opportunity to have stricter regulations than what was outlined in the articles, while this was possible under Directive 90/219 on contained use of GMMs. The containment Directive was primarily implemented at the national level, while deliberate release also involved the member states at the community level.

Directive 90/220/EEC depended to a high degree on cooperation between competent authorities of the member countries in decision making. It gave authorities the opportunity to comment on experimental releases in other member countries through the summary notification information format (SNIF) system that was established for this purpose. Countries receiving comments regarding applications for national release experiments were not obliged to follow the comments or recommendations received, but would be wise to take them into consideration.

When an EU country received a notification for commercial marketing release, the competent authority in the country receiving the application conducted a risk assessment based on the information in the notification. If a country intended to approve a notification, it had to send its positive assessment to the European Commission and the other member countries for comments. After a fixed period of time, discussions and voting in the EU committee of competent authorities, a decision on whether to approve the application or not had to be taken. The EU Council (representing ministers) would take a final decision if the EU committee is not able to come up with a final decision in favour or against the application. One of the major criticisms of this approval system within the EU was that if the Council does not act within three months (or in practice does not reach an agreement), the proposed measures have to be adopted by the European Commission (in other words, the decision is taken by the Commission). In most cases, at this stage of the decision procedure, the Commission was in favour of approving the marketing.

In 1993, marketing of GMOs as medical products for human and veterinary use was lifted out of the EU Directive 90/220/EEC and regulated by a separate product regulation (EEC 1993). Pharmaceuticals that are GMOs are managed by the European Agency for the Evaluation of Medicinal Products (EMEA) which began its activities in 1995. Regulation (EC) No. 2309/93 was replaced by a new Regulation (EC) No. 726/2004 which entered into force on 20 May 2004 (EC 2004).

Mainly due to disagreement between different EU authorities on how the deliberate release directive was operated, increased criticism was raised on limitations in the regulatory framework and insufficient attention to important risk-related issues, including lack of knowledge as basis for risk assessments; a ‘de facto moratorium’ against approvals of GMOs became the consequence in 1998. In parallel, there was also an ongoing controversy between the biotech industry, scientists, non-governmental organisations (NGOs), and authorities regarding safety, risk assessments and handling of GMO applications in Europe. This debate clearly did not escape governments’ attention. With changes in government in some major EU countries between 1995 and 1996, which also entailed stricter GMO policy, it was decided to revise the 90/220 Directive. Due to the regulatory revision processes, and the finalisation of the Cartagena Protocol on Biosafety, the drafting of new regulations on GM food and feed, and traceability and transboundary movement of GMOs began in the EU.

3. The EU regulations on GMOs after 2002

Although there are many similarities in the EU regulations before and after the revision of the 90/220 Directive, there have also been many changes, both through new legislation and new management regimes. The biosafety regulatory framework follows the GMO development process from research in contained use, to deliberate release and placing on the market, to labelling and traceability of GMOs as food, feed, or for processing, and to transboundary movement that implements obligations under the Cartagena Protocol on Biosafety in the EU.

The different regulations are:
1) Contained Use Directive 90/219/EEC (EEC 1990)
2) Medicinal Products for Human and Veterinary Use Regulation (EC) No. 726/2004 (EEC 1994)
3) Deliberate Release Directive 2001/18/EC (EC 2001)
4) GM Food/Feed Regulation 1829/2003 (EC 2003)
5) Traceability Regulation 1830/2003 (EC 2003)
6) Transboundary Movements Regulation 1946/2003 (EC 2003).

The main difference between Directives and Regulations in the EU is that Directives have to be implemented via national member states' laws, while Regulations are directly applicable. Many practical guidelines have also been developed on how to interpret different regimes under the regulations. I will not describe in detail any of these guidelines (for further information see either http://gmoinfo.jrc.it/ or http://www.biosafety.be).

In general, the policy of the EU in relation to GMOs tries to ensure that there are safety nets available. These are put into operation via risk assessments that are based on the Precautionary Principle, monitoring and reporting requirements, and public registers of GMO release and cultivation sites, traceability and co-existence.

Transparency is another key principle of the EU policy. This is ensured by having public registers of release and cultivation sites, labelling and traceability, and facilitating public participation. This is also the intention of the Aarhus Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters, which the EU and its member states have ratified (Aarhus Convention 1998), and the Aarhus Convention was also amended with respect to GMOs in 2005 (MOP-2).

3.1 Deliberate release in the EU

Directive 2001/18/EEC on deliberate release into the environment of GMOs that replaced Directive 90/220/EEC has been in force since 17 April 2001. Its objectives are the protection of human health and the environment, and it is based on the Precautionary Principle, which is explicitly stated in the objective of the directive. The Precautionary Principle will be dealt with later in Chapters 29 and 30.

The 2001/18 Directive sets up a mandatory pre-release authorisation procedure, which involves a case-by-case risk assessment. The risk assessments must consider the direct and indirect, immediate and delayed effects of GMOs on the environment and human health. They therefore recognise that the indirect and long-term implications of GMOs should also be taken into account. This Directive also establishes public registers of releases, including cultivation sites. Public participation is mandated in EU regulations, with opportunities for the public to comment on sub-legislation, and on each application (or notification) that is submitted by GMO applicants to the EU countries' authorities.

There is a time limit for an authorisation, which is 10 years. It is possible to renew applications after the period of authorisation. The renewal should, for example, be based on assessment of monitoring reports that have been carried out during the period of marketing and use. This is an important aspect, as approvals are not indefinite, and should take into account new scientific information and the results of monitoring. Monitoring (both case-specific and general surveillance) is mandatory, and a monitoring plan must be included in applications.

The Directive requires that unauthorised releases are terminated immediately. The Member State should also initiate remedial action if necessary, and inform its public, the EU Commission and other Member States in the case of any unauthorised release. The Directive allows for emergency measures to be taken when necessary.

There is an obligation in the 2001/18 Directive to phase-out antibiotic resistance marker genes (ARMGs) in GMOs by 2004 for those antibiotics used in commercial products, and by 2008 for experimental GMOs with ARMGs. However, this obligation only applies to ARMGs which may have adverse effects on human health and the environment, but it is not clear yet which these will be. The European Food Safety Authority (EFSA), and also a working group under the 2001/18 Directive, has evaluated the potential risks associated with specific ARMGs, taking into account their current usage in clinical and veterinary medicine. The likely occurrence of horizontal gene transfer (see Chapter 13) from genetically modified (GM) plants to microbes and also the potential impact of horizontal gene transfer, where naturally occurring resistance to the relevant antibiotics exists in the microbial gene pool, have also been evaluated to some degree. EFSA has produced an Opinion (statement) on this, which serves as guidance for member states.

The 2001/18 Directive outlines in its annexes many important issues linked to GMO application, management and regulation procedures in the EU. For example, Annex I A/B identifies techniques that, in accordance with the EU-countries' understanding, are used in development of GMOs, and which techniques or methods do not develop GMOs. Their understanding is, in principle, similar to the definition of Living Modified Organisms (LMOs) in the Cartagena Protocol on Biosafety in Article 3h (see Chapter 26). Annex II elaborates the principles for environmental risk assessment, and Annex III lists all the information required in notifications (applications). The Commission Decision 2002/623 establishes guidance notes on the objectives, elements, general principles, and methodologies of the environmental risk assessment referred to in Annex II to Directive 2001/18/EC.

Annex VII, regarding the monitoring plan is very important and a Council decision from 2002 establishes a guidance note supplementing Annex VII on monitoring (EC 2002). The issue of monitoring will be dealt with in Chapters 32 and 33.

3.2 GM Food/Feed in the EU

Regulation 1829/2004 on GM food and feed has applied since 18 April 2004. Its objectives are the protection of human and animal health, and the environment. It also ensures transparency, so that consumers are aware of the GMO content of a product.

The scope of the regulation applies to food and feed containing, consisting of, or produced or containing ingredients from GMOs, irrespective of the existence of transgenic DNA or the expressed proteins in the final product. GMO 'products thereof' therefore need to undergo a full authorisation procedure and have to be labelled accordingly.

The regulation mandates a mandatory pre-marketing authorisation procedure for GM food and feed. The time limit for any authorisation is 10 years. Risk assessment is conducted at the EU level (via the European Food Safety Authority, EFSA), and includes an environmental risk assessment in line with Directive 2001/18 and its annexes if the food and/or feed consists of or contains GMOs. If a GMO is likely to have dual use purposes, i.e. it is likely to be used for both food and feed, it cannot be released onto the market without approval for both purposes. This is particularly important in light of, e.g. the StarLink incident, whereby a GM corn only approved for feed use in the US entered the food chain, highlighting the difficulties in keeping the food and feed chains separate.

The regulation requires labelling of all GM food and feed irrespective of whether the transgenic DNA or protein can be detected in the final product. This is a form of consumer information labelling. Health-related labelling is also allowed for, where necessary.

The labelling threshold level that is set by the regulation is 0.9% (per GM ingredient) for adventitious or technically unavoidable presence of GM materials in the final product. There is a temporary threshold (0.5%) for non-authorised (or not yet authorised) GM materials (which expired 18 April 2007). This threshold is valid only if the GMO present in the food/feed is adventitious or technically unavoidable and if the GMO has already received a favourable EFSA opinion, including that the application has not been rejected and that the detection methods are

publicly available. Examples of GMO events that fall into this category are Bt11 and MON863 x MON 810.

3.3 Traceability in the EU
Regulation 1830/2003, on traceability and labelling of GMOs and traceability of food and feed products produced from GMOs, has been in force since 7 November 2003. Its objectives allow for the control and monitoring (from the 'field to fork') of GMO production and the marketing chain. Withdrawal of products, if they do not comply with the regulation, is therefore possible. This regulation governs labelling of GMOs, including traceability of undetectable GM food and feed products. The scope extends to food and feed containing, consisting of, or produced from GMOs. Labelling of GM food and feed coming from GMOs is regulated under 1829/2004.

At the heart of the traceability scheme is a documentation system that effectively means that at any point in the chain, one should know the origin of the product and where it will go to next ('one step forward – one step back'). The regulation requires record keeping for five years. Identification of the GMOs is based on unique codes. For GM plants, these codes are assigned by the OECD Unique Identifier system.

The only exception is for commodities that contain a range of GMOs. In such a situation, only a list of unique codes of all GMOs used to constitute the mixture is provided. The EU requires that the documentation accompanying shipments of GMOs for food, feed or for processing must indicate which and what kind of GMOs have been used to constitute that shipment. Article 18.2(a) of the Cartagena Protocol gives the possibility for different solutions regarding documentation accompanying shipments of GMOs destined for use as food, feed or for processing (see Chapter 26).

The labelling thresholds as discussed in the previous section apply to traceability. If the GMO content is below the threshold, then the traceability requirements do not apply. The scientific rationale behind the chosen threshold level has been discussed in many forums, and is, of course, arguable.

3.3 Transboundary Movements of GMOs in the EU
The scope of Regulation 1946/2003 is on the transboundary movements (export and import) of GMOs, which is but one small part of the EU's biosafety framework. This regulation implements the obligations under the Cartagena Protocol on Biosafety, and states that no export of GMOs destined for environmental release can be carried out by any European exporter without the advanced informed agreement, or prior informed consent, from the potential importing country.

The exporter is obliged to respect any decision of the importing country on the import of GMOs intended for food, feed or for processing and those intended for deliberate release. If the importing country requires that prior approval must be sought before GMOs for food, feed or for processing can be imported, then no export of such GMOs can occur without the approval of the party of import.

3.4 Co-existence in the EU
Directive 2001/18/EC also stipulates that Member States may take appropriate measures to avoid the unintended presence of GMOs in other products.
The European Commission has issued recommended guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming (EC 2003).

However, some Member States are calling for legally binding measures that apply EU-wide, rather than leaving the development and implementation of co-existence measures to each Member State. They feel that the Commission's recommendations do not go far enough in addressing the issue of possible transgenic contamination, e.g. through cross pollination,

agricultural practices or mixing of seeds at the farm level. Different EU countries have therefore chosen different solutions in implementing co-existence regimes. Some have enacted regulations and some have developed volunteer agreements between farmers, including strict rules for GMO farming, while others have introduced GMO free zones.

For list of references see Chapter 24 – Sustainability, social and ethical considerations in regulations

Chapter 23
Definitions of GMO/LMO and modern biotechnology

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Three different definitions – but the same legal interpretation?

There are many products coming from the field of gene technology that are not necessarily covered by today's national biosafety regulations and the Cartagena Protocol on Biosafety. There are also many possible ways of interpreting the different existing definitions of Genetically Modified Organism (GMO) and Living Modified Organism (LMO). This includes the understanding of what gene technology and modern biotechnology constitute, something that may give rise to different regulations, including differences in legal coverage at the national level. The combined knowledge of biology, molecular genetics, techniques, and methodologies in combination with legal understanding and interpretation is necessary to outline the practical consequences of the definitions.

In this chapter, I will take a closer look at the definition of GMO/LMO within the Cartagena Protocol, the EU directive 2001/18/EC and the Norwegian Gene Technology Act, and discuss possible similarities and differences in interpretation and understanding of what a GMO/LMO is. In the context of these definitions I mainly look at GMOs and LMOs as synonymous, but will also give some explanations as to possible different understandings of the two terms.

The interpretation of what an LMO is in the Cartagena Protocol context needs to be made in the linkage between the definitions of 'living modified organism', 'living organism' and 'modern biotechnology', and is as follows:

Cartagena Protocol; Article 3, Use of Terms

> *g) 'Living modified organism' means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;*
>
> *h) 'Living organism' means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;*
>
> i) 'Modern biotechnology' means the application of:
>
> a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
>
> *b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;*

The definition of a GMO in the EU directive 2001/18/EC was not changed during the revision of the old directive 90/220/EC. It also constitutes which techniques lead to a GMO outcome and which do not, and is formulated as follows:

EU Directive 2001/18/EC, Article 2, 1) 2, 2a, 2b and article 3

Article 2, Definitions

1) 'Organism' means any biological entity capable of replication or of transferring genetic material;

2) 'Genetically modified organism (GMO)' means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;

Within the terms of this definition:

a) Genetic modification occurs at least through the use of the techniques listed in Annex I A, part1;

b) The techniques listed in Annex I A, part 2, are not considered to result in genetic modification;

Article 3, Exemptions

1) This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex I B.

2) This Directive shall not apply to the carriage of genetically modified organisms by rail, road, inland waterway, sea or air.

ANNEX I A

TECHNIQUES REFERRED TO IN ARTICLE 2(2)

PART 1

Techniques of genetic modification referred to in Article 2(2)(a) are inter alia:

1) Recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;

2) Techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

3) Cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

PART 2

Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:

1) In vitro fertilisation,

2) Natural processes such as: conjugation, transduction, transformation,

3) Polyploidy induction.

ANNEX I B

TECHNIQUES REFERRED TO IN ARTICLE 3

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

1) Mutagenesis,

2) Cell fusion (including protoplast fusion) of plant cells of organisms, which can exchange genetic material through traditional breeding methods.

During the negotiations of the Cartagena Protocol, the EU member countries accepted the LMO definition in the negotiated text and interpreted this definition to be in accordance with the definition of a GMO in their directive 90/220/EC.
The same understanding was also acceptable for Norway during the negotiations, although the definition in the Norwegian Gene Technology Act is formulated differently from the one in the EU directive and the Protocol, and is as follows:

The Norwegian Gene Technology Act; Section 2, Technical area of application of the Act

The Act applies to the production and use of genetically modified organisms. The Act also applies to the production of cloned vertebrates and crustaceans. The provisions of the Act relating to genetically modified organisms also apply to substances and products that consist of or contain modified organisms. Unless the genetically modified organisms are used as parent organisms, the Act does not apply to the production with the aid of cell technology of:

a) Genetically modified plant cells when the same result can be obtained by means of traditional methods of cultivation, or

b) Animal cells in culture where the cell material has been obtained from different individuals of the same species and where the cells could have been produced by natural reproduction, and the use of such plant or animal cells.

If the purpose is not to produce cloned individuals, then the act does not apply to cloning of genes, cells or tissue. The Act does not apply to the production of non-genetically modified cloned animals that can occur naturally as a result of natural biological processes.

SECTION 4, DEFINITIONS

In this Act the following terms mean:

a) Microorganisms: any cellular or non-cellular microbiological entity that is able to reproduce or transfer genetic material;

b) Genetically modified organisms: microorganisms, plants and animals in which the genetic material has been altered by means of gene or cell technology;

c) Gene technology: techniques that involve the isolation, characterization, modification and introduction into living cells or viruses of DNA;

d) Cell technology: techniques for the production of living cells with new combinations of genetic material by the fusion of two or more cells.

What can be said to be common between the three definitions and how they may be interpreted?

All three definitions include introduction and/or injection of nucleic acids (or heritable material, DNA/RNA) into viruses, microorganisms, plants, and animals. Fungi are normally understood to be included under microorganisms, or often also commonly as plants (e.g. mushrooms). All the definitions have 'alteration', 'modification' or 'recombination' of genetic material as a central requisite. The usual interpretation is that introduction of any DNA/RNA into cells or organisms through the different molecular gene technologies and methodologies in use, or to be developed, is covered under all these definitions.

The definition of *Living Organism* within the Cartagena Protocol as *any biological entity capable of transferring or replicating genetic material* is very broad and includes, for example, cells and tissue cultures. The introduction of DNA/RNA into organisms or cells is an alteration in itself, and 'recombination' of the genetic material by the researchers before the introduction, into the organism or the cells, is not necessarily a prerequisite for the modification to be covered by the three definitions. The term 'transgenic organisms' (or cells), a term not used in any of these definitions, has its origin in that the genes used in the modification are of 'trans species origin', in other words, derived from another species. This is not a prerequisite for developing a GMO/LMO in the context of these definitions. In the European common understanding of the definition, it does not matter if the genes or nucleic acid involved in the modification originate from the same species or organism as the one being modified. The rationale for this is that any of the techniques in use introduce genetic material randomly into novel places within the genome or the cells, and the outcome may therefore result in, for example, that more than one gene copy, or small pieces of DNA, end up at random places in the genome of the cells. The introduction can therefore give unexpected genetic effects, independent of the source of the nucleic acids or the methods used (see Chapters 4 and 8). Due to the random insertion, the outcome of the modification event becomes variable, and a selection procedure has to be undertaken after the modification in order to identify any successful or useful transformation events. The definitions are also understood to include future molecular technologies (techniques of modern biotechnology) to be developed, as long as nucleic acids are involved. For instance, the usual understanding of recombination, alteration or modification of organisms also includes deletion, 'turning' or 'blocking' of genes and DNA/RNA base sequences. This understanding should also take care of, for example 'knock out', siRNA and antisense techniques used to alter chromosomes or expression of genes and regulation of novel or existing proteins within the organism or cells in question.

One exception that does not meet a GMO definition is fusion of cells where the cells (e.g. protoplasts) or the plants, could have been made by traditional breeding methods, or in

ways that can occur naturally. The main problem with this exception is that we rarely have accurate scientific knowledge in order to state which changes can occur naturally or not. Ordinary propagation of, for example, disease-free plant cells, protoplasts, callus cultures, and plants will not fall within the GMO definition, unless the original plant or cells are initially genetically modified.
Further to the definitions, there is no prerequisite that the introduced genes (nucleic acids) or traits in question, shall have to be heritable to the organism's progeny, in order to become a GMO/LMO. In the Cartagena Protocol, it is expressed that any biological entity capable of transferring or replicating genetic material, including viruses, viroids and sterile organisms, are included in the definition of LMOs. This understanding is also common when interpreting the EU directives and the Norwegian Gene Technology Act. All cells and organisms, replicate the genetic material of its chromosomes at species-, tissue- and cell-specific intervals. Viruses and viroids need to do this by using the cellular apparatus of their host organism after introduction or infection. It is therefore not a prerequisite for developing a GMO/LMO that the traits, or the genetic modification in question, can be transferred to the organism's progeny through sexual reproduction or other means. Whether and how reproduction or transfer of genetic material happens is important as basic knowledge for conducting risk assessments, but is not a prerequisite for developing a GMO/LMO within these definitions.

What constitutes a GMO?

From the aforementioned definitions it is clear that all living organisms that are genetically modified and have received foreign DNA/RNA through modern biotechnology methods or cell fusion (except when the results can be made through traditional breeding methods) are GMO/LMOs. This also accounts for modified sterile organisms, virus and viroids in accordance with the understanding of the three GMO/LMO definitions. This includes all types of genetically modified microorganisms, plants and animals; e.g. GM seeds and grains, plant tubers, spores, GM plant tissue, protoplasts or cells; in fact, all parts of an organism that can be propagated into a living organism again, or into cell and tissue cultures. Development stages such as, for example, insect larvae and pupae are included in these definitions, so are sperm, living eggs and cells from genetically modified animals that can, for example, be used in breeding or the making of cell/tissue cultures. Humans are exempted in accordance with the usual legal understanding of GMO/LMO definitions.

It is also important to note that in most cases these definitions are independent of the intended use of the GMO/LMO in question. The Norwegian Act is the only one of the three definitions that includes the purpose of developing cloned animals, but the Act exempts animal cloning that can occur through natural processes. If the cloned animals in question are genetically modified, all three definitions include them in the term GMO/LMO.

Are products arising from GMO/LMOs also GMO/LMOs?

During the negotiation of the Cartagena Protocol it was also agreed that products thereof; meaning products arising from LMOs, but not being living and viable, were not LMOs.

This includes, for example, processed products such as plant oil and tomato purée, but also ground seeds, plant flour, dead animals, and meat. GM processed products for animal and human consumption are therefore not LMOs in accordance with what is living under the definition of the Cartagena Protocol, and the same applies to the understanding of a GMO within the EU directives and the Norwegian legislation. If dead GM animals are used to make cell or tissue cultures in research, or in other ways in cloning or propagating of cells, then the outcome is covered by the GMO/LMO definition. Dead and living in a common or literal sense, are therefore not the same as living in the definition of LMO, where nucleic acids, plasmids, cells, and tissue can be viable and functional molecules and biological entities. It can therefore also be said that in some cases the intended use of a product thereof defines whether it is a GMO/LMO or not. This interpretation is the same and in accordance with the intentions of the EU and the Norwegian legislation. This understanding does not, however, mean that there are no national regulations covering these types of GM products thereof. In the EU, there are strict labelling requirements and approval systems for products produced from GMOs (regulations 1829/2003 and 1830/2003), and in Norway there are also labelling and approval requirements for food and feed products produced from GMOs. This includes a ban on GMOs, and food and feed products produced from GMOs, that contain antibiotic resistance marker genes. These types of products thereof are, however, not regulated by the Cartagena Protocol, but have to be dealt with at the national level.

New methods using recombinant plasmids or 'naked' DNA/RNA

Despite differences in opinions among the negotiators of the Cartagena Protocol, it was agreed that bacterial plasmids outside an organism, and modified through DNA/RNA technologies and thus becoming recombinant, were not GMO/LMOs. When recombinant plasmids are transferred into an organism, does the organism become a GMO/LMO? In most cases, molecular biologists and regulators will say 'yes', but with some new advances and approaches within the field of modern biotechnology, some might wish to argue 'no'. In the 'traditional' way of genetically engineering plants, with infecting plasmids within bacteria as the vector (e.g. *Agrobacterium tumefaciens*-mediated transformation of recombinant T-DNA), all will answer that the result is a GMO/LMO. The same will be the case when introducing recombinant plasmids or DNA/RNA strands into fertilized eggs, or at an early embryo development stage of animals, in order to make gene modified animals. The development of new scientific approaches, especially for 'gene therapies' and vaccines for animals has led to a discussion on this understanding between authorities, the biotechnology advisory board and some researchers in Norway. For a long time it was thought that 'naked DNA' or plasmids outside the nucleus of living cells, would rapidly be degraded and destroyed rapidly. This is not necessarily the case. There is therefore ongoing research in which recombinant plasmids (or other forms of 'naked' DNA/RNA, in other words, DNA/RNA outside cells, tissue or organisms) are used as a form of 'gene therapy' for animals (e.g. plasmids with incorporated growth hormone genes with the intention of making animals grow faster and larger after injection). There is also increased research activity in order to use recombinant plasmids as vaccines against animal and human diseases (with production of relevant proteins in animal or human cells in order to give an immune response – see Chapter 4 for methods and principles). Any medicinal or pharmaceutical product that constitutes GMO/LMOs or

mixtures of such, whether it is for human or veterinary usage, is clearly within the definition of a GMO/LMO (e.g. rabies virus vaccines), but recombinant plasmids as 'free' or 'naked' molecules are not. What the stability and fate of the recombinant plasmids is after injection/introduction into animals is therefore the issue of this discourse, independent of the purpose, whether it is 'gene therapy', vaccination or other intentions.

One argument to place this type of use within the definition of GMO/LMOs is that it is exactly the same methods used to make transgenic animals as those used to deliver recombinant plasmids for 'gene therapy' and vaccines. The main difference is that the delivery of the recombinant plasmids, or DNA/RNA strands, is at a later stage of development (juvenile or adult) than when making transgenic animals. One usual way of making, for example, transgenic fish, is that the recombinant DNA is delivered into the egg after fertilization. On the other hand, in 'gene therapy' and vaccine types of application, the researchers, for instance, inject the recombinant plasmids directly into animal tissue in order for the internal bio-chemical apparatus of the cell to produce the gene product (proteins, e.g. anti globulins or growth hormones), and produce an intended immune response or increased growth rate. When a recombinant plasmid is injected into somatic cells/tissues of an animal, nobody knows the results. The main problem is therefore similar to development of any GMO/LMO; nobody can a priori know the outcome of the plasmid introduction. It may lead to an expression of the transferred genes, or novel changes in the genome composition. There are many possible results that can occur through this type of application that raise concern, some of which are listed as follows:

- The plasmid, or its fragments, can end up in humoral or lymphocyte fluid or somatic cells in any tissue or organ in the animal body
- The DNA can be integrated into the host-cell chromosomes
- The DNA can be taken up by the animal's spermatozoa and thereby passed on to the offspring
- The plasmid can be taken up by microorganisms in the animal, and thereby unintentionally develop GMO/LMOs.

There is nothing in the three definitions of GMO/LMOs that indicates that this type of plasmids or 'naked DNA/RNA' use is exempted from the definitions. However, it can be stated that these GM applications are modern biotechnology within the definition of the Cartagena Protocol, and that in most cases they will result in genetically modified cells, and hence also lead to the development of GMO/LMOs. Relevant Norwegian authorities interpreted this type of use to be regulated within the Norwegian Gene Technology Act, but we find no discussion or clear interpretation of these approaches linked to the EU regulations and the Cartagena Protocol. In the IUCN Explanatory Guide to the Cartagena Protocol on Biosafety, section 206 and 207 on naked DNA and Plasmids, we find an interpretation that supports this view (IUCN 2003). It will anyway be up to the member countries of EU and the Parties to the Protocol to interpret whether these approaches fall within the GMO/LMO definitions or not. The main problem with these applications, as within many areas linked to GMO/LMOs, is the lack of relevant and appropriate

knowledge as basis for risk assessments. It is therefore essential to increase risk-relevant research activities within these areas to improve the basis for the risk evaluations that authorities' conduct.

Nano-biotechnology: within or outside the scope of the GMO/LMO definitions?

Nanotechnology is a fast emerging research field. It involves designing and building of molecules at the nano scale into many different types of products. Linked to this development, there is ongoing research where nano-particles are used, for example, to deliver drugs or biologically active molecules into organisms, for different types of treatments and use. If nano-particles and these methodologies are used to transport or carry recombinant plasmids or DNA/RNA into cells/tissues or organisms, they will fall within the definition of modern biotechnology of the Cartagena Protocol, and may result in a GMO/LMO. If nano-particles are used to alter, modify or regulate chromosomes or genes of cells without introducing recombinant plasmids or DNA/RNA, they may fall outside the scope of the GMO/LMO definitions, even if the results might be a similar outcome as when developing a GMO/LMO. Governments, authorities, regulators, and parties to the Protocol will have to discuss and decide whether to place these types of new advanced methodologies within the definition of GMO/LMOs or not. So far, it is not reported that GMO/LMOs have been created through this type of new nano-biotechnology methodologies, but this may happen in the near future.

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Chapter 24
Sustainability, social and ethical considerations in regulations

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1. Introduction

The issue of sustainability, social and ethical dimensions of gene technologies and the use of GMOs, is often a part of the national and international debate. Some countries consider some of these elements in their regulations. In the Cartagena Protocol on Biosafety we find, for example, in Article 26, the issue of how socio-economic considerations can be a part of decisions at the national level, and how the parties to the protocol are encouraged to cooperate in this regard.

In this chapter I will use the Norwegian Gene Technology Act as an example of how the issues of sustainability, social and ethical considerations can be addressed within GMO regulations.

The Norwegian Gene Technology Act has some unusual elements compared to the legislation of many other countries. Section One of the Act states:

> *The purpose of this Act is to ensure that production and use of genetically modified organisms, and production of cloned animals, takes place in an ethical and socially justifiable way, in accordance with the principle of sustainable development and without detrimental effects on health and the environment.*

This is further elaborated in paragraph two of Section 10 of the Act, where it is stated:

> *Deliberate release of GMOs may only be approved when there is no risk of detrimental effects on health or the environment. In deciding whether or not to grant the application, significant emphasis shall also be placed on whether the deliberate release represents a benefit to the community and a contribution to sustainable development.*

Norway is not a member of the EU, but has implemented some of the EU biosafety regulations due to the Agreement on the European Economic Area (EEA), to which Norway is a Party. An adaptation in the EEA agreement, secures the right to apply these elements when considering whether or not to approve deliberate release of GMOs.

2. The Precautionary Principle

The Precautionary Principle is not written down in the Norwegian Gene Technology Act itself, as we, for example, find it in Article 1 – objective – of the EU Directive 2001/18/EC. In Proposition No. 8, 1992-93, to the Norwegian Odelsting, where we find the legal interpretation of the Act and its consequences, it is stated that the Precautionary Principle shall be a basis for evaluation of safety and risks.

This is further elaborated in Appendix 4 of the newly revised Regulations on Impact Assessments under the Gene Technology Act. The details and questions listed in Appendix 4 of the Norwegian Impact Assessment Regulation are attached at the end of this chapter. There it is stated that the Precautionary Principle shall be used when evaluating possible hazards and damage for animal and human health and the environment. It is also emphasized that ethical considerations shall be ascribed importance in decisions taken in accordance with the Act (see separate sections on the Precautionary Principle in Chapters 7 and 17).

3. Impact assessment or risk assessment

The term ‘Impact Assessment’ is also something that is different from many other modern biotechnology regulations, where we usually find the term ‘Risk Assessment’. Some of the

differences in terminology usage and its consequences will be explained later in this section.

One of the intentions of the applications and approval procedures for deliberate release is to clarify uncertainty and to have appropriate and relevant risk related information. Pursuant to Section 11 of the Act, which states that an application 'shall contain an impact assessment setting out the risk of detrimental effects on health and the environment and other consequences of the release', no GMOs can be released for experimental or commercial purposes without a thorough impact assessment as a prerequisite.

'Other consequences of the release' is usually interpreted to refer to the purpose of the Act regarding ethics, social justification and the principle of sustainability.
The revised Regulations on Impact Assessment entered into force in January 2006 and include in Appendix 4 a comprehensive list of issues and questions regarding ethics, sustainability and social justification that shall be a part of the Impact Assessment. Appendix 4 may, if appropriate and correct information is made available, to a certain degree fulfil the purpose of the Act in relation to the assessments of ethics, sustainability and social justification. An important question is: Who will be responsible for providing this information, the applicants or the authorities?

In Directive 2001/18/EC, we can find elements of the Norwegian approach, since it is stated that in the regular reports from the European Commission to the European Parliament and the member countries, socio-economic implications shall be included. The Commission shall also every year report about ethical issues if they have been raised. This is, however, of no help to the Norwegian authorities that need this type of information linked to the specific case-by-case Impact Assessments.
Another way of presenting the aim and intention of the Norwegian Gene Technology Act and its Impact Assessment Regulation is reflected in Figure 24.1.

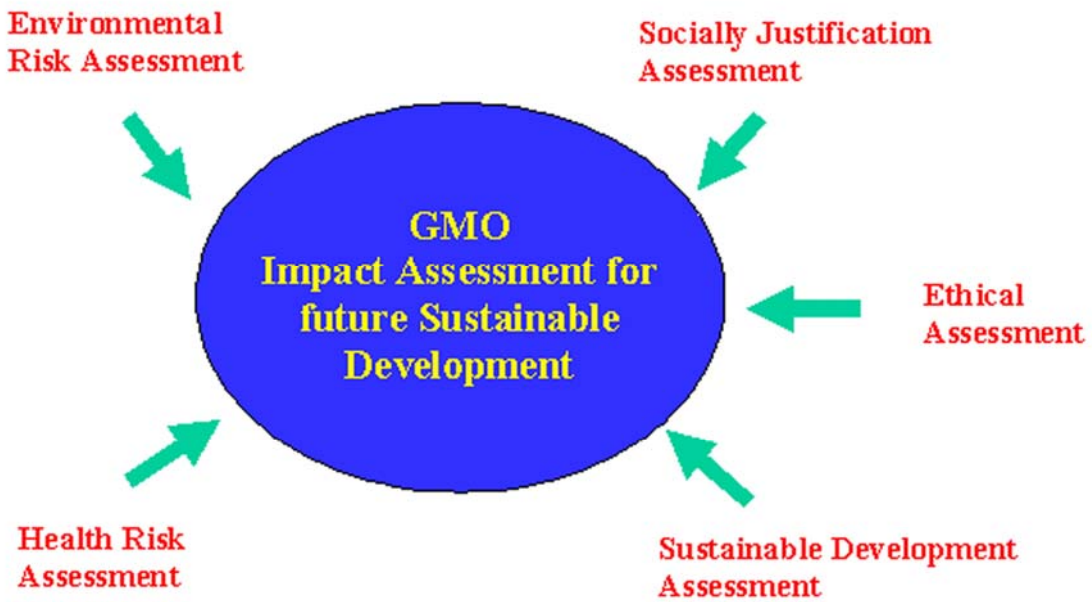

Figure 24.1. The aim and intention of the Norwegian Gene Technology Act.

On the left side of Figure 24.1 we find the traditional 'natural science' risk assessment issues and on the right side, what can be referred to as the 'social science' assessment issues, although sustainability issues in many cases also involve inputs from natural sciences. The intention of this procedure is that if appropriate and relevant knowledge is made available for

both the left and the right side, an overall or 'holistic' evaluation of the GMO in question is possible. An overall evaluation of risks for health or the environment, benefit for the community, and whether the GMO is ethically and socially acceptable, should therefore be possible to carry out. The figure is a simplified model, since experience has shown that it is difficult to operate this system in practical management. There is a need for broad collaboration with authorities in other countries, but also the applicant, in order to receive all necessary information for the assessment. Sustainability linked to GMOs is, in many respects, an international issue and should therefore be seen in a global context.

It could therefore, in addition to the national level, be appropriate to handle this issue under the Cartagena Protocol on Biosafety. In the Protocol, under Article 26 on socio-economic considerations, it is stated that Parties can take into account socio-economic considerations arising from the impact of LMOs, especially on the value of biological diversity to indigenous and local communities. Further, it is stated that Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts.

It is also important to note that in accordance with Article 10 of the Norwegian Gene Technology Act 'a product may not be approved for placing on the market until it has been satisfactorily tested in natural environments that will be affected by the intended use'. This is important, and is usually found in most countries' environmental GMO regulations. Risk assessments on the left side of Figure 24.1 should therefore always be closely linked to both general and specific environmental and ecological knowledge as a basis. The information on environmental and ecological conditions at the national level thereby becomes central for when to use the Precautionary Principle, especially when appropriate or important knowledge is lacking or omitted in applications for deliberate release.

Regarding the social science issues on the right side of Figure 24.1, the Norwegian Ministry of Environment requested in 1998 the assistance of the Norwegian Biotechnology Advisory Board (NBAB) in operationalizing the concepts of ethics, sustainable development and social justification in the Gene Technology Act. Their discussion document was the basis for Appendix 4 to the aforementioned Impact Assessment Regulation (NBAB 2000).

The Norwegian Biotechnology Advisory Board (NBAB) is appointed by the Norwegian Government and its mandate is to give advice to the regulatory authorities, Parliament and Government. It includes representatives from different stakeholders, e.g. non-governmental organizations, and scientists from relevant institutions and fields of research. In November 1999, the NBAB published their statement regarding how to interpret, in a practical way, sustainable development, social justification, and ethical and social considerations connected to applications for marketing of GMOs (see the report 'Sustainability, benefit to the community and ethics in the assessment of genetically modified organisms', which is currently available on the NBAB's homepage: www.bion.no).

The NBAB provided an interpretation of the Gene Technology Act, to establish the basis for their further work with the report:

> *Section 10 of the Gene Technology Act should be interpreted to mean that the requirements of sustainable development, benefit to the community and other ethical and social considerations, represent prerequisites that alone could carry decisive weight against granting an application, but that should also be considered in relation to, and weighted against the risk of detrimental effects, when such risk is low.*

With this understanding as a starting point, the NBAB developed a decision structure where evaluation of each GMO application should be based on the following general questions:

1. Danger of detrimental effects on health and the environment:
   a) What are the possible negative consequences?

b) What is the likelihood of such consequences occurring?

2. The Precautionary Principle:
   a) Is the risk assessment associated with justified uncertainty?
   b) Is there a possibility of substantial or irreversible harm?

3. Is it:
   a) In compliance with the principle of sustainable development?
   b) Of benefit to the community?
   c) Ethically and socially justifiable?

The first point is connected to the left side of Figure 24.1, and usually has broad coverage in most countries' GMO regulations. Point two, the Precautionary Principle, is now a part of the objective in the revised EU directive (2001/18/EC). We find the principle (or approach) as a basis for the Cartagena Protocol on Biosafety, where it also has gained practical interpretation in Articles 10(6) and 11(8), and in the risk assessment Annex III (point 4).

The NBAB stated that a common understanding is that the Precautionary Principle is one of many principles of the concept of sustainable development. In the international context we find the concept of sustainable development in the Rio Declaration and the Convention on Biodiversity (CBD), which was adopted at the UN's Earth Summit (UNCED) on sustainable development in 1992. The NBAB further states that sustainable development is building on a set of ideas connected to the following:

– The idea of the global effects of human activities
– The idea of ecological limits and that these limits have been exceeded in several areas
– The idea of meeting basic human needs
– The idea of just distribution between generations
– The idea of just distribution between wealthy and poor nations
– The idea of a new form of economic growth.

These six points serve as a structure for evaluating whether marketing of a GMO is in accordance with the demand for sustainable development. Many of the points are recognized as important issues in the global discussion regarding acceptance of GMOs in developing countries, and they are closely linked to the need for technology transfer and capacity building.

The NBAB explains that it is necessary to clarify the relationship between biological diversity (i.e. the diversity of genes, species and ecosystems) and ecological sustainability. Effects on biological diversity are a type of environmental risk that implies that assessment primarily should be done in relation to possible effects regarding health, environment and the Precautionary Principle. When these issues are brought into the discussion about sustainable development, it implies a change of focus in time and space. The NBAB was of the opinion that questions related to negative effects on health and environment, and the employment of the Precautionary Principle, apply primarily to local, national and regional relations. Assessments connected to sustainable development apply globally and also, to an extent, over a longer time scale (generations).

In connection with ethical considerations, the NBAB found it appropriate to distinguish between ethical norms and values associated with humans and those associated with environmental ethics ('the integrity of nature'). Based on a set of values, the procedure proposed by the NBAB outlined ethical reflections which aim to enable us to undertake assessments of what is right or wrong, in a more systematic and justifiable way. Further, the NBAB stated that ethical reflections in connection with moral dilemmas are often based on an intuitive experience of the situation as problematic, without actually being able to pinpoint

what is alarming. In many respects, this is the situation when dealing with the scientific knowledge regarding safety aspects of GMOs. Scientists working within different, but relevant, research fields often tend to interpret data connected to risk assessments differently, and make value judgements with a basis in their own research traditions and experiences. This makes it difficult for authorities when receiving advice in connection with decision making, because the emphasis on risks of possible negative effects will vary depending on whom you ask and their professional background, integrity and personal standpoint. The worst-case scenario may, of course, happen, even if the probability is low. It can therefore be easy for authorities to 'hide behind' the Precautionary Principle. Appropriate research and scientific knowledge about possible hazards are therefore of utmost importance also when dealing with ethical dilemmas connected with risks assessments. It is therefore the duty of the authorities to ensure that appropriate and required biosafety research is carried out as a basis for risk assessments.
Knowledge about the public opinion and values regarding these issues is important if this type of assessment is to reflect reality. It is therefore necessary to have meeting points for debate and discussions between politicians, authorities, scientists, the biotech industry, and the public. Debate and meeting points will enhance the authorities' knowledge of the different opinions within the society.

In Norway, as in many other European countries, all new applications for marketing release of GMOs are subjected to public hearings where different opinions may come forward. For many years, the NBAB has also arranged public meetings and consensus conferences where important biosafety related topics have been the main focus. This type of activity increases public knowledge about biosafety and GMOs, and the authorities and politicians gain important feedback related to the aforementioned outlined topics. Issues related to safety and use of GMOs have also been discussed at open conferences organized by the NBAB. These types of conferences take place in many countries, and usually involve different stakeholders and the general public, and commonly draw a large audience.

The procedures for addressing biosafety issues in accordance with the principles of sustainable development, ethical and social elements, as proposed by the NBAB, have not yet fully been applied in practical management, but will be applied in the near future due to the newly implemented Impact Assessment Regulation. It will therefore be interesting to see how this regulation will be used in the future management of GMO applications in Norway. It will also be interesting to see how the use of this regulation will influence public debate and further development of GMO regulations in Europe and other parts of the world.

The debate about acceptance of GMOs is not only an issue of natural science and risk assessment, but involves many cross-cutting research fields, ethics and socio-economic issues at both a national and a global scale. It is therefore important to engage in an open discussion regarding the types of issues that have been raised through the Norwegian approach towards GMO regulations.

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Appendix 4 to the Norwegian Impact Assessment Regulation:

EVALUATION OF ETHICAL CONSIDERATIONS, SUSTAINABILITY AND BENEFIT TO SOCIETY, CF SECTION 17 OF THE REGULATIONS

INTRODUCTION

This appendix explains what should be included in an account of other consequences of the production and use of genetically modified organisms pursuant to Section 17 of the regulations. To the extent necessary, such an account should as far as possible include all the elements listed in the Appendix. However, the Appendix is not exhaustive, and not all the elements will be relevant in every case.

The purpose of the Gene Technology Act, as set out in its section 1, is to ensure that the production and use of genetically modified organisms and the production of cloned animals take place in an ethically justifiable and socially acceptable manner, in accordance with the principle of sustainable development and without adverse effects to health and the environment. Section 10, second paragraph, of the Act lays down that the deliberate release of genetically modified organisms may only be approved when there is no risk of adverse effects on health or the environment, and that considerable weight is to be given to whether the deliberate release of genetically modified organisms will be of benefit to society and is likely to promote sustainable development. The comments on the objects clause of the Act in Proposition No. 8 (1992 to 1993) to the Odelsting make it clear that the precautionary principle is to be used as a basis in evaluating potential adverse effects on human and animal health and the environment, and that ethical considerations must be given considerable weight when making decisions on applications for approval pursuant to the Act. The comments on Section 10, second paragraph, make it clear that when applications for deliberate release pursuant to the Act are considered, any benefits to society and contributions to sustainable development are to be used both as independent criteria for the evaluation of applications and as criteria that may result in less strict application of the requirement that the release of genetically modified organisms must not have adverse effects on health or the environment. An evaluation of benefits to society and contribution to sustainable development should be based on the principles of cost-benefit analysis.

I PROCEDURE FOR THE EVALUATION

The evaluation should be organized as follows:

1) Risk of adverse effects on human and animal health and the environment:
   a) What are the possible adverse effects?
   b) How probable are these effects?

2) Precautionary principle:
   a) Is there justified uncertainty associated with the risk assessment?
   b) Is there a possibility of substantial or irreversible harm?

3) Will the project
   a) tend to promote or hinder sustainable development?
   b) have favourable or unfavourable social consequences
   c) be ethically justifiable?

In assessing the questions in item 3, it can be useful to distinguish between the following three elements:

– the characteristics of the product
– its production
– its use.

II RISK OF ADVERSE EFFECTS ON HUMAN AND ANIMAL HEALTH AND THE ENVIRONMENT

A. Checklist

1. Does the application provide sufficient documentation for evaluating possible adverse effects?
2. Is it reasonable to assume that there will be a major or significant risk to health or the environment?
3. Is it reasonable to assume that there will be major or significant adverse effects on health or the environment?
4. Is it reasonable to assume that there will be major or significant adverse cumulative effects on health or the environment?

B. Comments

If the answer to question 1 is no, the application shall be evaluated in relation to the precautionary principle. If the answer to one or more of questions 2–4 is 'yes', the application shall be refused. If the answer to all of questions 2–4 is 'no', the application shall be evaluated in relation to the precautionary principle.

III THE PRECAUTIONARY PRINCIPLE

A. Checklist

- Is there a reasonable degree of doubt about existing risk assessments, and is there a danger that the risk may be higher than these assessments indicate?
- Is there a reasonable degree of doubt about existing probability assessments, and is there a danger that the probability of adverse effects is higher than these assessments indicate?
- Is there a reasonable degree of doubt about existing impact assessments and is there a danger of even more serious effects on health and the environment than these assessments indicate?
- Is there a reasonable degree of doubt about possible serious cumulative effects on health or the environment?
- Is there a reasonable degree of doubt as to whether proposed mitigating measures and instruments will function as intended?

B. Comment

If the answer to one or more of these questions is 'yes', this indicates that the application can be refused with reference to the precautionary principle.

IV SUSTAINABLE DEVELOPMENT

A. Checklist

1. Global impacts
   - Will there be global impacts on biodiversity?
   - Will there be impacts on ecosystem functioning?
   - Will there be differences between the impacts of production and use in these respects?

2. Ecological limits
   - Will there be any impact on the efficiency of energy use?
   - Will there be any impact on the efficiency of other natural resource use?
   - Will there be any impact on the proportions of renewable and non-renewable resources used?
   - Will there be any impact on emissions of global and transboundary pollutants?
   - Will there be any particular impact on greenhouse gas emissions?
   - Will there be differences between the impacts of production and use in these respects?

3. Basic human needs
   - Will there be any impact on the degree to which basic human needs are met?
   - Will there be differences between the impacts of production and use in these respects?

4. Distribution between generations
   - Will there be any impact on the distribution of benefits between generations?
   - Will there be any impact on the distribution of burdens between generations?
   - Will there be differences between the impacts of production and use in these respects?

5. Distribution between rich and poor countries
   - Will there be any impact on the distribution of benefits between rich and poor countries?
   - Will there be any impact on the distribution of burdens between rich and poor countries?
   - Will there be differences between the impacts of production and use in these respects?

6. Economic growth
   - Will there be any impact on the use of energy and other natural resources for economic growth?
   - Will there be any impact on the global/transnational environmental impacts of economic growth?
   - Will the there be any impact on the distribution of economic growth between rich and poor countries?
   - Will there be differences between the impacts of production and use in these respects?

B. Comment
An evaluation of whether a project is in accordance with the principle of sustainable development must be based on an overall assessment and discussion of all these questions. However, not all the questions will be relevant in all cases.

V FAVOURABLE OR UNFAVOURABLE SOCIAL CONSEQUENCES

A. Checklist
1. Characteristics of the product
   - Is it reasonable to say that there is a demand or a need for the product?
   - Is it reasonable to say that the product will solve or help to solve a social problem?
   - Is it reasonable to say that the product is significantly better than similar products that are already on the market?
   - Is it reasonable to say that there are alternatives that are more suitable than this product for solving or helping to solve the social problem in question?

2. Production and use of the product
   - Will the product have a positive effect on industrial development and wealth creation, including new employment opportunities?
   - Will the product have a positive effect on industrial development and wealth creation, including new employment opportunities, in rural areas in particular?
   - Will the product have a positive effect on industrial development and wealth creation, including new employment opportunities, in other countries?

- Will the product tend to create problems for existing production that should be maintained?
- Will the product tend to create problems for existing production in other countries?

B. Comments
An evaluation of whether a product is of benefit to society must be based on a discussion of the answers to all these questions. However, not all the questions will be relevant in all cases.

VI ETHICAL CONSIDERATIONS

A. General considerations

1. Analysis of the situation
   - What alternatives are there?
   - Which parties are involved? How will they be disadvantaged by, or benefit from, the different alternatives?

2. Ethical reasoning
   - Which norms are applicable?
   - How can any conflict between these norms be resolved?

3. Implementation
   - How can the best alternative be implemented in practice?

B. Checklist

1. Ethical norms and values relating to people
   - Will approval or prohibition of the product and its production and use be in accordance with the moral views of the general population?
   - Will the product or its production and use come into conflict with the ideals of solidarity and equality between people, such as the need to show special consideration for weaker groups?
   - Decisions made by mainstream society can have a serious adverse impact on indigenous peoples, people who live in highly traditional cultures, and weaker groups. Special account should be taken of the need of these groups to be able to control their own processes of social change.
   - Will the marketing and sales, in particular, of the product come into conflict with ethical norms and values relating to people?

2. Eco-ethical considerations
   - Will the product and its production be in conflict with any intrinsic value assigned to animal species?
   - Will the production of the product cause unnecessary suffering to animals?
   - Will the production of the product involve crossing species barriers in ways that are materially different from those otherwise found in cultivation or in the wild, and that must be considered incompatible with the value assigned to the integrity of species?

C. Comment

An evaluation of other ethical and social considerations must be based on a discussion of the answers to all these questions. However, not all the questions will be relevant in all cases.

Chapter 25

The Cartagena Protocol on Biosafety: History, Content and Implementation from a Developing Country Perspective

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1. Introduction

The negotiations of the Cartagena Protocol on Biosafety were finalized on 29 January 2000 in Montreal, Canada. The Protocol came into force on 11 September 2003 (CBD Handbook 2005). A history of the Cartagena Protocol by a professional historian who was not involved in the negotiation process and could thus be expected to have objectively evaluated the roles played by the various protagonists has not been written. Given the short time since the negotiations were finalized and the Protocol came into force, such an objective history could not as yet have been expected. Time will show, in fact, if expecting a history of the Protocol is presumptuous.
Thus, the history I recall here necessarily reflects my own notes and unpublished reports as one of the main negotiators of the Protocol, though I have tried to resort to the documents produced by the various protagonists to help me become as objective as I can. Even thus, both because I know the issues intimately, and because I believe that developing countries carried a heavier load owing to their position of greater disadvantage, I will give more attention to the negotiations of the developing countries. Their load is heavier because of both their obvious limitation in well-trained human resources, and because of the greater complexity of their biodiversity, which increases the risks of gene introgression and thus complicates biosafety considerations. It is also more than likely that history from the perspective of developed countries is going to be well preserved and presented by their better endowed professionals and institutions.

The Biosafety Protocol is complex both because of the nature of regulating genetic engineering, and because of the compromises that had to be reached in order to accommodate a wide range of beliefs (ideologies) on the sanctity of, and acceptability of human-made modifications to, life. This complex situation was exacerbated by the wide range of perceived positions of advantage and disadvantage of human societies.

It is just over three years since the Protocol came into force. Therefore, its implementation is only just beginning. In any event, the implementation of the Protocol will remain difficult as long as the country that is the most active in genetic engineering, the United States of America, remains a non-Party. It should also be noted that, like all environmental agreements, the Cartagena Protocol on Biosafety lacks an enforcement mechanism comparable in power and influence to the Dispute Settlement Body of the World Trade Organization, let alone to the Security Council of the United Nations.

2. A Brief History of the Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety is an international law that emanated from the Convention on Biological Diversity. Therefore, its history starts with that of the Convention.

2.1. The Convention on Biological Diversity
A brief history of the negotiations that gave us the Convention on Biological Diversity is given in the Nairobi Final Act of the Conference for the Adoption of the Agreed Text of the Convention on Biological Diversity (UNEP 1992).

The United Nations Environment Programme (UNEP) Governing Council decided in 1987 to establish the Ad Hoc Working Group of Experts on Biological Diversity. This Group of Experts held three meetings between 1988 and 1990, and produced a final report.

On 25 May 1989, the UNEP Governing Council established the Ad Hoc Working Group of Legal and Technical Experts to negotiate an international law 'for the conservation and rational use of biological diversity'. In May 1991, the Ad Hoc Working Group became the Intergovernmental Negotiating Committee (INC) for a Convention on Biological Diversity.
The INC held a total of seven negotiation sessions from 1990 to 1992. It was transformed into a Conference to adopt the final text of the Convention on Biological Diversity on 22 May 1992. The Conference also adopted 4 resolutions and registered 14 declarations by States or groups of States.

Resolution 2, in Paragraph 2 (c), asks the UNEP Governing Council to 'consider requesting the Executive Director of the Programme [UNEP] to convene an Intergovernmental Committee on the Convention on Biological Diversity starting in 1993 to consider... the need for and modalities of a' biosafety protocol. This was intended to start the implementation of Article 19.3 of the Convention (the clause that requests Parties to consider such a need) before it came into force.

2.2. Report of Panel IV
To prepare for the implementation of Paragraph 2 (c), the then Executive Director of UNEP, Mustapha Tolba, established a group of experts to analyze the need for and modalities of a biosafety protocol. This group of experts was referred to as Panel IV. It was co-chaired by Veit Koester of Denmark and myself of Ethiopia. A total of 29 experts from 12 countries (including the European Economic Community) as well as 5 organizations participated in the three meetings of Panel IV. The Panel's report was submitted to UNEP on 28 April 1993 (UNEP 1993).

2.3. International Technical Guidelines for Safety in Biotechnology
The Departments of Environment of the Netherlands and the United Kingdom convened international experts in Ascot, England, on 9–10 March 1994 and developed the first draft of technical guidelines on safety in biotechnology. The draft was discussed by invited experts from 17 countries in May 1994. The representatives of the United Kingdom and the Netherlands were made focal points for receiving further comments on these guidelines (NME & UKDE 1994). The draft guidelines were promoted by UNEP and subjected to further consultations in the various regions (UNEP 1995a). A 'global consultation of government-designated experts on [the] international technical guidelines for safety in biotechnology' was then held in Cairo, Egypt, on 11–14 December 1995, and this meeting adopted the final text of the 'United Nations Environment Programme International Technical Guidelines for Safety in Biotechnology' (UNEP 1995b). Though the meeting was considered global, only 59 countries and the European Commission took part (UNEP 1995b). Based upon a decision made at this meeting, UNEP organized an international workshop on 31 October – 1 November 1996 in Buenos Aires, Argentina, to review the implementation of these Guidelines (UNEP 1996), which was attended by experts from 55 countries. The last statement in the recommendation from this workshop was a call to UNEP 'to review periodically the Guidelines' (UNEP 1996).

This workshop was soon followed by the Third Conference of the Parties to the Convention on Biological Diversity (COP III) on 4–15 November 1996, also in Buenos Aires. On 8 November, the Committee of the Whole of COP III decided to consider the negotiations on the biosafety protocol and progress on the implementation of the UNEP International Technical Guidelines for Safety in Biotechnology together as one item (UNEP 1997). The subsequent COPs focused only on the biosafety protocol negotiations and the UNEP Technical Guidelines on Safety in Biotechnology faded into oblivion as an issue for the COP to discuss.

2.4.Negotiations on the Cartagena Protocol on Biosafety

UNEP's Panel IV Report (UNEP 1993) had a majority view that called for negotiating a biosafety protocol, and a minority view that stated that there was no need for a biosafety protocol. The minority view was that of the representative from the United States of America, supported by two representatives of the Organisation for Economic Cooperation and Development (OECD).
By the time the Report of Panel IV was finalized, the Executive Director of UNEP who had established the Panel, Mustapha Tolba of Egypt, had been replaced by Elizabeth Dowdeswell of Canada.

Under Elizabeth Dowdeswell, UNEP tried to avoid the Panel IV Report from being considered in the meetings of the Intergovernmental Committee on the Convention on Biological Diversity (ICCBD), which was the body that had been created to prepare the ground for the implementation of the CBD while it was awaiting entry into force (UNEP 1992).

The Interim Secretariat of the CBD in UNEP under Dowdeswell oversaw the functioning of the ICCBD. The document prepared by the Interim Secretariat for the work of the ICCBD on biosafety deliberately left out mentioning the Panel IV Report. The first meeting of the ICCBD, which took place in Geneva, Switzerland, 11–15 October 1993, focused only on capacity building and international cooperation in biosafety (CBD Int. Sec. 1993). The reason can be revealed by studying the document on biosafety presented to the 2nd session of the ICCBD, which took place in Nairobi, Kenya, on 20 June – 1 July 1994 (UNEP 1994). In its 19th paragraph, this document suggested the ICCBD should define the term 'protocol' and added that it 'may then proceed to consider whether or not a protocol is needed; whether it is an immediate need or whether its development is envisaged for the future'. However, in its 18th paragraph it states: 'As familiarity with LMOs increases and experience accumulates ... the patterns of regulation will likely evolve from initial stringency to less stringent requirements'. In its 20th paragraph, it states: 'If a protocol is not needed at all or if it is only needed in the future, the Committee [ICCBD] may wish to consider whether other instruments such as voluntary codes of conduct and guidelines could be considered'. To make this view palatable, the document's 14th and 21st paragraphs emphasize the need for capacity building in developing countries.

The UNEP International Guidelines for Safety in Biotechnology were, therefore, promoted by Dowdeswell's UNEP in order to stifle the call for a biosafety protocol made by UNEP's own Panel IV. This was realized by the environmental NGOs and by developing countries which therefore supported the Panel IV Report and called for starting negotiations on a biosafety protocol.

Both UNEP's attempt to prevent negotiations on biosafety from starting and the calls for them to start continued during the First Conference of the Parties to the CBD, which took place in Nassau, the Bahamas, on 28 November – 9 December 1994. From among the NGOs, Third World Network, Greenpeace, the Community Nutrition Institute, and Friends of the Earth distributed a statement to this effect on 5 December 1994. On 6 December, they again distributed a similar statement, this time joined also by Accion Ecologica, condemning particularly Australia, Austria,

Canada, the European Union, Finland, Japan, New Zealand, Norway, Sweden, and Switzerland for the terms of reference of the Open-ended Ad Hoc Group of Experts on Biosafety which COP I established. According to these NGOs, the terms of reference did 'not address the question of modalities, but rather entered into a never ending process considering the need' for a biosafety protocol.

A Panel of Experts on Biosafety, established by the Secretariat of the Convention to prepare for the meeting of the Open-ended Ad Hoc Group of Experts on Biosafety, presumably in order to bypass the Panel IV Report, met in Cairo, Egypt, on 1–5 May 1995. This Panel's report made no mention of the report of its predecessor, Panel IV (CBD report 1995). Its Paragraph 35 states: 'The adoption of an international framework, such as guidelines, regulations, codes of conduct or a protocol, does not of itself insure safety'. Its overall tone is that of letting things be: that of not taking any immediate international action.

2.4.1. Negotiations in the Open-ended Ad Hoc Group of Experts on Biosafety
Following Decision I/9 of COP I, an Open-ended Ad Hoc Group of Experts on Biosafety met in Madrid, Spain, on 24–28 July 1995. Experts from 83 countries, 21 from Africa, and one regional organization (the European Community) participated in a decisive debate that shaped the future of biosafety. Dr Emilio Munoz of Spain was elected as the chair of the meeting. Dr Luiz Antonio Barreto de Castro of Brazil representing Latin America and the Caribbean, myself representing Africa and Dr Sugiono Moelzopawiro of Indonesia representing Asia were elected as vice-chairpersons. The four of us constituted the Bureau of the meeting.

The meeting examined the report of the Cairo Panel of Experts (CBD report 1995). It soon became clear that this document was not acceptable to all delegates. In fact, of all the parties to the CBD, only Australia and Canada were fully in favour of this document. They were fully supported by the United States of America, which, though not a Party, was most active in canvassing opinion.

As the meeting continued, the call for recommending to COP II an authorization of negotiations on a biosafety protocol grew. Because of the role I had played in Panel IV as its co-chairman, virtually all delegates from developing countries rallied behind me to make this call (UNEP 1993).

Feeling the need to break this unity among delegates from developing countries, some of the delegates from the United States pointed out rightly, that the call for negotiating a protocol was strongest from Africa. However, they explained it as an unjustified ignorant fear from the most backward continent about this avant-garde technology called modern biotechnology. This started to cause defections from the call for a protocol. At the same time, Professor Elaine Ingham of Oregon State University in the United States explained her research results on the genetically engineered soil bacterium *Klabsiella planticola.* She pointed out how this normally useful bacterium had been rendered dangerous to plant life by genetic engineering. After that, the developing country delegates rallied around me again and the call for a protocol grew louder. Of the industrialized countries, New Zealand, Germany, Japan, and South Korea sided with Australia and Canada. Finally, after much debate, they accepted a decision that recommended to COP II that a biosafety protocol be negotiated.

The main issues to be covered by the protocol were also identified (CBD elaboration 1996). However, the industrialized countries, with a few notable exceptions, did not want socio-economic considerations and liability and redress to be included in the protocol.

2.4.2. Negotiations in the Open-ended Ad Hoc Working Group on Biosafety
The Open-ended Ad Hoc Working Group on Biosafety was established by COP II through Decision II/5. The first meeting of the Working Group was in Aarhus, Denmark, on 22–26 July 1996 (CBD report 1 1996). On the first day, the African Group elected me as its spokesman. The various regional groups also elected their spokespersons.

The meeting reviewed and elaborated the items identified by the Madrid meeting of the Open-ended Ad Hoc Groups of Experts. The question of whether or not to include socio-economic issues and liability and redress divided the G77 and China. Brazil, South Korea, Costa Rica, and Argentina took the stand of the industrialized countries that these two items should not constitute a part of the protocol. Therefore, at the suggestion of Amarjeet Ahuja of India and myself, the developing countries with the exception of the aforementioned four, formally pushed for these two issues as essential, and the G77 and China stopped functioning as a group in any meaningful manner in the subsequent biosafety negotiations.

The African Group asked me to draft a biosafety protocol on behalf of Africa. Upon returning to Ethiopia, I initiated the drafting of the protocol. Under my chairmanship, experts from four institutions developed the working draft. Once funding was secured, the African Group met in Addis Ababa on 23–25 October 1996 to revise and adopt the draft protocol. Early on during the meeting, the South African representative tried to steer the African Group towards a minimalist direction so that the protocol would be weak. He was not followed by any delegate from another African country. I realized that he did not want the South African delegation to formally separate from the African Group for fear of a political backlash at home, but would continue causing as much difficulty as he could during negotiations. Neither did I want the African Group to formally exclude the South African delegation because that would have reduced our political impact as a Group. Managing his disruptive tactics was the greatest difficulty I had in leading the African Group. In spite of his attempts, the African Group that met in Addis Ababa adopted the text of a draft protocol. I submitted this draft protocol in the name of the African Group to the CBD Secretariat at the COP III in Buenos Aires on 4–15 November 1996.

The second meeting of the Open-ended Ad Hoc Working Group on Biosafety took place in Montreal, Canada, on 12–16 May 1997 (CBD report 2 1997). The submissions of views by governments on the provisions of the protocol were discussed by the meeting (CBD IGS 1997, CBD compilations 1997). Except for the submission of the African Group, which was in the form of legal text, the remaining submissions were descriptive in nature. Though these views showed the diversity of thinking, they could not be used to start negotiations. The meeting was, therefore, basically for exchanging opinions. The meeting established a Contact Group to consider how the definitions of key terms should be formulated.

The third meeting of the Open-ended Ad Hoc Working Group took place in Montreal, Canada, on 13–17 October 1997. More detailed submissions by governments were the basis of the negotiations (CBD GS 1997). South Africa submitted its own separate text, but would still not formally declare that it disagreed with, and was splitting from, the African Group. Therefore, it continued to disrupt African Group meetings from within. The meeting of the Working Group widened the scope of the work of the Contact Group to include negotiating on Annexes. It also established (divided into) two Sub-Working Groups and started negotiating on the consolidated text of country submissions, trying to produce an agreed text. Delegates could move in and out of the Sub-Working Groups and the Contact Group as their interests dictated.

The fourth meeting of the Open-ended Ad Hoc Working Group took place in Montreal, Canada, on 5–13 February 1998 (CBD report 4 1998). The negotiations, which continued in the two Sub-

Working Groups and two Contact Groups already established, became highly polarized. A second Contact Group was also created, to focus on financial and institutional issues. The delegation of the United States of America tried to divide the African Group by calling us for consultations on sub-regional bases. Attempts by South Africa to organize the delegates from the Southern African Development Community to speak to the United States delegation on their own failed, because the other delegates refused to speak to the United States delegation except as the African Group. However, we had consultations with the European Community and with other regions as an African Group.

The fifth meeting of the Ad Hoc Open-ended Working Group took place also in Montreal, Canada, on 17–28 August 1998. Many states submitted final portions of their proposed detailed wording for the provisions of the protocol, as had already been done by the African Region (CBD IGS 1997; CBD report 5 1998). As a consequence, the first draft text of the protocol, albeit full of brackets, was compiled and the negotiation process became clearly defined (CBD report 5 1998). So, too, did the divisions among states and groups of states. It became clear that the industrialized countries, as a bloc, were blocking any negotiations on liability and redress by simply refusing to comment on the issue. Therefore, the delegates of developing countries also refused to comment on any issue other than liability and redress. After one day of near total silence, this forced the industrialized countries to agree to seriously negotiate on liability and redress, and the negotiations continued. The question of whether products of LMOs should be regulated by the protocol also became divisive. The African Group and most developing countries wanted products of LMOs to be covered by the protocol. The issue continued unresolved to the end of the negotiations.

Upon returning home, now that the negotiating text was available, I commented on the implications of each bracketed text, pointed out what our preference should be, and sent these comments to my other African colleagues. Because African delegates had read my comments and thought about the issues, taking a common position during the subsequent negotiation session became easier.

The Chairman of the Working Group rightly gauged the divisions among delegations to be very wide, and the time left too short. Trying to hasten the negotiations, he called a meeting of the Bureau of the Working Group – which included the elected representative from each Region – and a selected number of other delegates on 21 and 22 October 1998 in Montreal. This was dubbed the ‘Extended Bureau’. The Extended Bureau discussed ways of hastening the negotiations. The most intriguing suggestion was proposed by the European Union, which ‘strongly urges states and regional integration organizations to operate as many of the provisions as possible of the protocol’ (CBD Working Group 1998). If passed, this would have reduced the protocol from an international law to a suggested procedure. This draft decision was not tabled at the subsequent meeting of the Working Group and, in the final analysis the meeting of the Extended Bureau did not help much.

The sixth and final meeting of the Open-ended Ad Hoc Working Group on Biosafety took place in Cartagena, Colombia, on 14–22 February 1999. The negotiations were scheduled to be finalized at that meeting. Negotiations continued in the two Sub-Working Groups and two Contact Groups. The South African delegate’s disruption of the African Group had been noted in South Africa and he was consequently dropped from the delegation. The negotiating text had hundreds of brackets and it seemed certain that no consensus text would be produced from it. LMO commodities, products of LMOs, socio-economic issues, the Precautionary Principle, and the scope of the protocol remained divisive. On 15 February, the Chairman asked the Regional Groups to elect his Friends of the Chair from among their members, but he handpicked

Mohammed Mahmoud El Ghaouth of Mauritania and Darryl Dunn of New Zealand as his Vice Chairmen to help him chair the meetings of the Friends of the Chair. On the evening of 15 February, the Friends of the Chair had its first meeting. The Chairman had said that his Friends of the Chair were to advise him and not to negotiate. In practice, these two functions became indistinguishable.

On 16 February 1999, formal negotiations which were open to all delegates continued. In the evening, the Chairman, with the Friends of the Chair, reviewed progress, and found it to be minimal. On 17 February, the Chairman, following a suggestion by one of the Bureau Members, informed the Bureau that anything agreed in the Sub-Working Groups and Contact Groups would not be re-opened in Plenary, which would merely endorse the agreement. I pointed out to him that this would not be transparent and was thus undemocratic especially since most developing countries were represented by single delegates who could not be present in the Sub-Working Groups and Contact Groups simultaneously. However, he and his Vice-Chairman, El Ghaouth, tried to implement it, contributing to the failure of the negotiations. In the evening, he announced that he would produce a Chairman's text the next day.

This new text galvanized the groupings into even greater confrontation. Especially, three strong groupings emerged. The developing countries, with the exception of Mexico, Argentina, Chile and Uruguay, created the Like-minded Group of Developing Countries and elected me as their chief negotiator. Canada, Australia, Argentina, Uruguay, Chile, and the United States of America had already formed the Miami Group. These two groups were the furthest apart on most substantive issues. As a result of these groupings, the usual UN Regional groups could no longer continue. Mexico joined with Japan, Switzerland, Norway, and New Zealand to form the Compromise Group. The Central and Eastern European Group remained intact. These two groups became rather quiet in the confrontation. The European Union stayed very active, with its position on most of the divisive issues being in between those of the Miami and the Like-minded Groups.

On Saturday 20 February 1999, the chief negotiators of the various Regional Groups, with their advisors, met twice over the new draft under the Chairmanship of Veit Koester, with the Environment Minister of Colombia, H.E. Juan Mayr Maldonado, facilitating the negotiations. On Sunday 21 February, the Chairman produced a revised text. It did not bring the parties any closer. During the night, H.E. Juan Mayr Maldonado chaired negotiations between the chief negotiators of the Miami, European and Like-minded Groups. The chief negotiator of the European Group offered what he called 'a package' to the Miami Group. The chief negotiator of the Miami Group also had a list of changes he wanted in the Chairman's revised text. The suggested changes from both Groups wanted the provision on the Precautionary Principle to be deleted. Both sets of proposals were unacceptable to me. The proposal of the Miami Group was also unacceptable to the chief negotiator of the European Union, and vice versa. So, the negotiations failed.

In spite of unusual and extraordinary attempts by the Chairman to push his new draft protocol through, it was resoundingly rejected early on the morning of 22 February 1999, and the negotiations by the Open-ended Ad Hoc Working Group on Biosafety were formally abandoned. It was agreed, however, that the text would be presented to the Extraordinary COP of the CBD. A formal Extraordinary Session of the Conference of the Parties, chaired by H.E. Juan Mayr Maldonado, the Minister of Environment of Colombia, had been planned to approve the text of the finalized protocol. Instead, the Extraordinary COP had to restart the negotiations almost from scratch, though it took into consideration the draft protocol passed on to it by the Ad Hoc Working Group on Biosafety.

2.4.3. Negotiations in the Extraordinary Sessions of the Conference of the Parties to the Convention on Biological Diversity

The meeting of the Extraordinary COP started only five minutes after the negotiations of the Open-ended Ad Hoc Working Group ended in the early morning of 22 February 1999. Therefore, the outgoing Chairman of the negotiations, Veit Koester, gave only a short verbal report.

At the end of the meeting, the Chairman of the Extraordinary COP convened another negotiation session involving the chief negotiators of the Miami Group, the European Union and the Like-minded Group. The chief negotiator of the European Union again came up with his previous package, which, among other problems, accepted the deletion of the Precautionary Principle, as did the chief negotiator of the Miami Group with his set of proposed changes. The two continued discussing their respective proposals as if I did not exist. It looked as if they were about to agree. At this stage, even the European Union's package contained a provision for virtually unregulated import and export of LMO commodities, with the provision that it would be reviewed at the first meeting of the Parties after the Protocol had come into force. Therefore, I rejected both sets of proposals. I told the two chief negotiators that ignoring the developing countries had completely died with the colonial era. The Chairman tried to break the deadlock by introducing an enabling clause similar to that of the European Union, which, he thought, would have made it possible for me to accept the Miami Group's position on LMO commodities. The enabling clause stated that the issue of LMO commodities would be renegotiated after the protocol came into force. However, the Miami Group's chief negotiator rejected it. I was also going to reject it, but one rejection was good enough and I kept quiet.

On 23 February 1999, the last day of negotiations, the chief negotiators of all the Regional Groups (i.e. not only those of the Miami, European Union and Like-Minded Groups) met under the chairmanship of H. E. the Minister of Environment of Colombia. It became clear that there would be no agreement. The Miami Group promised to come back to the negotiations after one year with a new proposal that took into account the difficulties on LMO commodities expressed by me on behalf of the Like-minded Group.

Nonetheless, informal consultations continued by all parties. In an informal discussion with John Herity of Canada, I had suggested that, if the Miami Group knew the nature of the agricultural systems in developing countries, where most crop gene pools are found, their delegations would appreciate our problems more clearly. He took up the challenge. Therefore, delegates from the Miami Group of countries visited Ethiopia on 2–6 September 1999 and toured farms, homesteads and grain markets. They left Ethiopia saying that this was going to help them to come up with an acceptable proposal for the next negotiation session.

In the meantime, I wrote an analysis of the negotiations in Cartagena and of the latest text of the draft Protocol, pointing out what I thought we should fight for, and distributed it to the delegates of the Like-minded Group. This helped consolidate the views of the Group.

On 15–19 September 1999, the Chairman of the Extraordinary COP invited delegations to informal consultations in Vienna. These consultations showed more clearly the difficulties that the protagonist Regional Groups had with one another's positions. It also helped further consolidate the somewhat amorphous Like-Minded Group, which I continued to lead. I helped this consolidation process along by writing an analysis of the informal negotiations of Vienna and of the draft text of the protocol and distributing it to the members of the Like-Minded Group before the final negotiations by the Extraordinary COP.

On 30 November – 3 December 1999, the Miami Group, led by the United State of America, tried to have a decision on trade in LMO commodities passed at the Seattle Ministerial Session of the World Trade Organization (WTO). I went to Seattle and lobbied primarily for African delegations

to oppose this. Others also lobbied the delegations they could access. The result was that the WTO did not pass any decision on trade in LMO commodities. In fact, the ministerial negotiations collapsed and I am convinced that the issue of LMO commodities contributed its share to this. Probably as a result of all this, the Miami Group decided to negotiate on LMO commodities seriously in the Biosafety Protocol.

The final negotiations by the Extraordinary COP were in Montreal on 24–28 January 2000, and the text of the Cartagena Protocol on Biosafety was agreed. This final negotiation session of the Extraordinary COP was also difficult.

The negotiations on LMO commodities were primarily between the Miami Group and the Like-minded Group. The European Union negotiators, presumably confident that their internal laws were robust enough to protect them from unwanted LMO commodities, were, though, supportive of the Like-minded Group, not willing to fight much on the issue. On the other hand, they were very keen on having clear provisions on labelling LMOs and LMO products in the protocol. The Miami Group continued to oppose labelling, the Precautionary Principle and the treatment of products of LMOs as an issue. Both the Miami and European Union Groups continued to oppose any meaningful negotiations on liability and redress.

The Like-minded Group had wanted to join forces with the European Union to push for labelling. However, the prevaricating attitude of the Brazilian delegation had prevented us so far. In this session, the Brazilian delegation also became supportive of labelling. Nevertheless, I decided to hold back on the issue until I was sure that the European Union delegation would support the Like-minded Group until the negotiations on LMO commodities were finalized, and they did. The result was the somewhat clumsy compromise we struck with the Miami Group that is now in Article 11 of the Cartagena Protocol.

The Like-minded Group also wanted the Scope of the Protocol (Article 4) to include all LMOs and not to explicitly exclude any categories of LMOs. Though we realized, since the Miami and European Groups were united on the issue, that pharmaceuticals (Article 5 of the Protocol) and transit and contained use (Article 6 of the Protocol) would not fully be subject to the advance informed agreement procedure (Articles 7–10 of the Protocol) under the Protocol as we had wanted. We did not want these exceptions to be made at the level of the Scope of the Protocol (Article 4). We wanted to make it always possible for the Protocol to consider all LMOs. Therefore, because the Miami Group wanted a separate provision on LMO commodities and the European Union wanted labelling, we managed to obstinately bargain with them both to accept an all inclusive Scope (Article 4).

The European Union negotiators had changed their position since the failed negotiations of Cartagena and, with the support of the Like-minded Group they pushed for the Precautionary Principle (Articles 10.6 and 11.8 of the Protocol). The Miami Group thus had to accept the Precautionary Principle. I think the preceding collapse of the WTO negotiations in Seattle, together with the unity between the European Union and the Like-minded Groups, forced them to give up their wish not to subject the Protocol to the Precautionary Principle.

As I have already pointed out, LMOs that are transiting through a country are not subject to the advance informed agreement procedure (Article 6.1 of the Protocol). The Like-minded Group did not want this exception. The compromise that was forced on us was that of being allowed to prohibit through the Biosafety Clearing-House those specific LMOs that a state considered particularly dangerous. This will require capacity to access information through the Biosafety

Clearing-House and alertness to place objectionable LMOs in the Biosafety Clearing-House. Such capacity is often lacking in developing countries and it has to be developed.

Excluding contained use from the advance informed agreement procedure was unacceptable to the Like-minded Group. Norway proposed that agreement could be reached on the issue if the definition of what 'contained use' would constitute were to be legally left to the country of import. This made it possible for Article 6.2 to be formulated in a way that we could accept. Unlike the Miami and European Union Groups, we wanted LMO pharmaceuticals for human use also subjected to the advance informed agreement procedure. We were convinced that there were (and there still are) no 'other relevant international agreements or organizations' that are responsible for LMOs that are pharmaceuticals for humans. Therefore, these LMOs must be governed by the Cartagena Protocol. It thus became possible for us to accept Article 5 of the Protocol as a compromise. This will, however, require alertness in the World Health Organization and possibly in other forums so that rules on LMO pharmaceuticals for humans that violate the advance informed agreement procedure are not adopted anywhere. It should be noted that, because Article 5 of the Protocol is restricted to pharmaceuticals for humans, pharmaceuticals for animals have to be subjected to the advance informed agreement procedure (Articles 7–10) of the Protocol.

The Miami Group and some members of the European Union opposed the position of the Like-minded Group on products of LMOs. Therefore, we were forced to give it up. However, the Risk Assessment Annex (Paragraph 5 of Annex III) enables the assessment of risks posed by LMO products. This, in combination with national laws on environment and health, can fill the gap created.

The most important deficiency of the Protocol as far as developing countries are concerned is in the absence of provisions to govern liability and redress. A promise was made to continue negotiations after the coming into force of the Protocol (Article 27), and the Like-minded Group felt that this promise was all that the negotiations of the Protocol could yield at that time, and accepted the negotiations of the Protocol as finalized.
Another issue left pending by the Protocol, in spite of the push by the European Union towards the end of the negotiations, supported by the Like-minded Group, was that of packaging and labelling (Article 18, Paragraphs 2(a) and 3). This was the last issue to be negotiated before the Cartagena Protocol on Biosafety was adopted by the Extraordinary COP at *c.* 6 a.m. on 29 January 2000 after an all night session.

2.5 Negotiations on Issues Left Pending by the Cartagena Protocol on Biosafety
Article 27 of the Protocol stipulates that, at its first meeting, the Conference of the Parties to the Convention on Biological Diversity serving as the meeting of the Parties to the Cartagena Protocol (COP-MOP) shall 'adopt a process with respect to the appropriate elaboration of international rules and procedures in the field of liability and redress for damage resulting from transboundary movement of LMOs'. Through Decision BS-1/8, the first COP-MOP, which convened in Kuala Lumpur, Malaysia, on 23–27 February 2004, adopted the terms of reference of an Open-ended Ad Hoc Working Group of legal and technical experts to negotiate a liability and redress regime for the Protocol (CBD Handbook 2005). The Working Group has already held two negotiations sessions in Montreal in May 2005 and February 2006. Article 27 of the Protocol expects the Working Group to complete its negotiations within four years, i.e. before February 2008.

The second issue left pending at the adoption of the Protocol has two components. The first component (Article 18.2 (a) of the Protocol) requires the COP-MOP to take a decision on the

detailed labelling of LMO commodities 'no later than two years after the date of entry into force of this Protocol'. The Protocol entered into force on 11 September 2003. A labelling scheme should therefore have been finalized at the second meeting of the COP-MOP, which took place in Montreal on 30 May – 3 June 2005. However, New Zealand and Brazil prevented the meeting from reaching a consensus on labelling requirements for LMO commodities.

Therefore, the issue was taken up again at the third meeting, which took place in Curitiba, Brazil, on 13–17 March 2006. Brazil had changed its previous position and asked for a labelling system that gave sufficient detail, as had all the other Parties except for New Zealand and the members of the Miami Group, which are all non-Parties. New Zealand has laws that prohibit LMO commodities from entering its territories. The New Zealand delegation was, therefore, apparently acting on behalf of the Miami Group; as a Party to the Protocol, New Zealand could block consensus, but as non-Parties the Miami Group of countries could not. Dismayed by this, non-governmental organizations in New Zealand launched a campaign, and as a result, many letters were written to the Prime Minister of New Zealand by concerned people and organizations from all over the world. In this way, the New Zealand delegation at the meeting in Curitiba was forced into silence. Therefore, in spite of attempts by the Miami Group to prevent it, a decision requiring detailed labelling of LMO commodities was adopted (CBD Decision BS/111/10 2006).
The second component (Article 18.3 of the Protocol) requires the COP-MOP to evaluate the need for and modalities of developing standards for the packaging and transport of LMOs. The completion of this requirement is not time-bound. The process started at the third meeting in Curitiba, and it may be a few years before it is finalized (CBD Decision BS/111/10).

3. A Brief Evaluation of the Appropriateness of the Provisions of the Cartagena Protocol on Biosafety

In evaluating the provisions of the Cartagena Protocol on Biosafety, I need first to state the premises I start from. My premises are the following:

- Since once released into the environment LMOs cannot be recalled, a strict adherence to the Precautionary Principle is required in biosafety.
- Since, on the whole, the number of living species increases towards the equator, unforeseen impacts of LMOs also increase towards the equator. This means that risk assessment becomes more complex towards the equator.
- Since the countries with the least scientific capacities are found towards the equator, mistakes in both risk assessment and risk management are likely to increase towards the equator.
- Since the poorest countries are found towards the equator, mistakes in managing LMOs are likely to be most devastating towards the equator.
- Since, on the whole, the number of species increases towards the equator, the possibilities of solving perceived problems of development by choosing from among the diverse varieties of the species available rather than trying to create transgenic organisms increase towards the equator.

This makes genetic engineering less relevant towards the equator.
Starting from these premises, I can point out the following difficulties with the main provisions of the Protocol.

3.1 General Provisions

Article 2.4 starts by stating that any Party can take action more protective than the Protocol. However, it weakens this possible action by qualifying it. It specifies that such action must be consistent with the objective and the provisions of the Protocol. The objective of the Protocol (Article 1) is broad and would thus allow a lot of room. However, the provisions are, of necessity,

much more detailed and they thus arguably restrict the more protective action that can be taken. Article 2.4 also allows international law, e.g. on trade, to restrict the more protective action that can be taken.

3.2 The Scope
The scope of the Protocol (Article 4) is good. However, Article 5 weakens it when it comes to LMOs that are pharmaceuticals for humans. It does so by making it essential, especially for the countries with least wealth and scientific capacity, to actively watch the World Health Organization and other international institutions lest they institute procedures or laws that bypass the advance informed agreement procedure. Article 6 similarly weakens the scope with regard to LMOs in transit and LMOs for contained use.

3.3 LMO Commodities
Though the provisions of Article 11 of the Protocol make the advance informed agreement the basis of decision taking in importing LMO commodities, the notification process takes place via the Biosafety Clearing-House (Articles 11.1, 11.6). This requires a well developed capacity even in a poor importing country. It would have helped poor importing countries if LMO commodities had been treated in the same way as LMOs intended for direct release into the environment (Articles 7–10).

3.4 Simplified Procedure
Article 13 of the Protocol allows the simplification of the advance informed agreement procedure in dealings between Parties that want to do so. This leaves poor developing countries vulnerable to pressure to accept simplified procedures from rich and powerful countries that produce LMO commodities and thus weakens the applications of the Precautionary Principle.

3.5 Bilateral, Regional and Multilateral Agreements and Arrangements (Article 14) and Non-Parties (Article 24)
Article 14 of the Protocol, especially in combination with Article 24, can also be used to lower protection in weaker countries. For example, Mexico, though a Party to the Protocol, insisted on including a paragraph that it interprets as exempting it from the labelling requirements finalized in the third meeting of the COP-MOP. This was because Mexico is a member of the North American Free Trade Agreement and thus has to import unlabelled LMO commodities from the United States of America and Canada. It can be expected that the United States will insist on similar concessions when it makes bilateral or multilateral agreements with other Parties to the Protocol.

3.6 Confidential Information
Article 21 of the Protocol allows for an exporter of a LMO to ask any of the items of information it supplies the importer to be kept as confidential. Though the same Article allows the importer to refuse keeping information that is necessary for biosafety as confidential, it makes this refusal conditional upon giving reasons. Again, this may subject a poor importing country to complications that it has little capacity to deal with. The consequence is then likely to be that it will accept treating information that is important for ensuring biosafety as confidential.

3.7 Socio-economic Considerations
Article 26 of the Protocol allows importing Parties to ‘take into account, consistent with their international obligations, socio-economic considerations arising from the impact of’ LMOs when deciding to import or reject those LMOs. For a poor developing country, socio-economic considerations should have a very high weight in decision taking on whether to import a given

LMO or not, especially when that LMO is a commodity. However, Article 26 diminishes this weight by invoking 'international obligations'.

4. Concluding Remarks

The Cartagena Protocol on Biosafety is the first environmental international law negotiated to pre-empt possible problems with the entirely new technology, recombinant DNA technology. This is why it is also the first environmental international law that is based on the Precautionary Principle. It is not surprising, therefore, that the process of negotiating it has been very divisive. It is equally not surprising that it satisfies nobody completely. Time will show whether negotiating it has set a good precedent to ensure the safety of emerging new technologies.

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Chapter 26
Cartagena Protocol on Biosafety

LIM LI LIN
THIRD WORLD NETWORK

This chapter will address and highlight some of the key substantive principles and provisions of the Cartagena Protocol on Biosafety. It does not comprehensively cover all aspects of the Protocol, and is not meant to provide legal interpretation of the Protocol. It will, however, underscore some of the rights and obligations of Parties under the Protocol, and address its interpretation and implementation at the national level.

1. Introduction

The Cartagena Protocol on Biosafety entered into force on 11 September 2003. It is a legally binding international agreement under the United Nations' Convention on Biological Diversity (CBD) (see Box 26.1).

Box 26.1. What is a protocol? (adapted from Mackenzie et al. 2003)

A protocol is a binding international instrument, separate from, but related to, another treaty. It is a separate instrument: a protocol must be individually negotiated, signed and eventually ratified. It is only binding on States that become Parties to it. It thus has its own Parties, and creates separate rights and obligations for them, as any other treaty.

The unique characteristic of a protocol is that it is related to a 'parent' treaty, through substantive, procedural, and institutional links. Most importantly, a protocol under a specific treaty must comply with the parent treaty's provisions authorizing and regulating the adoption of protocols under its auspices. Any protocol adopted as a result of these 'enabling' provisions in the parent treaty must comply with them. In particular, it may not deal with subjects which are beyond the purview of these provisions, or if these provisions are not restrictive in this regard, with subjects which are beyond the purview of the parent instrument. Such enabling provisions usually restrict (as is the case for the Cartagena Protocol) participation in a protocol to Parties to the parent treaty.
In addition, the parent treaty usually defines basic institutional and procedural links between the two instruments, for example it may indicate that provisions in the treaty itself (e.g. related to dispute settlement) will also apply to any protocol adopted under it.

The protocol itself may, however, add further links to the parent treaty, for example by designating mechanisms existing under the treaty (e.g. the Conference of the Parties) also to serve the protocol. This is the case for the Cartagena Protocol.

The Cartagena Protocol on Biosafety is the first international law to specifically regulate genetic engineering, and this largely reflects the global climate of concern about the safety, health and environmental risks of genetically modified organisms (GMOs), along with the wider political and socio-economic implications of this technology. For the first time in international law, there is an implicit recognition that GMOs are inherently different from naturally occurring organisms, and carry special risks and hazards, hence the need to have a legally binding international instrument. The Protocol recognizes that GMOs may have biodiversity, human health and socio-economic impacts, and that these impacts should be risk assessed or taken into account when making decisions on GMOs. Precaution is the basis for the Protocol itself, and is operationalized in decision-making and risk assessment.

The entry into force of the Protocol was an important defining moment in global biosafety regulation. It followed years of negotiations, from when the need for a biosafety protocol to address the risks of genetic engineering was first articulated in Article 19(3) of the CBD in 1992, to its adoption by more than 130 countries in the year 2000 in Montreal.

The Protocol's entry into force means that it is legally binding in the international legal system and in the legal systems of countries that have ratified, approved, accepted, or acceded to it (depending on a country's legal system). As of March 2007, there are 140 Parties to the Protocol. The Protocol enters into force in a country 90 days after it deposits its instrument of ratification, approval, acceptance, or accession with the United Nations Secretary General.

The first Conference of the Parties serving as the Meeting of the Parties to the Protocol (COP-MOP 1) was held in Kuala Lumpur, Malaysia, 23–27 February 2004. COP-MOP 2 was held in Montreal, Canada, 30 May–3 June 2005, and COP-MOP 3 was held in Curitiba, Brazil 13–17 March 2006. The COP-MOP is the Protocol's supreme decision-making body, which negotiates and adopts decisions that take forward the development, interpretation and implementation of the Protocol. COP-MOP decisions are binding on the Parties. Subsequent COP-MOPs will be held every two years, back to back with the Conferences of the Parties (COPs) of the CBD. The next COP-MOP will be held in Bonn, Germany in May 2008.

Prior to the Protocol's coming into force, the Intergovernmental Committee of the Cartagena Protocol (ICCP) met three times to move forward the work of the Protocol in the interim.

1.1 The different perspectives and interests

The Protocol negotiations were very difficult and divisive; although scheduled to conclude after six meetings of the Working Group on Biosafety (1995–1999) in February 1999 in Cartagena, Colombia, the talks collapsed (see Chapter 25). The United States-led Miami Group (comprising also Canada, Australia, Argentina, Chile, and Uruguay – the major producers of GMOs and their allies) could not agree to provisions on the transboundary movement of genetically engineered commodities. The provisions would

have required the prior informed consent of the importing Party before the GMOs are shipped to the respective countries. These commodities are the bulk of traded GMOs, and the Miami Group was determined that they should be excluded from the Protocol. On the other hand, developing countries felt very keenly the need to have an internationally binding legal instrument on biosafety, based on the principle of precaution, which would regulate the movement of *all* GMOs between countries.

During the negotiations, the overwhelming majority of these countries forged a negotiating bloc known as the Like-Minded Group of Developing Countries. As importers of GMOs, and as countries most vulnerable to their ecological and socio-economic impacts, they presented a united front.

At that time, most developing countries had no laws or regulations on biosafety and lacked the capacity, and technological and financial resources to regulate genetic engineering (this is still the case in many developing countries). As public rejection of GMOs in Europe and other parts of the world gathered momentum, the fear of becoming a dumping ground for GMOs was real.

It was thus imperative to place the onus on exporting countries to seek the prior informed consent of importing countries, instead of simply allowing GMOs to pass unregulated through the global market and across national boundaries. Furthermore, the lack of scientific certainty and gaps in scientific knowledge, mounting scientific evidence of hazards, and revelations of flawed approval systems in producer countries highlighted the urgent need for international regulation. The Protocol establishes the foundations of international law on the regulation – primarily of the transboundary movement – of GMOs. While many aspects of biosafety regulation are best addressed by national legislation, aspects relating to transboundary movement are difficult to regulate domestically. An international law is therefore necessary.

None of the Miami Group countries have so far become Parties to the Protocol. The United States is the leading producer of GMOs in the world but it is not even a Party to the CBD, and cannot become a Party to the Protocol unless it first becomes a Party to the CBD. Nevertheless, a number of significant GMO producing countries, such as Brazil, China, India, and South Africa, have become Parties.

2. Objective of the Cartagena Protocol on Biosafety

> 'In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human heath, and specifically focusing on transboundary movements'.
>
> Article 1, Cartagena Protocol on Biosafety

A number of points can be made with regard to the objective of the Protocol.

First, the precautionary approach as contained in the Rio Declaration is clearly identified to be the basis of the Protocol, and the objective of the Protocol is taken to be in accordance with the precautionary approach in Principle 15. (The preamble of the Protocol also reaffirms the precautionary approach contained in Principle 15.):

> 'In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.'
>
> Principle 15 of the Rio Declaration

Thus, precaution is the basis for the international regulation of GMOs. A full discussion of the Precautionary Principle in GMO regulations, and in the Protocol, is contained in Chapters 29 and 30 respectively.

The idea is to ensure that there is an adequate level of protection in the undertaking of all activities, in particular, the transboundary movement of living modified organisms (LMOs). Protection against adverse effects on biological diversity, 'taking also into account risks to human health', is the objective of the Protocol.

Clearly, protection from risks to human health is part of the objective of the Protocol. The Protocol always uses this language formulation whenever making reference to impacts on human health. This reflects the compromise that was reached on this issue, between the majority of developing countries that wanted the protection of human health to be included as an objective of the Protocol and those that only wanted the Protocol to ensure protection of biological diversity. It is clear from this formulation that impacts on human health as a result of adverse effects on biological diversity are captured, while direct impacts on human health (e.g. from consuming a GMO) may also arguably be captured.

2.1 'Living modified organisms' ('LMOs')

The term 'living modified organism' ('LMO') is used in the Protocol to mean 'any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology' (Article 3 (g)). This means that only living organisms that contain novel combinations of genetic material, and which have been produced using the techniques of modern biotechnology are defined as 'LMOs', and are within the scope of the Protocol. (See Chapter 23 on 'Definitions of GMO/LMO and modern biotechnology' for a discussion on possible similarities and differences in interpretation and understanding of GMOs/LMOs. For a thorough discussion on whether an organism is an LMO under the Protocol see Mackenzie et al. 2003).

Many countries use the terms 'LMO' and 'GMO' interchangeably, and consider that the terms refer to the same thing. A number of countries use the term 'GMO' in their national laws, and interpret the definition of LMO in the Protocol to be consistent with the definition of GMO in their national laws. Laws in the European Union and a number of other countries use the term 'GMO' to refer to LMOs covered by the Protocol. Malaysia, for example, made a written declaration on signing the CBD that the term 'LMO' would be understood as meaning 'GMO'. The definition of LMO in the Protocol is instructive

on this point.

What are clearly excluded from the definition of LMO, and by using the term 'living organism', are products from LMOs, which are not living, and which are therefore not covered by the scope of the Protocol. This includes, for example, oil produced from genetically modified (GM) canola or meat from GM animals.

3. General scope of the Protocol

A key fight during the course of the Protocol negotiations was for the inclusion of 'products thereof' in the general scope of the Protocol. This was strongly advocated by the Like-Minded Group of Developing Countries. 'Products thereof' include products derived from LMOs such as processed foods containing GM soya, cotton clothing made from GM cotton, etc. However, 'products thereof' are excluded from the scope of the Protocol and, as such, remain largely unregulated internationally.

However, there are two references to 'products thereof' in the Protocol. First, in the Risk Assessment Annex (Annex III) of the Protocol 'Risks associated with LMOs or products thereof, namely, processed materials that are of LMO origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment', and in Article 20.3 (c) which requires relevant information on 'products thereof' in the context of risk assessments or environmental reviews to be made available to the Biosafety Clearing House, where appropriate.

The Protocol's scope applies to the 'transboundary movement, transit, handling, and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health' (Article 4). The terms 'transboundary movement, transit, handling and use' are wide enough to include all activities related to LMOs under the scope of the Protocol.
The general scope of the Protocol in Article 4 provides for a comprehensive scope, covering all LMOs, and does not specifically exclude any category of LMOs.

3.1 GM pharmaceuticals: Within the scope of the Protocol?

Article 5 states that, 'Notwithstanding Article 4 and without prejudice to the right of a Party to subject all LMOs to risk assessment prior to the making of decisions on import, this Protocol shall not apply to the transboundary movement of LMOs which are pharmaceuticals for humans that are addressed by other relevant international agreements or organizations'.

Clearly, LMOs that are pharmaceuticals for animals are within the scope of the Protocol. Less clear is whether or not biopharm crops and animals (e.g. edible vaccines or plant/animal 'factories' that produce pharmaceutical compounds) are considered 'LMOs which are pharmaceuticals for humans'. In any case, these biopharm crops are clearly LMOs as defined by the Protocol, and Article 5 does not explicitly exclude biopharm

crops and animals.

The Protocol does not apply to only the transboundary movement of LMOs which are pharmaceuticals for humans and which must also be 'addressed by other relevant international agreements or organizations'. All three elements (there must be transboundary movement, involving LMOs which are pharmaceuticals for humans, and the LMOs in question must be addressed by other relevant international agreements or organizations) must be satisfied for the exemption from the general scope of the Protocol to apply.

In other words, the transboundary movement of some LMOs which are pharmaceuticals for humans may be excluded from the scope of the Protocol depending on whether or not they are addressed by other international agreements or organizations. However, a lot depends on the interpretation of the terms used in this provision, such as for example, 'addressed', 'relevant' and what would constitute an international agreement or organization for the purposes of this provision.

With regard to 'other international agreements', the Protocol allows for Parties to enter into bilateral, regional and multilateral agreements, and arrangements regarding the intentional transboundary movements of LMOs, but these must be consistent with the objective of the Protocol, and must not result in a lower level of protection than that provided for by the Protocol.

It is envisaged that 'other relevant international organizations' is meant to refer to the World Health Organization (WHO). However, WHO does not 'address' GM pharmaceuticals as such, to take into account the special hazards and risks of GMOs. Furthermore, it only sets standards for human health and safety and does not take into account impacts on the environment and biological diversity, which is the main focus of the Protocol.

Nevertheless, the Protocol explicitly preserves the right of Parties to subject all LMOs, including those that are pharmaceuticals for humans, to risk assessment prior to the making of decisions on import.

4. Main principles and provisions

A number of key principles underpin the Protocol. The principle of prior informed consent applies and there should be no transboundary movement without the prior knowledge and authorization of the importing Party. The onus is on the exporters and exporting Parties to notify and furnish relevant information to the importing Party before an LMO crosses national boundaries. An importing Party makes its own decision based on risk assessment and applying precaution, and national sovereignty in decision making is therefore one of the principles that the Protocol establishes. The right to say 'no' is also clearly established.

Precaution is operationalized in the decision-making procedures:

> Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a LMO on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the LMO … in order to avoid or minimize such potential adverse effects.

Precaution is also established as a principle in risk assessment:

> Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.

4.1 Advance informed agreement (AIA) procedure

The Protocol has a special focus on transboundary movements. The procedure by which transboundary movements of LMOs are regulated is known as the advance informed agreement (AIA) procedure which involves a few steps. Firstly, the Party of export notifies or requires its exporters to notify the Party of import if there is an intention to export a LMO. The notification must include at least the information required in Annex I (Information Required in Notifications under Articles 8, 10 and 13) of the Protocol. The notification is then acknowledged by the Party of import. The Party of import may make a decision on the notification according to its domestic regulatory framework, which must be consistent with the Protocol or proceed according to the decision procedure in the Protocol.

The decision by the Party of import is based on risk assessment and precaution, and the Party of import may take into account socio-economic considerations when making its decision. A Party is obliged to consult its public in the decision-making process, and must make the results of such decisions available to the public. A Party may make the following decisions: unconditional approval, approval with conditions, prohibition of the import, request for additional relevant information, or extension of the time period for making a decision.

There are time periods specified in the AIA procedure. The Party of import is required to acknowledge receipt of the notification within 90 days, and has a total of 270 days from the time it receives the notification to communicate its decision on the transboundary movement.

However, the issue of time frames was very contentious during the Protocol negotiations and a number of flexibilities have been built into the provisions. The Party of import may make its decision according to its domestic regulatory framework, which may not necessarily strictly adhere to the time periods specified in the Protocol, but which must be ‘consistent with’ the Protocol. Moreover, the clock stops (i.e. the time keeping is suspended) once the Party of import has requested for additional relevant information. The decision by the Party of import may also be to extend the time period.
A failure to acknowledge receipt of the notification does not imply the consent of the Party of import. Neither does a failure by the Party of import to communicate its decision within the specified time period imply that it has consented to the transboundary movement.

COP-MOP 1 adopted a decision on procedures and mechanisms for facilitating decision-making by importing Parties, which provides some guidelines and procedures to assist importing Parties.

4.1.1 Applicability of the AIA procedure

The AIA procedure under the Protocol does not apply to all LMOs. It applies only to the first intentional transboundary movement of LMOs for intentional introduction into the environment (e.g. planting and field testing) of the Party of import. Under the Protocol, subsequent exports will not be subject to the AIA procedure.

Under the Protocol, LMOs in transit (i.e. that are passing through the territory of a third party) are excluded from the AIA procedure. This simply means that the AIA procedure under the Protocol does not apply between the Party of export and the Party of transit. The AIA procedure will still apply between the Party of export and the Party of import for that shipment.

However, the right of a Party to regulate LMOs in transit is explicitly preserved under the Protocol. It must be noted that it is only the AIA procedure that does not apply to LMOs in transit, and all other provisions in the Protocol still apply.

Under the Protocol, the transboundary movement of LMOs destined for contained use (defined as specific measures that limit the contact and impact of LMOs on the external environment) undertaken in accordance with the standards of the Party of import are also excluded from the AIA procedure.

Article 6 (2) on contained use states:

> Notwithstanding Article 4 and without prejudice to any right of a Party to subject all LMOs to risk assessment prior to decisions on import and to set standards for contained use within its jurisdiction, the provisions of this Protocol with respect to the AIA procedure shall not apply to the transboundary movement of LMOs destined for contained use undertaken in accordance with the standards of the Party of import.

This means that if there is transboundary movement of a LMO that is destined for contained use, and the contained use is undertaken in accordance with the standards of the Party of import, only then can this category of LMO be exempted from the AIA procedure.

The right of Parties to subject all LMOs to risk assessment prior to decisions on import and to set standards for contained use within their jurisdiction is explicitly preserved. As with transit, all other provisions in the Protocol, apart from the provisions relating to the AIA procedure, will still apply.

4.2 Procedure for LMO-FFPs

LMOs intended for direct use as food or feed, or for processing (LMO-FFPs) are also excluded from the AIA procedure. These LMOs make up the bulk of traded GMOs, but they are not subject to the AIA procedure. These LMOs include, for example, GM foods, GM animal feed and GM microbes used in industrial production. For this category, an alternative system, based on information sharing via the Biosafety Clearing House (BCH)

(a website database administered by the CBD Secretariat in Montreal) applies.

When a Party makes a final domestic decision (e.g. for commercialization or placing on the market) on LMOs intended for direct use as food or feed, or for processing, that may be subject to transboundary movement, minimal information (specified in Annex II – Information Required Concerning LMO-FFPs under Article 11) must be posted on the BCH within fifteen days. This is the basically the extent of the obligation of the potential exporting Party.

A Party may take a decision on the import of LMO-FFPs under its domestic regulatory framework which must be consistent with the objective of the Protocol. This explicitly preserves the right of Parties to regulate LMO-FFPs in much the same way as other LMOs, according to an AIA-like procedure (bilateral notification and case-by-case decision making), at the national level.

How, to regulate LMO-FFPs, if at all, in the Protocol was the subject of much debate. It was argued by the majority of developing countries that the intended use of the LMO, even though for food, animal feed or for processing, would not ensure that the LMO did not end up being, for example, planted or released into the environment, which might entail risks to the environment and biological diversity. Hence, developing countries had wanted LMO-FFPs to be subject to the same AIA procedure as other kinds of LMOs. This was resisted by the Miami Group in particular, and the resulting procedure for LMO-FFPs, while preserving the rights of Parties to regulate LMO-FFPs according to their domestic regulatory framework, is a compromise.

Parties which are developing countries or which are economies in transition may, if they do not have a domestic regulatory framework, declare that their decision prior to the first import of LMO-FFPs will be taken according to a risk assessment in accordance with the risk assessment annex of the Protocol, and that the decision will be made within a predictable timeframe, which will not exceed 270 days.

Again, this allows for an AIA-like notification and decision-making procedure for countries without domestic regulatory frameworks. A potential importing Party that does not communicate this decision is not assumed to have agreed to or refused the import of LMO-FFPs. Precaution is also given operational meaning under this procedure.

The multilateral nature of the notification procedure for LMO-FFPs is vastly different from the bilateral nature of the AIA procedure for other LMOs. The burden is placed on potential importing Parties to constantly monitor the BCH for any notifications for domestic approvals in producer Parties. Potential importing Parties may have to initiate procedures for risk assessment and decision making without knowing whether a given LMO will ever be exported, or whether it will be exported to them. The burden of regulation of LMO-FFPs has thus been shifted from exporting Parties onto other Parties, and from international to domestic regulatory procedures.

5. Other key provisions in the Protocol

5.1 Risk assessment and risk management

Risk assessments are mandatory for decision making under the AIA procedure. It is the duty of the Party of import to ensure that risk assessments are conducted. The Party of import may also require the exporter to undertake the risk assessment as well as require the notifier (Party of export or exporter) to pay for the risk assessment. The rights of Parties to require risk assessment or to regulate LMOs according to their domestic regulatory frameworks which may require risk assessment is preserved for decision making for LMOs which fall outside the AIA procedure.

Risk assessments are carried out in order to identify and evaluate possible adverse effects on biological diversity and human health. In general, risk assessment includes identifying potential adverse effects, assessing the likelihood that the adverse effect may occur, and evaluating the magnitude of the consequences should the potential adverse effect occur. An adverse effect that is not very likely to occur may still carry a high risk if the consequences are severe and irreversible.

Under the Protocol, risk assessments must be carried out in a scientifically sound manner, taking into account recognized risk assessment techniques. Risk assessments are to be based, at a minimum, on the information provided in the notification, and other available scientific evidence, and carried out in accordance with the Risk Assessment Annex (Annex III) of the Protocol.

Risk management addresses the issue of how to regulate, manage and control the risks that may have been identified in the risk assessment process. Parties must establish and maintain appropriate mechanisms, measures and strategies for this purpose. These measures should be imposed to the extent necessary to prevent adverse effects on biological diversity and human health.

Parties must endeavour to ensure that LMOs have undergone an appropriate period of observation either corresponding with their life cycle or generation time, before the LMOs are utilized. Depending on the LMO concerned, the life-cycle time may vary from seconds to centuries. The generation time (from germination to producing progeny) would, in most cases, be shorter.

5.2 Socio-economic considerations

The Protocol recognizes that LMOs may have socio-economic impacts. Parties are entitled to take into account socio-economic considerations arising from the impact of LMOs on biological diversity when taking decisions on imports of all LMOs, as well as in decision making at the national level. This must, however, be consistent with Parties' other international obligations. In particular, the value of biological diversity to indigenous and local communities is highlighted, and Parties are encouraged to cooperate on research and information exchange on any socio-economic impacts, especially on indigenous and local communities.

5.3 Public awareness, education and participation
Public consultation in decision making is mandatory under the Protocol, in accordance with national laws and regulations. The results of such decisions must also be made available to the public. Parties are also under an obligation to promote and facilitate public awareness, education and participation on the impact of LMO activities on biological diversity and human health, and to endeavour to ensure that public awareness and education include access to information on imported LMOs.

5.4 Review of decisions
Parties may at any time review and change their decisions regarding imports of LMOs, in the light of new scientific information on potential adverse effects on biological diversity and human health. The Party must inform the notifier and the BCH, and provide reasons for the decision.

An exporting Party or a notifier may also request an importing Party to review an AIA decision where it considers that either there has been a change in circumstances that may influence the outcome of the risk assessment on which the decision was based, or additional relevant scientific or technical information has become available. The Party of import must then respond within 90 days providing reasons for its decision.

Under the Protocol, the AIA procedure only applies to the first intentional transboundary movement of LMOs for intentional introduction into the environment. However, the Party of import may also exercise its discretion to require a risk assessment for subsequent imports.

5.5 Unintentional transboundary movements and emergency measures
When a Party knows of an occurrence in its territory that has led or may lead to an unintentional transboundary movement that is likely to have significant adverse effects on biological diversity or human health, it must take appropriate measures to notify the BCH and other countries that have been affected or which may potentially be affected. It may also be required to notify relevant international organizations. The Party is under an obligation to immediately consult the countries that have been or may be affected in order to enable them to determine the appropriate response and initiate necessary action, which includes emergency measures.

Notification of such unintentional transboundary movement should include information on the quantities and characteristics and/or traits of the LMO; on the circumstances, estimated date of the release and the use of the LMO in the originating Party; about the possible adverse effects on biological diversity and human health; and on possible risk management measures. A contact point for further information should also be provided. Under customary international law, non-Party states are also under obligation to notify and consult other affected or potentially affected countries. However, they will not be bound by the specific procedures established under the Protocol for unintentional transboundary movements.

5.6 Illegal transboundary movement of GMOs
Parties must adopt appropriate measures to prevent and penalize (if appropriate) import and export of any LMOs that are in contravention of domestic measures implementing the Protocol, which are illegal transboundary movements. In such cases, the affected Party may request the Party of origin to either repatriate or destroy the LMO in question at its own expense. Parties must make available information about cases of illegal transboundary movements pertaining to it, to the BCH.

5.7 Handling, transport, packaging, and identification of transboundary shipments
Parties are to take necessary measures to require that all LMOs that are subject to transboundary movement within the scope of the Protocol are handled, packaged and transported under conditions of safety, taking into consideration relevant international rules and standards.

5.7.1 Identification of LMO-FFPs
The issue of identification of LMO-FFPs was very contentious during the Protocol negotiations and nearly caused the negotiations to collapse for the second time in Montreal in 2000. The compromise was to mandate the COP-MOP to make a decision on the detailed requirements, no later than two years after the Protocol enters into force. This meant that the decision had to be taken by COP-MOP 2, which was held in 2005. However, negotiations collapsed then, and no decision was taken until COP-MOP 3 in 2006.

The issue that was difficult to reach agreement on was about how shipments of LMO-FFPs should be identified. The majority of countries wanted such shipments to be clearly identified as containing LMOs that are not intended for intentional introduction into the environment, while the Miami Group countries would only agree to identify such shipments as ‘may contain’ LMOs not intended for intentional introduction into the environment. This was the compromise settled on and the last issue decided upon during the early hours of the morning when the Protocol was finally adopted.

On the contain/may contain issue, the COP-MOP 3 decision specifies that in situations where the identity of the LMO is known through ‘means such as identity preservation systems’, the shipment must be identified as one that ‘contains’ LMOs that are for direct use as food or feed, or for processing. A two-stage approach is set out for cases where the identity of the LMO shipment is not known.

In cases where the identity of the LMO is not known through ‘means such as identity preservation systems’, the shipment can be identified as one that ‘may contain’ one or more LMOs that are intended for direct use as food or feed, or for processing. This requirement is subject to review and assessment at COP-MOP 5 (2010), ‘with a view to considering a decision’ at COP-MOP 6 (2012) to ensure that the shipment is identified as one that ‘contains’ LMO-FFPs. This means that the ‘may contain’ language should no longer be an option after the interim period.

In both cases, where the shipment is identified as one that ‘contains’ LMOs as well as where the shipment is one that ‘may contain’ LMOs, the documentation accompanying

them must include the following details:

- that the LMOs are not intended for intentional introduction into the environment
- the common, scientific and, where available, commercial names of the LMOs
- the transformation event code of the LMOs or, where available, as a key to accessing information in the BCH, its unique identifier code
- the internet address of the BCH for further information

5.7.2 Identification of LMOs destined for contained use

For LMOs destined for contained use, they must be clearly identified as LMOs, and requirements for their safe handling, storage, transport, and use must be specified. The contact point for further information as well as the name and address of the individual and institution to whom the LMOs are being delivered are information that must also be included. COP-MOP 1 adopted a decision which specifies more details on these requirements.

5.7.3 Identification of LMOs for deliberate release and other LMOs within the scope of the Protocol

For LMOs that are intended for intentional introduction into the environment as well as other LMOs within the scope of the Protocol (e.g. LMOs in transit), documentation information must clearly identify them as LMOs, specify their identity and relevant traits and/or characteristics, and any requirements for safe handling, storage, transport, and use. The contact point for further information must be included as well as the name and address or the importer and exporter. In addition, a declaration that the transboundary movement is in conformity with the requirements of the Protocol must be included. COP-MOP 1 adopted a decision which specifies more details on these requirements.
COP-MOP 1 also adopted a decision providing examples of templates that could accompany shipments of LMOs destined for contained use and for intentional introduction into the environment as well as for other LMOs within the scope of the Protocol. In addition, the COP-MOP 1 decision addresses unique identification systems, particularly the system that has been developed by the Organisation for Economic Co-operation and Development (OECD) for GM plants.

5.8 Information sharing and the Biosafety Clearing-House

The Protocol establishes a Biosafety Clearing House (BCH) to function as a mechanism for the procedure that applies for LMO-FFPs and as a means through which information relevant to the implementation of the Protocol is made available by the Parties. It also serves to facilitate the exchange of scientific, technical, environmental, and legal information and experience with LMOs and to assist Parties to implement the Protocol. Parties must make available to the BCH any information required to be made available to the BCH under the Protocol as well as any existing laws, regulations and guidelines for implementation of the Protocol; information required for the AIA procedure; any bilateral, regional and multilateral agreements and arrangements; summaries of risk assessment or environmental reviews of LMOs generated by its regulatory process, including where appropriate, relevant information regarding ‘products thereof’; final decisions on import or release of LMOs; and reports to the COP-MOP on measures taken to implement the Protocol, including on implementation of the AIA procedure.

5.9 Confidential information

References to information sharing in the Protocol are usually qualified by a reference to respect confidential information as specified by Article 21. It must be noted that confidentiality is only vis-à-vis the public or a third party, and that no information can be withheld from the competent national authority. The notifier may identify information that it has submitted to the Party of import that it would like to be treated as confidential, providing justification upon request. The Party of import then decides on whether or not the information identified by the notifier qualifies as confidential information.

If the Party of import decides that the information identified does not qualify as confidential information, it must consult the notifier and must inform the notifier of its decision before releasing the identified information to the public. The Party of import should provide reasons upon request, as well as have an opportunity for consultation and for an internal review of the decision before it discloses the information to the public. Parties shall protect information received under the Protocol and deemed as confidential and must ensure that it has procedures in place to protect such information. It must do so in a manner no less favourable than its treatment of confidential information on LMOs that are domestically produced. The Party of import shall not use such information for commercial purposes unless it has the written consent of the notifier.

If a notifier withdraws a notification, the Party of import shall respect the confidentiality of commercial and industrial information including research and development information, as well as information on which there is no agreement as to its confidentiality. The Protocol specifies that the following information should never be considered as confidential: the name and address of the notifier; the general description of the LMO; the summary of the risk assessment of the effects on biological diversity and human health; and any methods and plans for emergency response.

5.10 Capacity building

The Protocol recognizes the special needs and vulnerabilities of developing countries, in particular the least developed and small island developing States, and Parties with economies in transition. To this end, Parties are required to cooperate in developing and strengthening human resources and institutional capacities in biosafety, including biotechnology to the extent that it is required for biosafety, including through existing global, regional, subregional, and national institutions and organizations and through facilitating private sector involvement, as appropriate.

Financial resource needs and access to and transfer of technology and know-how should be fully taken into account in accordance with the CBD. Cooperation in capacity building should include scientific and technical training in the proper and safe management of biotechnology, in the use of risk assessment and risk management, and the enhancement of technological and institutional capacities in biosafety. This should be subject to the different situations, capabilities and requirements of the Parties.

COP-MOP 1 adopted the ‘Action Plan for Building Capacities for Effective Implementation of the Protocol’ as well as the Coordination Mechanism for the

implementation of the Action Plan. The Action Plan identifies key elements that require concrete action; the processes/steps that should be undertaken; implementation at the national, subregional, regional, and international levels; monitoring and coordination of different actors undertaking capacity-building initiatives; and identifies a possible sequence of actions and activities identified in the Action Plan. An updated Action Plan was adopted at COP-MOP 3.

The Coordination Mechanism consists of the Liaison Group on capacity building for biosafety, biosafety capacity building databases in the BCH, an information sharing and networking mechanism consisting of the biosafety information resource centre and the biosafety capacity building network, coordination meetings and workshops, and a reporting mechanism.

COP-MOP 1 also adopted a number of guidance documents, on the 'Role of Different Entities in Supporting Capacity Building', an 'Implementation Tool Kit', as well as a 'Set of Indicators for Monitoring Implementation of the Action Plan for Building Capacities for the Effective Implementation of the Protocol'.

A related mechanism for capacity building under the Protocol is the Roster of Experts, which was established by a decision of the Extraordinary COP to the CBD the adopted the Protocol, to provide advice and other support, to conduct risk assessment, make informed decisions, develop national human resources, and promote institutional strengthening associated with the transboundary movements of LMOs to developing country Parties and Parties with economies in transition in fields relevant to risk assessment and risk management related to the Protocol.

5.11 Liability and redress

The issue of liability and redress was one of the most contentious during the Protocol negotiations. The majority of developing countries wanted operational provisions included in the Protocol, while the Miami Group did not want any provisions on liability and redress included in the Protocol. The compromise was to include text in the Protocol that mandates further work to develop a liability and redress regime.

Accordingly, COP-MOP 1 adopted a process to elaborate international rules and procedures in the field of liability and redress for damage resulting from transboundary movements of LMOs. Five Working Group meetings are to be held, and the regime should be adopted in May 2008 at COP-MOP 4, in line with the mandate to endeavour to complete the process within four years. As of March 2007, three Working Group meetings have already been held, and the subsequent negotiations are scheduled to be held in October 2007 and March 2008.

(See Chapter 31 'Liability and redress for damage arising from genetically modified organisms: Law and policy options for developing countries'.)

5.12 Non-Parties and bilateral, regional, multilateral agreements and arrangements

The Protocol also specifies that any transboundary movement of LMOs between Parties and non-Parties should be consistent with the objective of the Protocol. As an

international law, the Protocol cannot bind countries which are not Parties, but can only place obligations on countries which are Parties. Therefore, Parties are under the obligation to ensure that transboundary movement of LMOs between them and non-Parties is consistent with the objective of the Protocol.

Parties may enter into bilateral, regional and multilateral agreements and arrangements with non-Parties regarding such transboundary movements. Parties are also required to encourage non-Parties to adhere to the Protocol and to contribute appropriate information to the BCH on LMO transactions in their territory. COP-MOP 1 adopted a guidance document on the transboundary movement of LMOs between Parties and non-Parties. Parties may also enter into bilateral, regional and multilateral agreements and arrangements with each other on intentional transboundary movements of LMOs. These agreements and arrangements must be consistent with the objective of the Protocol, and must not result in a lower level of protection than that provided for in the Protocol. Parties must inform each other through the BCH of any such agreements or arrangements that they have entered into before or after the date of entry into force of the Protocol. However, if an agreement or arrangement was entered into before the date of entry into force of the Protocol, but it is not consistent with its objective and results in a lower level of protection than the Protocol, the Protocol will take precedence over that agreement or arrangement.

The provisions of the Protocol will not affect intentional transboundary movements between the Parties that take place pursuant to such agreements and arrangements, provided that they are consistent with the objective of the Protocol and do not result in a lower level of protection. Only intentional transboundary movements shall not be affected, and other provisions of the Protocol which do not relate to transboundary movements will apply.

5.13 Compliance with the Protocol

The compliance mechanism under the Protocol is separate from and without prejudice to the dispute settlement procedures and mechanisms under the CBD. It is meant to promote compliance of the Parties with their obligations under the Protocol.

COP-MOP 1 established procedures and mechanisms on compliance, which spelt out the objective, nature and underlying principles, and also established a Compliance Committee, and specified its functions and procedures. The decision also addresses information and consultation, measures to promote compliance and address cases of non-compliance, and review of the procedures and mechanisms.

COP-MOP 2 adopted the rules of procedure for Compliance Committee meetings. However, on the issue of voting, there was no agreement on taking a decision by a two-thirds majority, and this issue is still unresolved.

5.14 Relationship with other international agreements

(For a full discussion, see Chapter 27, ‘The WTO Agreements: An Introduction to the Obligations and Opportunities for Biosafety’.)

5.15 Review
The Protocol will be reviewed five years after its entry into force, i.e. at COP-MOP 4 in 2008, and at least every five years thereafter to evaluate its effectiveness, including an assessment of its procedures and annexes.

6. National implementation of the Protocol

The Protocol sets minimum standards for the regulation of LMOs – Parties may take action that is more protective of the conservation and sustainable use of biological diversity than that called for in the Protocol. However, the action must be consistent with the objectives and provisions of the Protocol and be in accordance with the Parties' other obligations under international law.

Parties are also under an obligation to take the necessary and appropriate legal, administrative and other measures to implement their obligations under the Protocol. This means that national measures such as a national biosafety law should be put in place to implement Protocol obligations.

7. Conclusions

The Protocol contains many important principles, which are now established in international law. However, it is a negotiated text with deficiencies for biosafety. While strengthening the Protocol and rectifying its deficiencies should be the long-term goal, it is critical that national governments, and developing countries in particular, formulate domestic biosafety laws that improve on the scope and standards set by the Protocol, and which also comprehensively regulate the domestic development and use of GMOs.
As an international law that is binding on countries that are Party to it, the Protocol presents obligations on and opportunities for sovereign countries. As a negotiated text, many flexibilities for interpretation and implementation are available for countries to utilize, putting real biosafety at the heart of national regulation.

In conclusion, the Protocol is just the start of the long and difficult road to effective international regulation of genetic engineering. Much more needs to be done, and countries must act to ensure that real biosafety becomes a reality.

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Chapter 27

The WTO Agreements: An Introduction to the Obligations and Opportunities for Biosafety

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The Cartagena Protocol on Biosafety is an extremely important development in the international regulation of genetically modified organisms (GMOs) and genetic engineering. It is the first international law to specifically regulate GMOs and genetic engineering. However, there are also other international laws and forums that are part of the international regulatory framework, which set up standards relevant for biosafety and which will have a relationship with the Cartagena Protocol.

This chapter covers the biosafety-related World Trade Organization (WTO) Agreements, which are legally binding for its Members. It examines the key relevant obligations contained in these agreements, and the opportunities for biosafety to be ensured.

The three forums that are recognized by the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) as international standard-setting bodies – the Codex Alimentarius Commission for food safety, International Plant Protection Convention (IPPC) for plant health, and the World Organization for Animal Health (OIE) for animal health and zoonoses – are considered in Chapter 28. Because these bodies are recognized by the SPS Agreement as standard-setting bodies, a WTO Member's measures that conform to their standards, guidelines and recommendations are presumed to be WTO-consistent. It is thus important to be aware of their developments in relation to biosafety.

However, it is also important to note that the SPS Agreement list of the three forums is not exhaustive. This means that international biosafety standards can be set in other relevant international organizations. In addition, standard-setting bodies should also be guided by the principles and standards established under the Cartagena Protocol on Biosafety. Although the standards from the three forums are guidelines, in practice they are often incorporated as national standards.

Since the WTO is the only international organization with a formal and enforceable dispute settlement system, it could have the effect of creating a legal hierarchy through its decisions with respect to United Nations agreements, which was not the intention of countries that negotiated the trade agreements and the establishment of the WTO. This 'relationship' issue was a key part of the Cartagena Protocol negotiations.

A problem that has arisen is the substantial interpretations of the WTO Agreements by dispute settlement panels and the Appellate Body (where appeals are made on panel decisions) of the WTO. These have included adjudication of conflicting provisions in two WTO Agreements. Under the WTO system, it is the General Council comprising all Members that is supposed to provide authoritative interpretation. However, in practice, the interpretations contained in the recommendations of the Appellate Body tend to become the final pronouncements of the issues concerned. Trade experts sit on WTO dispute panels, while trade lawyers are members of the Appellate Body.

Where there are possible conflicts between WTO and other agreements the situation raises even more concerns as it would mean that the WTO could be effectively adjudicating on those other agreements. An example is some observations about the Precautionary Principle made by the Panel in the case involving the European Communities' approval and marketing process of biotechnology products,[1] and the decision by the Panel to not consider the Biosafety Protocol.

1. General Agreement on Tariffs & Trade (GATT) 1994

In essence, WTO rules are disciplines on Member States' rights to take actions that affect trade, and this includes their rights to regulate biotechnology and adopt biosafety measures.
GATT 1994 applies to all measures affecting any product in international trade among WTO Members, including GMOs and genetically modified (GM) products. It should be read together with GATT 1947. The key disciplines are in three provisions:

- *Article I* on Most Favoured Nation requires that any advantage, favour, privilege, or immunity offered by any Member to any product originating in or destined for any other country shall be accorded immediately and unconditionally to the 'like product' originating or destined for the territories of all other Members.

- *Article III* (National Treatment) prohibits WTO Members from taking measures that directly or indirectly discriminate between the like products on the basis of their country of origin.

- *Article XI* (Quantitative Restrictions) prevents WTO Members from instituting or maintaining prohibitions or quantitative restrictions (such as quotas or import licences) on the import of products from other WTO Members.

Two important and unsettled issues on interpreting these Articles are relevant for biosafety regulation. First, there is no determination on whether GMOs and GM products and conventional products are 'like products' (e.g. GM soya and conventional soya).

Secondly, there is no agreement among Members on whether and how production and processing methods (PPMs) are regulated under the WTO Agreements. Developing countries that are WTO Members are wary of the *general* inclusion of PPMs in the WTO as these could be disguised trade protectionism.

The biosafety argument distinguishes genetic engineering as a production method that is fundamentally different from a conventional method, with potential risks inherent in the former. Thus, a soya variety that is produced from genetic engineering can be subject to trade restrictions necessary for biosafety, compared to a variety that is produced conventionally. This is the position that the majority of developing countries took in pressing for the Cartagena Protocol on Biosafety. However, PPMs in the WTO context and legal jurisprudence remain unsettled.
In any event, Article XX of GATT contains several general exceptions to these disciplines, including allowing for trade-restricting measures:

I. 'necessary to protect human, animal or plant life or health' under Article XX(b)
II. 'relating to the conservation of exhaustible natural resources if such measures are made effective in conjunction with restrictions on domestic production or consumption' under Article XX(g).

[1]European Communities – Measures Affecting the Approval and Marketing of Biotech Products

This means that Members may adopt or enforce such measures, even though they restrict trade. There are, however, limits on measures taken under Article XX. These measures must not imply 'arbitrary or unjustified discrimination between countries where the same conditions prevail or a disguised restriction on international trade'.

The body of WTO-related rules does not contain general exemptions of an environmental nature, nor does it provide a special status for multilateral environmental agreements such as the Cartagena Protocol on Biosafety. This is why the provision on general exceptions in Article XX is of crucial importance.

A biosafety measure would fall within Article XX provided it meets certain criteria (see Section 6).

2. Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement)

A WTO Member intending to apply measures to restrict trade for the protection of the life or health of humans, animals or plants has to comply with the Agreement on the Application of Sanitary and Phytosanitary Measures. The SPS Agreement deals with sanitary and phytosanitary measures that 'may, directly or indirectly, affect international trade' (Article 1.1). These measures include laws, regulations, requirements, procedures, and decrees.

The SPS Agreement is actually an elaboration of the rules for the application of the provisions of GATT 1994 which relate to the use of sanitary and phytosanitary measures, in particular the provisions of Article XX(b).

Annex A of the SPS Agreement provides definitions, including on the sanitary or phytosanitary nature of a measure. A sanitary or phytosanitary measure is any measure applied:

- to protect animal or plant life or health within the territory of the Member from risks arising from the entry, establishment or spread of pests, disease, disease-carrying organisms, or disease-causing organisms
- to protect human or animal life or health within the territory of the Member from risks arising from additives, contaminants, toxins, or disease-causing organisms in foods, beverages or feedstuffs
- to protect human life or health within the territory of the Member from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests
- to prevent or limit other damage within the territory of the Member from the entry, establishment or spread of pests.
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WTO Members are allowed to set their own standards, as long as the measures are applied only to the extent necessary to protect human, animal and plant life or health; are based on scientific principles and maintained with sufficient scientific evidence; are not a disguised trade restriction; do not arbitrarily or unjustifiably discriminate between Members where identical or similar conditions prevail (but can discriminate where different conditions prevail); and are not more trade-restrictive than required to achieve an appropriate level of protection, taking into account technical and economic feasibility.

WTO Members are encouraged to use international standards, guidelines and recommendations where these exist, although they may use measures that result in higher levels of protection, if there is scientific justification (i.e. they have conducted an examination and evaluation of

available scientific information and have decided that the international standards are not sufficient to achieve their appropriate level of protection). Alternatively, there needs to have been a risk assessment conducted according to the SPS Agreement provisions as a basis for a sanitary or phytosanitary measure taken, for that measure to be regarded as achieving the appropriate level of protection from the risk concerned.

The SPS Agreement also covers measures relevant to the *operation* of sanitary and phytosanitary measures. These are requirements for the 'operation of control, inspection and approval procedures, including national systems for approving the use of additives or for establishing tolerances for contamination in foods, beverages or feedstuffs'. These operational measures include undue delays in a sanitary or phytosanitary-related approval process.[2] This was the key issue in the case of *European Communities – Measures Affecting the Approval and Marketing of Biotech Products.*[3]

3. Agreement on Technical Barriers to Trade (TBT Agreement)

The Agreement on Technical Barriers to Trade covers all industrial and agricultural products, and regulates measures affecting trade which are technical regulations and technical standards (including packaging, marking and labelling requirements) and that are not sanitary or phytosanitary measures as defined in Annex A of the SPS Agreement.
The TBT Agreement tries to ensure that the regulations, standards, testing, and certification procedures (which vary from country to country) do not create unnecessary obstacles to international trade.

It allows a WTO Member to have national regulations, which should not be more trade-restrictive than necessary to fulfil a legitimate objective which includes national security; prevention of deceptive practices; protection of human health or safety, animal or plant life or health or the environment.

WTO Members can take measures necessary to ensure their own standards are met. They are encouraged to apply relevant international standards when these are available, but Members are not required to change their level of protection as a result.

The TBT Agreement covers (i) formulation of technical regulations by governments and these are mandatory; (ii) formulation of standards by the standardizing bodies of governments and these are voluntary standards; and (iii) procedures to assess or determine conformity with these regulations and standards. These are defined in Annex 1 of the TBT Agreement.

4. Relationship between GATT, SPS and TBT Agreements

While there is some controversy over the relationship between the GATT, SPS and TBT Agreements, it is clear under Article 1.4 of the SPS Agreement ('*Nothing in this Agreement shall affect the rights of Members under the Agreement on Technical Barriers to Trade with respect to measures not within the scope of this Agreement.*') and Article 1.5 of the TBT Agreement ('*The provisions of this Agreement do not apply to sanitary and phytosanitary measures*') that TBT measures which at the same time are sanitary or phytosanitary measures, are regulated under the SPS Agreement, not the TBT Agreement.

[2]See SPS Agreement, Article 8 and Annex C.
[3]DS 291(Complainant: United States)/DS292 (Complainant: Canada)/DS293 (Complainant: Argentina).

Furthermore, Article 2.4 of the SPS Agreement presumes that measures that are compatible under the SPS Agreement conform to GATT 1994: '*Sanitary or phytosanitary measures which conform to the relevant provisions of this Agreement shall be presumed to be in accordance with the obligations of the Members under the provisions of GATT 1994 which relate to the use of sanitary or phytosanitary measures, in particular the provisions of Article XX(b)*. This is not necessarily true in the reverse, so GATT-compatible measures may violate the SPS Agreement.
There is thus a hierarchy to the WTO Agreements related to biosafety, with seeming priority given to the most specific agreement applicable to any given measure. The SPS Agreement is the most specific agreement, dealing with plant, animal and human health protection. The TBT Agreement is less specific in that it regulates measures affecting trade which are technical and industrial standards (including packaging, marking and labelling requirements), and that do not fall under the SPS Agreement. GATT 1994 is much more general and overarching, and applies to all measures affecting any product in international trade, including GMOs and GM products.
A country that is a WTO Member would need to examine the compatibility of its biosafety measures under each Agreement. Which Agreement applies to a biosafety measure would depend on the objective of that measure. For example, in the case of labelling of GM food, if the policy objective is to protect human health, then this is an SPS measure, so it would fall under the purview of the SPS Agreement. If it is not an SPS measure, then one would have to ask whether it is a TBT measure (e.g. if a measure's objective is to prevent deceptive practices by informing the consumer) and if so, it would come under the TBT Agreement. If a measure does not fall specifically under the TBT Agreement, it would still have to comply with GATT 1994, especially Article XX.

5. Biosafety measures

Biosafety measures include pre-marketing approval procedures, monitoring obligations, restrictions and conditions, and bans or moratoria. These could be considered sanitary or phytosanitary measures, if their purposes relate to the protection of human, plant or animal life or health, and so fall under the SPS Agreement.

WTO Members need to ensure that any biosafety measure that is put in place to protect human, animal or plant life or health is consistent with the SPS Agreement.

Article 3.2 of the SPS Agreement states that sanitary and phytosanitary measures which '*conform to international standards, guidelines or recommendations shall be deemed to be necessary to protect human, animal or plant life or health, and presumed to be consistent with the relevant provisions of this Agreement and of GATT 1994*'. The international technical standard-setting bodies that are expressly recognized by the SPS Agreement are the Codex Alimentarius Commission for food safety, the International Office of Epizootics (known by its French acronym, OIE, and now known as the World Organization on Animal Health) for animal health and zoonoses, and the International Plant Protection Convention (IPPC) for plant health.
According to the WTO Appellate Body in *European Communities – Hormones*,[4] a WTO Member's measure that conforms to international standards, guidelines and recommendations are presumed to be WTO-consistent (although it is a rebuttable presumption). This measure should embody the international standard completely. If a Member imposes a measure that adopts some, but not necessarily all, of the elements of the international standard (i.e. 'based on'), it may not benefit from the presumption of consistency set up in Article 3.2.

[4]Appellate Body report on EC-Hormones, paragraphs 170–172.

Standards/guidelines relevant to biosafety have already been set by the Codex Alimentarius Commission (Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; and Codex Guideline for the Conduct of Food Safety Assessment of Foods Produced using Recombinant-DNA Microorganisms), and the IPPC (International Standards for Phytosanitary Measures No. 11: Pest Risk Analysis for Quarantine Pests, Including Analysis of Environmental Risks and Living Modified Organisms).

Article 3.1 of the SPS Agreement says that SPS measures should '*be based on international standards, guidelines or recommendations, where they exist*', except as otherwise provided for. In particular, Article 3.3 states that Members may introduce or maintain SPS measures '*which result in a higher level of sanitary or phytosanitary protection than would be achieved by measures based on the relevant international standards, guidelines or recommendations*'.

In other words, while the adherence to international standards, guidelines or recommendations is encouraged, a Member still has the right to set higher standards. This is possible 'if there is a scientific justification', or 'as a consequence of the level of sanitary or phytosanitary protection a Member determines to be appropriate' in accordance with certain criteria as contained in Article 5 (which deals with assessment of risk and determination of the appropriate level of protection), as discussed in the following, Sections 6–8.

Note that for the purposes of Article 3.3, there is a scientific justification if, on the basis of an examination and evaluation of available scientific information, a Member determines that the relevant international standards, guidelines or recommendations are not sufficient to achieve its appropriate level of sanitary or phytosanitary protection.

6. Tests for biosafety measures

Articles 2 and 5.1 of the SPS Agreement stipulate that while Members have the right to take sanitary and phytosanitary measures necessary for the protection of human, animal or plant life or health, such measures have to be applied only to the extent necessary to protect human, animal or plant life or health, are based on scientific principles, and are supported by scientific evidence. Sanitary and phytosanitary measures must be based on a scientific risk assessment, including consideration of the risks of the use of a product under real-life conditions.
The risk assessments that are undertaken are specific to the product in question. For example, a risk assessment would not be for all GMOs, but for a specific transgenic event in a specific GM crop. Minority opinions can be taken to account. The Appellate Body of the WTO has cautioned that a 'risk assessment' need not come to a 'monolithic conclusion':

> We do not believe that a risk assessment has to come to a monolithic conclusion that coincides with the scientific conclusion or view implicit in the SPS measure. The risk assessment could set out both the prevailing view representing the 'mainstream' of scientific opinion, as well as the opinions of scientists taking a divergent view. Article 5.1 does not require that the risk assessment must necessarily embody only the view of a majority of the relevant scientific community. … In most cases, responsible and representative governments tend to base their legislative and administrative measures on 'mainstream' scientific opinion. In other cases, equally responsible and representative governments may act in good faith on the basis of what, at a given time, may be a divergent opinion coming from qualified and respected sources.[5]

[5]EC-Asbestos Dispute, page 64.

Thus, the risk assessment need not necessarily be based on a majority opinion, and minority opinions can also be taken into account. Dispute settlement panels have not insisted that the science relied upon represents a mainstream scientific opinion, as long as it is based on respected and qualified sources. This is because the WTO does not decide on scientific issues, as its main task is to prevent unfair trade practices.

Under the SPS Agreement, there needs to be a rational relationship between a risk assessment and a biosafety measure. This arguably means that a mandatory pre-marketing approval procedure on a case-by-case basis would not violate the SPS Agreement. However, a general ban on GMOs may, in all likelihood, violate the SPS Agreement, as such a general ban is not product specific. This step may only be taken if it can be argued and supported by scientific evidence that GMOs are inherently dangerous.

6.1 Non-discrimination

A key trade principle that operates in the WTO is that of non-discrimination. The SPS Agreement, in Article 2.3, states that '*Members shall ensure that their sanitary and phytosanitary measures do not arbitrarily or unjustifiably discriminate between Members where identical or similar conditions prevail, including between their own territory and that of other Members. Sanitary and phytosanitary measures shall not be applied in a manner which would constitute a disguised restriction on international trade*'.

Under the National Treatment principle, biosafety measures must not distinguish between foreign and domestic products. Likewise, under Most Favoured Nation treatment, Members should not apply a measure that would constitute a means of arbitrary or unjustifiable discrimination among WTO Members. Thus, biosafety laws should not distinguish between different Members, i.e. an importing country cannot ban GM products from, for example, the US, but allow for the import of the same products from, for example, the EU.

Furthermore, Article 5.5 of the SPS Agreement states that Members should '*avoid arbitrary or unjustifiable distinctions in the level it considers to be appropriate in different situations, if such distinctions result in discrimination or disguised restriction on international trade*'. With regard to biosafety measures, different levels of protection apply in different situations, i.e. between a GM product and its conventional counterpart. As such, it must be ensured that these do not result in discrimination or disguised restriction on international trade.

In a situation where a product is derived from a GMO, but is chemically similar to, and indistinguishable from, its conventional counterpart, the question may arise as to whether different levels of protection should apply. However, what is important here is that the 'like products' test should be applied. (See discussion in Section 9 on 'like products'.)

A biosafety measure that is specifically aimed at discrimination, i.e. intentional protectionism, would violate the SPS Agreement. If it can be shown that a measure is not intended to protect markets, then this would not violate the non-discrimination principle.

6.2 Necessity

Any biosafety measure will also be questioned as to whether it is the least trade-restrictive measure. Article 5.6 of the SPS Agreement states that measures should be '*not more trade-restrictive than required to achieve their appropriate level of sanitary or phytosanitary protection*'. Under the SPS Agreement, the measure is not more trade restrictive than required unless there is another measure reasonably available, taking into account technical and economic feasibility, that achieves the appropriate level of sanitary or phytosanitary protection and is

significantly less restrictive to trade. There must be a reasonable relationship between the risk assessment and the design of the measure.

If one can argue that a ban on GMOs or GM products is necessary because there is, for example, a serious risk to human health, and support this claim with scientific evidence, then this should be WTO-consistent. However, if the objective of a measure is to protect a consumer's right to know, the WTO may conclude that labelling is a less strict measure than a ban.
Again, what is an 'appropriate level of protection' can be higher than the standards set by international organizations (see Section 5 in this chapter) as long as the sanitary and phytosanitary tests are satisfied.

7. What biosafety measures are allowed under the SPS Agreement?

Considering the discussion so far in this chapter, the establishment of a mandatory pre-marketing approval procedure will arguably comply with the SPS Agreement, if it fulfils the following requirements: a case-by-case scientific risk assessment, non-discrimination, and is not more trade restrictive than necessary.

A general import ban on GMOs or GM products will likely violate the SPS Agreement, unless it can be scientifically demonstrated that GMOs are inherently dangerous. Individual bans may be justified if the scientific evidence and risk assessment call for it. In general, a WTO Member would have to demonstrate that any import bans (i) have a rational basis, (ii) are in support of a legitimate policy objective, (iii) are no more trade restrictive than necessary to achieve that objective, and (iv) are not being applied in an arbitrary or discriminatory manner.
Temporary bans are allowed if they are provisional measures as allowed for under Article 5.7 of the SPS Agreement,[6] which is, in essence, the Precautionary Principle in action. A precautionary measure, which must be applied provisionally, may be taken subject to the following specific conditions:

(i) It must be imposed in respect of a situation where relevant scientific information is insufficient
(ii) It must be adopted on the basis of available pertinent information
(iii) The Member must then seek to obtain the additional information necessary for a more objective assessment of the risk
(iv) The WTO Member taking the measure must review the measure within a reasonable period of time.

Whether or not a general ban on GMOs or GM products can be allowed under Article 5.7 is uncertain. However, it is arguable that individual product bans of specific GMOs can be justified, if there is a rational relationship between a risk assessment and such a biosafety measure.
The Appellate Body in *Japan-Agricultural Products II*[7] said that these four requirements are cumulative in nature and equally important for determining consistency with this provision.

[6] 'In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organisations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time'.
[7] Appellate Body Report on Japan-Agricultural Products II, paragraph 89. In this case, the Panel examined whether the measure at issue met with these four requirements. See Panel Report on Japan-Agricultural Products II, paragraphs 8.56, 8.57 and 8.60.

Whenever *one* of these four requirements is not met, the measure concerned is inconsistent with Article 5.7.

With regard to the final obligation, the WTO Appellate Body has accepted that this should be established on a case-by-case basis depending upon the specific circumstances of the case, including the difficulty of obtaining the additional information necessary for the review, and the characteristics of the measure. Thus, it does not seem to imply a fixed or necessarily brief period for review, but rather the time it takes for new scientific knowledge to become available and this would arguably be different for each case.

8. Economic considerations

Risk assessment under the SPS Agreement can involve a mix of scientific and economic considerations. Procedures under the SPS Agreement will differ, depending on whether the risk is to animal or plant life or health, or instead to human life or health. When assessing risks to animals and plants, Members are to take into account relevant economic factors (Article 5.3). There is no similar reference to economic concerns in relation to impacts on human health.

Article 5.3 of the SPS Agreement reads as follows: '*In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks*'.

Moreover, Annex A (Definitions) of the Agreement defines risk assessment as '*The evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied, and of the associated potential biological and economic consequences; or the evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease-causing organisms in food, beverages or feedstuffs*'.

9. GM product labelling and the TBT Agreement

The purpose of any biosafety labelling obligation will determine whether it comes under the TBT or SPS Agreements. There could be two purposes for labelling; the first is to inform consumers to prevent deceptive practices, and the second is to inform consumers who suffer from certain allergies (as an example of health impact). The second category may be an SPS measure as it aims to protect human health, while the first purpose clearly falls under the TBT Agreement. The European Community's regulation on traceability and labelling is an example of a labelling scheme which has the purpose of informing consumers. It does not deal with safety considerations as GM products on the market would have already gone through a pre-market safety assessment.

The key question of any labelling regime will be whether it is WTO-compatible. To be WTO-compatible, the measure must meet the criteria as stipulated under Article 2.1 of the TBT Agreement:

> Members shall ensure that in respect of technical regulations, products imported from the territory of any Member shall be accorded treatment no less favourable than that accorded to like products of national origin and to like products originating in any other country.

This means that the principles of National Treatment, Most Favoured Nation and 'like products' apply.

Products should not be accorded less favourable treatment than 'like' products. There is considerable case law as regards the issue of 'like products'. The WTO has developed tests to determine if a product is 'like' another, based on the following criteria: (i) the physical properties of the product (e.g. detectable versus undetectable GM products); (ii) the extent to which the product is able to serve the same or similar end uses; (iii) the international classification of products for tariff purposes.

These criteria applied to some of the GM products would imply that they are alike to conventional products (e.g. GM soybean oil where the GM DNA is undetectable could be considered as being 'like' conventional soybean oil).

However, WTO panels have insisted on a fourth criterion – the extent to which consumers perceive and treat the product as an alternative means of performing particular functions in order to satisfy a particular want or demand. This implies that consumer perception is of considerable importance when it comes to deciding whether a product is different from or like another product. If the product is like another, with no physical difference, but consumers perceive it as different, then under WTO law, Members may treat it as different. This implies that Members can treat GM soybean oil differently from conventional soybean oil. Consumer perception could be demonstrated by data showing that consumers do view the products differently, for example, through opinion polls and surveys.

The Appellate Body has also found that 'evidence relating to the health risks associated with a product may be pertinent in an examination of 'likeness'' (*European Communities –Measures Affecting Asbestos and Asbestos-Containing Products*, WT/DS135/AB/R, Report of the Appellate Body adopted 5 April 2001, paragraph 113).

Furthermore, it is arguable, that as the Cartagena Protocol on Biosafety gains wide acceptance internationally, it may provide a basis for concluding that GMOs, or certain GMOs, are not 'like' their non-GMO counterparts.

Article 2.2 of the TBT Agreement also stipulates that 'technical regulations shall not be more trade-restrictive than necessary to fulfil a *legitimate objective*'. This means that the necessity of the measure must be shown. Nonetheless, the legitimate objectives are, inter alia, national security requirements, *prevention of deceptive practices*, protection of human health or safety, animal or plant life or health, or the environment. The legitimate objective of prevention of deceptive practices indicates that consumer information labelling of GMO products is consistent with the TBT Agreement.

10. WTO-Biosafety Protocol relationship

The general issue of the relationship between the WTO Agreements and multilateral environmental agreements remains unclear. The WTO Committee on Trade and Environment is the only inter-governmental forum that has discussed the issue for a number of years.

The WTO Agreements were adopted before the Cartagena Protocol on Biosafety was adopted and entered into force. Under international law, the interpretation of treaties is governed by the Vienna Convention on the Law of Treaties. The rule is that a later agreement supercedes an earlier one, and an agreement on a specific subject prevails over a general one. Since the

Cartagena Protocol on Biosafety was enacted after the WTO Agreements and deals specifically with biosafety, in a conflict of laws, it could be argued that the Protocol as a more specific agreement, and a more recent law, overrules the WTO Agreements.

However, due to the compromises made during the Protocol's negotiations, the language in relation to the Protocol's relationship with other international agreements is ambiguous. While the Protocol does not address this issue in its substantive provisions, the Preamble of the Protocol recognizes that trade and multilateral environmental agreements should be mutually supportive. This reflects a general rule of treaty interpretation that agreements between the same States and covering the same subject matter should be interpreted in such a way that promotes their compatibility.

The Protocol further emphasizes, on the one hand, that it shall not be interpreted as implying a change in the rights and obligations of a Party under existing international agreements and, on the other, that this is not intended to subordinate the Protocol to other international agreements; these anticipate cases where the spirit of 'mutual supportiveness' is not sufficient to avoid or resolve a conflict between the Protocol and any 'existing' or 'other' international agreement. The two paragraphs counterbalance each other, and leave little specific guidance as to how to resolve any conflict that may arise between the Protocol and other international agreements, particularly the WTO Agreements.

As the language is relegated to the Preamble, it carries far less weight than a substantive provision. Preambular language in international agreements, however, sets the framework for their interpretation.

There are also specific provisions in the operative text of the Protocol that refer to 'other international obligations'. For example, Article 2(4) on the right of Parties to take more protective domestic biosafety action qualifies this right – such action has to be 'in accordance with its other obligations under international law'. Article 26 of the Protocol on socio-economic considerations also makes reference to consistency with the other international obligations of Parties.

Thus, the relationship between the Protocol and other international agreements is not really addressed. If a country is Party to both the WTO and the Protocol, then mutual supportiveness between the two must be ensured, though in practice tensions may be expected. If a country is Party to one agreement but not to the other, then mutual supportiveness is even more elusive. A lot will depend on the forum where any dispute is arbitrated. The United States, the largest producer and exporter of GMOs and their products, cannot be a Party to the Protocol as it is not a Party to the parent convention, the Convention on Biological Diversity (CBD). Since it is unlikely that the United States will ratify the CBD, any dispute initiated by it may ultimately be brought to the WTO, as has been the case in *European Communities – Measures Affecting the Approval and Marketing of Biotech Products*. Although the Panel in this specific case chose not to consider the Protocol, it is still an open question as to the extent to which the WTO will take into account the Cartagena Protocol on Biosafety.

At the same time, the Compliance Committee that was set up at the First Conference of the Parties serving of the Meeting of the Parties to the Protocol in 2003 will be important in overseeing the implementation of the objectives and principles of the Protocol, and in providing a forum for arbitration or dispute resolution. The CBD itself provides for a dispute resolution procedure, which is also applicable to the Protocol.

11. Some conclusions

While the broader issue of the relationship between the WTO Agreements and multilateral environmental agreements is still unclear, the WTO Agreements do allow some biosafety measures to be taken, as long as certain criteria are met, i.e. the measures are based on scientific evidence (with risk assessment); are not discriminatory; and are not more trade-restrictive than necessary.

Where scientific evidence is insufficient, provisional biosafety measures may be taken on the basis of available pertinent information, provided additional information is subsequently sought for a 'more objective assessment of risk' and the measures are reviewed 'within a reasonable time'.

Standards or phytosanitary measures which conform to international standards, guidelines or recommendations set by the relevant international standard setting bodies (such as Codex, IPPC, OIE) are presumed to be consistent with the SPS Agreement and GATT 1994.
The provision of consumer information through labelling of GM products is WTO-consistent if it serves to prevent deceptive practices and, in the case of undetectable GM products, if consumers perceive such products as being different from the conventional counterparts.

Any country faces challenges at the national level when implementing a wide range of international instruments, which may sometimes seem competing. It is important that countries understand what the WTO Agreements say, what their obligations are, what exceptions are available and what the opportunities for biosafety are. Equally important is an understanding of the rights of a sovereign country, including those as afforded under the Cartagena Protocol on Biosafety. This will help to avoid the WTO's 'chilling effect', whereby Members are reluctant to act strongly for environment and health for fear of allegations of being 'WTO-inconsistent' and the WTO's binding dispute settlement mechanism. Countries would also have to coordinate their internal mechanisms to meet their obligations, not just under the Cartagena Protocol on Biosafety but also under the international standard setting bodies that are dealing with biosafety, such as the Codex Alimentarius, IPPC and OIE, which are given a prominent role in the WTO and which actively shape national responses.

At the international level, the political landscape is also important. There are efforts currently being made by many developing countries to reform the WTO and to assert their rights under the various Agreements. The debate on biosafety is benefiting from increasing scientific inputs specifically targeted at biosafety, and international law is being made and implemented as the debate progresses. As more countries become more knowledgeable on biosafety and cooperate to implement biosafety measures, this will shape the discourse on biosafety and the interpretation of the relevant international instruments.

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Chapter 28

International Standard Setting on Biosafety: An Introduction to Some Other International Agreements and Forums

LIM LI CHING
THIRD WORLD NETWORK

1. The international regulatory regime governing biosafety

The Cartagena Protocol on Biosafety, as the first international law to specifically regulate genetically modified organisms (GMOs) and genetic engineering, is an extremely important development in the international biosafety regulatory regime (see Chapter 26). There are, however, also other international laws and forums that are part of the international regulatory regime and which establish standards for biosafety.

In Chapter 27, the biosafety-relevant World Trade Organization (WTO) Agreements, which are legally binding for its Members, were discussed. This chapter will describe in further detail the three bodies that are recognized by the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (1994) (SPS Agreement) as international standard-setting bodies – the Codex Alimentarius Commission for food safety, the International Plant Protection Convention (IPPC) for plant health, and the World Organisation for Animal Health (OIE) for animal health and zoonoses – and some of the key elements of their work in relation to biosafety.

The standards, guidelines and recommendations established by these international standard setting bodies are explicitly recognized in the SPS Agreement as international standards, guideline and recommendations, on which WTO Members shall base their sanitary or phytosanitary measures. Often, countries adopt these standards, guidelines and recommendations at the national level, but very importantly, the SPS Agreement has flexibilities for Members to introduce or maintain higher standards if there is scientific justification for doing so. Furthermore, according to Article 3(2) of the SPS Agreement (1994), 'sanitary or phytosanitary measures which conform to international standards, guidelines or recommendations shall be deemed to be necessary to protect human, animal or plant life or health, and presumed to be consistent with the relevant provisions of this Agreement and of GATT 1994'. Thus, the standards, guidelines and recommendations established by the three bodies, are presumed to be WTO consistent, potentially shielding WTO Members that conform to such standards from challenge at the WTO's Dispute Settlement Body.

For matters not covered by the above three organizations, the SPS Agreement recognizes as international standards, guidelines and recommendations, the appropriate standards, guidelines and recommendations promulgated by other relevant international organizations open for membership to all Members, as identified by the SPS Committee. This means that international biosafety standards can be set in other relevant international organizations. Moreover, standard-setting bodies should also be guided by the principles and standards established under the Cartagena Protocol on Biosafety.

There are also other international efforts to set up standards and guidelines for GMOs, which are not discussed in this chapter. These include the UNEP International Technical Guidelines for Safety in Biotechnology and the FAO Draft Code of Conduct on Biotechnology as it relates to genetic resources for food and agriculture. In addition, the International Organization for

Standardization (ISO) has developed international standards related to the detection methods for GMOs and derived products in foodstuffs.

2. Codex Alimentarius Commission

The Codex Alimentarius Commission has 175 member governments (including the European Community). It was created in 1963 to develop food standards, guidelines and related texts such as codes of practice under the Joint Food and Agriculture Organization/World Health Organization Food Standards Programme. The main purposes of this Programme are to protect the health of consumers, to ensure fair trade practices in the food trade, and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. Thus, the Commission basically provides for the international regulation of food matters.

The standards, guidelines and recommendations established by the Codex Alimentarius Commission relate to 'food additives, veterinary drug and pesticide residues, contaminants, methods of analysis and sampling, and codes and guidelines of hygienic practice'. They are non-binding, but are recognized in the SPS Agreement as international standards, guidelines and recommendations for food safety.

2.1 Ad–hoc Intergovernmental Task Force on Food Derived from Biotechnology

In 1999, governments established the Ad-hoc Intergovernmental Task Force on Food Derived from Biotechnology to deal with the issue of genetically modified (GM) food or in the language used by the Codex Alimentarius Commission, 'food derived from biotechnology', in particular their health and nutrition implications. One key mandate of the Task Force was to elaborate standards, guidelines or other principles, as appropriate, for foods derived from biotechnology. The Task Force worked for four years, and adopted the following in 2003 (Codex Alimentarius Commission 2004):

- Principles for the Risk Analysis of Foods Derived from Modern Biotechnology
- Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants
- Guideline for the Conduct of Food Safety Assessment on Foods Produced using Recombinant-DNA Microorganisms.

2.2 Some significant elements of the Codex Principles and Guidelines

The Principles and Guidelines adopted by the Task Force recognize that a pre-market safety assessment (the part of a risk assessment that identifies whether a hazard, nutritional or other safety concern is present) should be undertaken on a case-by-case basis for GM foods. They also acknowledge that there are unintended effects related to GM foods that have to be risk assessed, prior to their market approval (Haslberger 2003). This is in addition to an evaluation of their potential direct health effects such as toxicity and allergenicity.

The unintended effects (reflected by the loss or modification of acquired or existing traits) that need to be evaluated arise from the process of insertion of DNA sequences into the plant genome (Codex Alimentarius Commission 2004). This may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. New or changed patterns of metabolites may also result. Moreover, environmental factors and genetic background may affect the expression of the transgenes (Haslberger 2003).

Notably, the Guidelines broaden risk assessment to encompass not only the health effects of GM foods, but also the indirect effects of GM foods on human health, for example, as mediated

through the environment. Under such an approach, herbicide residues from GM herbicide resistant crops (Codex Alimentarius Commission 2004) or potential risks associated with gene flow, for example, of a transgene coding for the production of biopharmaceuticals (Haslberger 2003), also need to be considered.

The Task Force also clarifies that the concept of 'substantial equivalence' is not a safety assessment in itself, but is only a starting point for any GM food safety assessment, to identify similarities and differences between the GM food and its conventional counterpart (Codex Alimentarius Commission 2004). This is in line with the limitations increasingly associated with the concept (for example, see the analysis by the Royal Society of Canada's Expert Panel on the Future of Food Biotechnology (Expert Panel on the Future of Food Biotechnology 2001)).
In relation to the use of antibiotic resistance marker genes, the Guidelines discourage their use and instead recommend that alternative transformation technologies be used in the future development of GM plants or GM microorganisms (Codex Alimentarius Commission 2004). This is because the possibility of horizontal gene transfer to intestinal microorganisms or human cells (see Chapter 13) is an occurrence that cannot be completely discounted. For food derived from GM plants, the Task Force recommends that 'If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in food. Antibiotic resistance genes used in food production that encode resistance to clinically used antibiotics should not be used in foods'. For food produced using GM microorganisms, the Task Force makes several recommendations, including the avoidance of genes in the genetic construct that could provide a selective advantage.

Legislation in the European Union already implements this, as there is an obligation in its Directive 2001/18 (see Chapter 22) to phase-out antibiotic resistance markers in GMOs by 2004 in the case of GMOs placed on the market and by 2008 for experimental GMOs. This applies to antibiotic resistance marker genes that may have adverse effects on human health and the environment. The Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority in 2004 evaluated the potential risks associated with specific antibiotic resistance marker genes, taking into account their current usage in clinical and veterinary medicine, and has issued guidance on this issue for EU Member States (Scientific Panel on Genetically Modified Organisms 2004).

The Codex Guidelines further recommend that foods derived from GM plants or produced using GM organisms that have been intentionally modified to alter their nutritional quality or functionality should be subjected to additional nutritional assessment and may require additional testing (Codex Alimentarius Commission 2004). The nutrient profile may change, affecting the nutritional status of individuals consuming the food, or there could be unexpected alterations in the nutrients. The need for stringent risk assessment on such GM foods is becoming more urgent, as there are more GM crops with such modifications in the pipeline, which regulatory authorities will have to assess. In response to this, the Task Force is currently undertaking work to develop a guideline on food safety assessment of foods derived from GM plants modified for nutritional or health benefits (see Section 2.3).

The Codex Principles also underline that risk management should take into account the uncertainties identified in the risk assessment, and that measures could include food labelling conditions for marketing approvals and post-market monitoring (Codex Alimentarius Commission 2004). In particular, post-market monitoring may be needed to verify conclusions about the absence or possible occurrence, impact and significance of potential health effects, and

to monitor changes in nutrient intake levels to determine human health impact (for GM foods likely to significantly alter nutritional status). (See also Chapters 32 and 33 on monitoring.)

2.3 Ongoing work of the Task Force

In July 2004, government members of the Codex Alimentarius Commission approved the re-establishment of the Ad-hoc Intergovernmental Task Force on Food Derived from Biotechnology. When the Task Force met in 2005, it agreed to initiate new work on the following:

- A guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals
- An annex to the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants regarding food safety assessment of foods derived from recombinant-DNA plants modified for nutritional or health benefits.
- Two Working Groups were established for this purpose:
- A physical Working Group to prepare a Proposed Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals, co-chaired by Australia and Japan
- An electronic Working Group led by Canada to formulate a scoping document on the Proposed Draft Annex on Food Safety Assessment of Food Derived from Recombinant-DNA Plants Modified for Nutritional or Health Benefits.

The Working Group on GM animals met twice in 2006 and discussed draft text. Among the contentious issues were how to address non-food safety concerns, such as environmental risks, animal welfare and ethical issues, and the use of antibiotic resistance marker genes (ICTSD 2006a). A Joint FAO/WHO Expert Consultation was held in early 2007 to seek scientific advice on the Proposed Draft Guideline and in particular to address questions related to the development and use of marker and reporter genes, and the non-heritable applications of recombinant DNA techniques to the production of animals, such as the safety of GM vaccines and gene therapy. In relation to the proposed draft annex regarding food safety assessment of foods derived from GM plants modified for nutritional or health benefits, Canada sent out a questionnaire to Codex delegations and interested organizations, to gather information, in order to assist in drafting the document. The scope of the work is, with respect to any additional safety and nutritional considerations, related to the assessment of foods derived from GM plants with enhanced nutrition. Regrettably, it does not cover plants expressing pharmaceuticals or other non-food related substances, the rationale being that the primary purpose of these plants is not food use but rather as factories to produce industrial or pharmaceutical compounds.

The Biotechnology Task Force met again in November 2006 and continued discussions on the two issues (GM animals and GM crops modified for nutritional and health benefits). A physical Working Group was established to elaborate the proposed draft annex on the safety assessment of foods derived from GM plants modified for nutritional or health benefits. In addition, several discussion papers were tabled, including on 'Safety Assessment of Foods Derived from Animals Exposed to Protection against Disease through Gene Therapy or Recombinant-DNA Vaccines'. Moreover, the United States requested the inclusion of a new item to the agenda and proposed new work on 'Food Safety Assessment of Low-Level Presence of Recombinant-DNA Plant Material in Food Resulting from Asynchronous Authorizations'. This deals with the low-level presence of unapproved transgenic material in food, in other words, transgenic contamination. At the November 2006 meeting, a Working Group to deal with this issue was established, chaired by the United States, Germany and Thailand. It will draft an annex on 'Low-Level Presence of

rDNA Plant Material to the existing Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants' (ICTSD 2006b).

The United States' proposal was revised to remove reference to 'asynchronous approvals', which had implicitly assumed that the importing country would eventually approve the contaminant (nothing in the document actually questions the right of an importing country to reject the contaminated shipment), and the focus expanded to include a requirement for supplying adequate data and information on the shipment and the contamination (such as the primers and other detection methodologies needed to detect the contamination) (personal communication, Philip L. Bereano and Michael Hansen 2006). The annex will not replace a full risk assessment under the Guideline for any GM foods that would be marketed in a country. It also does not preclude countries from having zero tolerance for unapproved GMOs and exporters must still meet a country's relevant import requirements.

There was disagreement in terms of the scope of the work, with the United States targeting GM plants under development being field-tested or plants that are no longer used commercially but may still be present in the food supply, and the European Union preferring to limit the work to cases where a GM plant has been approved in one country but not another (ICTSD 2006b). In the end, the terms of reference for the Working Group are to develop recommendations to the Task Force on performing a safety assessment in situations of low-level presence in which the GM plant has been authorized for commercialization for food by one or more countries, but the importing country has not determined its food safety, and on the requisite data and information sharing systems to facilitate this process. The work of this Working Group will undoubtedly attract much interest, given the increasing number of cases of transgenic contamination of food supplies that have occurred, the latest being of unapproved experimental GM rice.

2.4 Other biosafety-related work of Codex

The Codex Committee on Food Labelling has been discussing a Draft Proposed Guideline for the Labelling of Food and Food Ingredients Obtained through Certain Techniques of Genetic Modification-Genetic Engineering for many years now. It has yet to come to agreement on an international standard for mandatory labelling, largely due to opposition from the United States, Canada and Argentina, the major GM crop producing countries. Nonetheless, the draft standard on GM labelling has support from the majority of countries, both developed and developing. In May 2006, to try and move the discussion forward, a new Working Group was established to prepare guidance on GM food labelling. It will consider all relevant issues in order to identify the main problems, and take into account the experience of countries that have established relevant regulations on mandatory and voluntary labelling, including communication aspects. Some 40 countries already have laws requiring labelling of GM food. The Working Group met in January 2007 in Oslo, and is co-chaired by Norway, Ghana and Argentina.

Other biosafety-relevant discussions at Codex include the ongoing discussions on risk analysis under the Committee on General Principles, which also touch on issues such as precaution, risk assessment, risk management, and risk communication; discussions on traceability/product tracing under the Committee on General Principles and the Committee on Food Import and Export Inspection and Certification Systems; and discussions under the Committee on Methods of Analysis and Sampling on the criteria for the detection and identification of foods derived from biotechnology.

3. International Plant Protection Convention

The International Plant Protection Convention (IPPC) is an international treaty that sets phytosanitary standards for plants. It has 158 Parties (as of 20 December 2006) and the secretariat is hosted by the UN Food and Agriculture Organization.

The IPPC aims to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control. The international standards, guidelines and recommendations developed under the auspices of the Secretariat of the IPPC in cooperation with regional organizations operating within the framework of the IPPC are recognized by the SPS Agreement as international standards, guidelines and recommendations for plant health. Phytosanitary measures that conform to IPPC standards, guidelines and recommendations are deemed necessary to protect plant life or health and are presumed WTO consistent. International standards for phytosanitary measures (ISPMs) are developed through the work programme of the Commission on Phytosanitary Measures. Non-contracting parties to the IPPC are encouraged to observe these standards.

3.1 Pest Risk Analysis for Quarantine Pests including Analysis of Environmental Risks and LMOs
In April 2004, the then Interim Commission on Phytosanitary Measures endorsed a supplement on pest risk analysis for genetically or living modified organisms (LMOs), resulting in an integrated standard: *ISPM No. 11: Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms*. It includes guidance on evaluating potential phytosanitary risks to plants and plant products posed by LMOs.
Annex 3 of ISPM No. 11 (ISPM 2004) identifies the potential phytosanitary risks from LMOs when associated with some characteristic or property related to the genetic modification, as including the following:

(a) Changes in adaptive characteristics which may increase the potential for introduction or spread, for example, alterations in:

- tolerance to adverse environmental conditions (e.g. drought, freezing, salinity, etc.)
- reproductive biology
- dispersal ability of pests
- growth rate or vigour
- host range
- pest resistance
- pesticide (including herbicide) resistance or tolerance

(b) Adverse effects of gene flow or gene transfer including, for example:

- transfer of pesticide or pest resistance genes to compatible species
- the potential to overcome existing reproductive and recombination barriers resulting in pest risks
- potential for hybridization with existing organisms or pathogens to result in pathogenicity or increased pathogenicity

(c) Adverse effects on non-target organisms including, for example:

- changes in host range of the LMO, including the cases where it is intended for use as a biological control agent or organism otherwise claimed to be beneficial
- effects on other organisms, such as biological control agents, beneficial organisms, or soil fauna and microflora, and nitrogen-fixing bacteria, that result in a phytosanitary impact (indirect effects)

- capacity to vector other pests
- negative direct or indirect effects of plant-produced pesticides on non-target organisms beneficial to plants

(d) Genotypic and phenotypic instability including, for example:

- reversion of an organism intended as a biocontrol agent to a virulent form

(e) Other injurious effects including, for example:

- phytosanitary risks presented by new traits in organisms that do not normally pose phytosanitary risk
- novel or enhanced capacity for virus recombination, trans-encapsidation and synergy events related to the presence of virus sequences
- phytosanitary risks resulting from nucleic acid sequences (markers, promoters, terminators, etc.) present in the insert

3.2 Some significant elements of the IPPC standard

ISPM No. 11 (ISPM 2004) harmonizes and standardizes the way countries analyse risks that LMOs may post to plant health. A country may use the standard to determine which LMOs pose a threat and if necessary can subsequently (as a last resort) prohibit or restrict their import and domestic use. The standard is not just restricted to GM plants, but also covers other LMOs that may be harmful to plants, such as GM insects, fungi and bacteria. Direct and indirect effects on plants or plant products are both considered.

The standard includes the assessment of the risks of LMOs to plants, in so far as they are pests of plants (e.g. if a GM plant subsequently becomes a weed). Phytosanitary risks may result from certain traits introduced into the organism, such as those that increase the potential for establishment and spread, or from inserted gene sequences that do not alter the pest characteristics of the organism but that might act independently of the organism or have unintended consequences. In cases of phytosanitary risks related to gene flow, the term 'pest' is understood to include the potential of a LMO to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk, rather than the LMO acting as a pest in and of itself.

Under the assessment process, LMOs are essentially considered a potential phytosanitary risk/quarantine pest, until decided otherwise. Thus, for LMOs, the aim of the first, initiation stage is to identify those LMOs that have the characteristics of a potential pest and need to be assessed further, and those which need no further assessment under ISPM No. 11.
Furthermore, in most cases, the parent organism is not normally considered to be a plant pest but an assessment may need to be performed to determine if the genetic modification (i.e. gene, new gene sequence that regulates other genes, or gene product) results in a new trait or characteristic that may present a plant pest risk.

Even if it is determined that the LMO does not need further assessment under the standard, the IPPC recognizes that this only relates to the assessment and management of phytosanitary risks and that LMOs may present other risks (to the environment, or to human or animal health) not falling within its scope. It thus encourages the notification of relevant authorities if potential non-phytosanitary risks come to light.

Once an LMO is determined to be a potential pest, it then goes through a pest risk assessment process, involving three inter-related steps:

Pest categorization, to determine whether the criteria for a quarantine pest are satisfied. This would include defining the identity of the pest, which requires information regarding characteristics of the recipient or parent organisms, the donor organism, the genetic construct, the gene or transgene vector, and the nature of the genetic modification.

Assessment of the probability of introduction and spread, including an analysis of both intentional and unintentional pathways of introduction, and intended use. The probability of gene flow and gene transfer should be considered, when there is a trait of phytosanitary concern that may be transferred, as should the probability of expression and establishment of that trait. Moreover, the survival capacity without human intervention of the LMO should also be assessed. Other factors to be considered include specific cultural, control or management practices for GM plants, genotypic and phenotypic instability, and the proposed production and control practices related to the LMO in the country of import.

Assessment of potential economic consequences (including environmental impacts); in the case of LMOs, this relates to the pest nature (injurious to plants and plant products) of the LMO. Additionally, the potential economic consequences that could result from adverse effects on non-target organisms that are injurious to plants or plant products, as well as the economic consequences that could result from pest properties, should be considered.

The analysis of unintentional pathways of introduction is particularly significant with respect to LMOs, as experience has shown that these can play significant roles, no matter what the intended use. For example, in the Cartagena Protocol on Biosafety, a regulatory distinction is made between how LMOs for intentional introduction into the environment and those intended for direct use as food or feed, or for processing, are treated. This distinction is actually an artificial one, given that a GM grain intended for use as food or feed, or for processing, may also germinate and end up in the environment. It is thus important also to consider unintentional pathways with equal weight as intentional pathways of introduction.

With regard to economic impact, while some scientists argue that the assessment of potential economic consequences are not part of scientific risk assessments, it is clear from the IPPC standard that these have to be taken into account. The WTO SPS Agreement (1994), which the IPPC standard has a relationship with, states in Article 5.3:

> *In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks.*

(See also Chapter 27 for a discussion on the biosafety relevant WTO Agreements.) Moreover, 'risk assessment' is defined in the SPS Agreement (1994) as:

> *The evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied, and of the associated potential biological and economic consequences; or the evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease-causing organisms in food, beverages or feedstuffs.*

The conclusions from the pest risk assessment are then used to decide whether pest risk management measures should be taken. These measures should be cost-effective and feasible, not more trade restrictive than necessary, be applied to the minimum area necessary, allow for alternatives if the effect of different measures are the same, and be non-discriminatory. No additional measures should be imposed if existing measures are effective.

In addition to options such as inspection and testing, and restrictions on end use, distribution, and periods of entry of a commodity, measures may also be applied to restrict the import of consignments, if the plants are considered to be pests. Moreover, the measures may include procedures for the provision of information on the phytosanitary integrity of consignments (e.g. tracing systems, documentation systems and identity preservation systems). This issue had been intensively discussed under Article 18.2(a) of the Cartagena Protocol on Biosafety, when in 2006 a decision was adopted on the identification requirements for shipments of LMOs intended for direct use as food or feed, or for processing (see Chapter 26).

Importantly, if no satisfactory measure is available to reduce risk to an acceptable level, ISPM No. 11 (ISPM 2004) acknowledges that the final option may be to prohibit importation of the relevant commodities. This is viewed as a measure of last resort. Nonetheless, the implementation of phytosanitary measures are not considered permanent, and should be monitored, reviewed and modified if necessary.

4. World Organisation for Animal Health

The World Organisation for Animal Health (OIE) is an intergovernmental organization, and as of May 2006, totalled 167 Member Countries. It is recognized by the SPS Agreement as the international organization responsible for standard-setting related to animal health. Within this mandate, it aims to safeguard world trade by publishing health standards for international trade in animals and animal products.

4.1 Ad Hoc Group on Biotechnology

In May 2005, at the 73rd General Session, OIE members adopted Resolution No. XXVIII: Applications of Genetic Engineering for Livestock and Biotechnology, which requested the constitution of an Ad Hoc Group on Biotechnology.
Members also asked the OIE to develop and adopt standards, recommendations and guidelines (ICTSD 2005) for:

- research on the use of live attenuated vaccines in animal health
- use of DNA vaccines
- animal health risks linked to cloning
- assessing the health of embryos and production animals derived from cloning, and associated safety of cloned production animals and their products
- exclusion of unapproved animals and products from the livestock population and segregation from the feed and food supply
- identification, testing, and certification for international trade in production animals and their products for which biotechnology procedures have been employed.

The work of the Ad Hoc Group is ongoing.

5. Conclusion

The work of the three standard-setting bodies described in this chapter is part of the international regulatory system for biosafety. It is important for countries to be aware of the developments in

these other international agreements and forums, and to ensure coordination and coherence at the national level when developing biosafety law, policy and regulation.

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Chapter 29
The Precautionary Principle in GMO regulations

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1. Introduction

The Precautionary Principle has been accepted by many national governments as a basis for policy making, and it has become important both in international environmental law and international treaties (Freestone & Hey 1996: CBD 2000; EU 2000). Initially, the Precautionary Principle was developed to restrict marine pollution discharges in the absence of proof of environmental damage, and entered international policy with the Conferences on the Protection of the North Sea (in London 1987, The Hague, 1990, Bremen, 1994; Esbjerg, 1995) (Ducrotoy 1997).

With regard to GMO regulations, a precautionary approach plays an important role in the Cartagena Protocol on Biosafety (see Chapter 26), an international agreement mainly regulating the safe transfer, handling, use, and trans-boundary movement of GMOs.
Article 1 specifies the objective of the Protocol:

> *In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.*

Accordingly, the Protocol allows countries to use the Precautionary Principle to limit the use and release of GMOs in situations of scientific uncertainty with regard to potentially adverse ecological and health effects.

We also find the Precautionary Principle in regulations such as the Norwegian Gene Technology Act of 1993 and the EU directive 2001/18/EC on deliberate release into the environment of GMOs (see Chapters 22 and 24). The Norwegian Gene Technology Act has included the Precautionary Principle in its preparatory work as well as in Appendix 4 of the newly revised regulations on Impact Assessments under the Gene Technology Act, where it is stated that the Precautionary Principle shall be used when evaluating possible hazards and damage for animal and human health and the environment. In the EU directive the Precautionary Principle is included in the objectives of the Act.

The Precautionary Principle is a normative principle for making practical decisions under conditions of scientific uncertainty. Its employment entails the identification of risk, scientific uncertainty and ignorance, and it involves transparent and inclusive decision making processes (Raffensperger &Tickner 1999). However, the application of the Precautionary Principle in risk assessment and management of GMO use and release is at present a subject of heated scientific and public controversies. In the view of the critics, the use of the Precautionary Principle places additional regulatory burden on GMO utilisation, and thereby reduces returns from innovation, limits utilisation of GMOs worldwide and provides disincentives for research. On the other hand,

advocates of the Precautionary Principle want to enhance safety procedures and to separate trade and environmental interests in decision making, and are often linking this to lack of knowledge and omitted biosafety research.

2. The Precautionary Principle

The Precautionary Principle is a normative principle for making practical decisions under conditions of scientific uncertainty. It has four central components: it is supposed to 1) initiate preventive action as a response to scientific uncertainty, 2) shift the burden of proof to the proponents of a potentially harmful activity, 3) explore alternative means to achieve the same goal, and 4) involve stakeholders in the decision making process (Kriebel et al. 2001). The actual content of the Precautionary Principle, however, and the practical implications of its implementation in policy issues are controversial (Raffensperger & Tickner 1999; Morris 2000). Several formulations of the Principle, ranging from ecocentric to anthropocentric, and from risk-adverse to risk-taking positions, have been put forward (see Boxes 29.1 and 29.2). A weak version of the Precautionary Principle is often grounded in narrow utilitarian ethics, and its application involves risk/cost-benefit analyses. In this context, the Principle may be used as an option to manage risks when they have been identified through risk analysis. For instance, the Rio Declaration employs the weighing of costs and benefits (Box 29.1), and similar wording has been reproduced in the preamble of the Convention on Biological Diversity and in Article 3 of the Framework Convention on Climate Change.

Strong versions of the Precautionary Principle embrace inherent values of the environment and often are founded in ecocentric views or duty-based concerns for non-human beings and ecosystems (see Chapter 7 for further elaboration). A strong version is active in nature and obliges regulators to take action, for instance by implementation of risk management procedures. The Wingspread Statement is considered to represent a strong version of the Precautionary Principle (Box 29.2).

Box 29.1 Weak version of the Precautionary Principle

The Rio Declaration:

In order to protect the environment, the precautionary approach should be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation (Agenda 21, 1992).

Whatever version/formulation one uses, the implementation of the Precautionary Principle presupposes:

I. Some threat of harm must have been identified
II. Scientific uncertainty exists with regard to the potential harm
III. There are criteria to guide proactive and precautionary measures.

Box 29.2 Strong version of the Precautionary Principle

The Wingspread Statement:

When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically (Raffensperger & Tickner 1999).

2.1 Implementation of the Precautionary Principle as a Response to the Threat of Harm

The implementation of the Precautionary Principle requires that indications of adverse impacts are being documented in some way, and that risk-associated research is initiated (Myhr & Traavik 1999; Foster et al. 2000). First of all, the sources and types of scientific uncertainties should be identified. At present, scientific information on environmental and health effects is limited, both from the industry and from public research institutions, due to lack of biosafety related research. Several aspects of scientific uncertainty in regard to GMO use and release are presented elsewhere in this book: see Chapters 8–15.

When one is making decisions the presence of scientific uncertainty complicates the weighing of benefits against both immediate and long-term costs. Technological and economical approaches, such as risk-cost-benefit analyses, may be used to specify the uncertainties within a reduced scientific framework. However, such approaches cannot cope with complex biological and ecological processes that, for instance, GMOs are going to be used and released into. The decision makers might be prone to rely on short-term considerations of risk, and thereby not include adverse effects with a low probability or long-term hypotheses of risk in the decision. Hence, both technological and economical approaches tend to function as less restrictive standards of safety, in so far as risk and uncertainty are being permitted as long as there are benefits. In this context, uncertainty is often defined simply as lack of knowledge that can be reduced by further research.

Recognising that uncertainty is more than unknown probabilities or insufficient data, different taxonomies of uncertainty have been developed (Wynne 1992; Dovers et al. 1996):
Hazard can be related to a specific adverse event. Risk represents the relationship between probability and consequences, hence a condition where the possible outcomes are identified and the relative likelihood of the outcomes is expressed in probabilities.
Uncertainty refers to situations where we do not know or cannot estimate the probability of hazard, but the hazards to be considered are known. The uncertainty may be due to the novelty of the activity, or to the variability or complexity involved. For instance, even if the frequency of horizontal gene transfer has been studied extensively before the use and release of GMOs, there will be selective forces influencing the outcome and causing different results than that obtained in laboratory experiments.

Ignorance represents situations where the kind of hazard to be measured is unknown, i.e. completely unexpected hazards may emerge. This has historically been experienced with BSE or mad-cow disease, dioxins and pesticides, among others. With regard to GMOs, there may emerge, for instance, unprecedented and unintended non-target effects. Non-target effects include the influence on and interactions with all organisms in the environment, and may be either direct or indirect. Direct effects concern eco-toxic effects on other organisms, for instance, adverse effects on insects resulting from larval feeding on insect-resistant plants, or effects on soil organisms. Indirect effects concern effects on consumer health, contamination of wild gene pools or alterations in ecological relationships (see Chapters 8–15 in this book for further elaboration).
Indeterminacy, or ‘great uncertainty’, describes the inevitable gap between limited experimental conditions and reality, where the consequences of an activity can never be fully predicted. The structures and dynamics of biological systems cannot be described by their parts solely, as genes and proteins, but concern interactions with each part of the system and the composite effects from abiotic (non-living) factors as well (Kitano 2002). Therefore, it is crucial that methods for detection and monitoring are initiated with the purpose of following up the performed risk assessment, to map the actual health and environmental effects, and to identify unexpected adverse effects. Long-term monitoring provides baselines against which to compare future changes, and it gives input data to improve regulation systems (Cranor 2003).

2.3 Implementation of the Precautionary Principle Involves Acknowledgement of Scientific Uncertainty

The first step for scientists is to become aware of the role they play in the production of information and the subsequent political use of this information (Myhr &Traavik 2002). At present, the proponents, sceptics and opponents use different evidence to describe or interpret the data (or lack of data) with regard to the potential consequences of GMO use and release in various ways. Such factual divergences cause disagreement about which facts are relevant, and what research needs to be initiated (Levidow 2003). In addition, in most cases proponents of an activity will challenge the significance of evidence and argue that the opponents have a credibility problem. Consequently, there is a need to consider how to deal with the present uncertainty accompanying the use and release of GMOs. For instance, how to approach statistics (see Chapter 17 and approaches that define and systematise the uncertainty involved, such as the W&H (Walker and Harremöes) framework), may help to use scientific knowledge more efficiently in directing further research and in guiding risk assessment and management processes.

3. Threshold for evidence

A threshold of scientific plausibility of potential harm must exist before a precautionary measure can be initiated. For instance, Article 15(1) of the Cartagena Protocol on Biosafety states:

> *Risk assessments undertaken pursuant to this Protocol shall be carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognized risk assessment techniques. Such risk assessments shall be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of living modified organisms on the conservation and sustainable use of biological diversity, taking also into account risks to human health.*

The references to 'available scientific evidence' and 'scientifically sound manner' can be seen as a predetermined qualitative term, while, for instance, the EC communication on the Precautionary Principle (EC 2000) and the Report of the Expert Group on the Precautionary Principle of the World Commission on the Ethics of Scientific Knowledge and Technology (UNESCO 2005) have chosen to focus on the quality of the information. By demanding scientific evidence before employing the Precautionary Principle, the Biosafety Protocol requires documentation indicating that the GMO causes harm to health or the environment. Does this mean that one needs scientific evidence for lack of scientific certainty?

There is an important difference between demanding scientific evidence for potential harm versus only focusing on scientific uncertainty. Strong versions of the Precautionary Principle as well as the UNESCO version allow that presence of scientific uncertainty and indications of harm are enough for acceptance of employment. Hence, the demand for 'scientific evidence' represents an ambiguity in the formulation of the Protocol, especially if one compares this with what is stated in Article 10 of the Protocol:

> *Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the living modified organism in question as referred to in paragraph 3 above, in order to avoid or minimize such potential adverse effects.*

The demand for 'scientific evidence' and risk assessments to be undertaken in a 'scientifically sound manner' involves a misrepresentation of the current lack of knowledge and may cause uncertainty to be downplayed, especially if these terms have implications for how to interpret Article 10. This ultimately raises the question: What will be the role of lack of scientific certainty when risk assessment is going to be carried out in a scientifically sound manner?

For instance, the different scientific disciplines that are involved in the epistemic debate employ competing models or different analogies for basic assumptions to frame the scope for further research. Molecular biologists would refer to the practice and precision of doing laboratory research, and plant biologists would compare safety with the history of conventional plant breeding, while ecologists would refer to the adverse experiences based on the introduction of novel species into new environments. Such factual divergences cause disagreement about which facts are relevant and what research needs to be initiated (Levidow 2003).

A reference to qualitative terms may also cause non-mainstream arguments to be downplayed. For instance, not many years ago horizontal gene transfer (HGT) was considered to have such low a frequency that it was regarded as insignificant. However, it is now gaining increased attention and has become an important topic for risk-associated research related to GMO use and release.

3.1 The 'Familiarity Principle' and Substantial Equivalence versus the Precautionary Principle

The OECD (1993a) introduced the 'Familiarity Principle', stating that GE used in order to produce new agricultural strains 'does not exceed the risk of conventional techniques'. Criteria for determining familiarity include knowledge of and experience with any or all of the following: the crop plant, the environment, the trait, pleiotropic genetic modification of the crop or trait, and interactions among the crop, the trait, and the release environment. The Familiarity Principle is founded on the assumption that there does not seem to be any reason to expect more serious problems arising from GMOs in agriculture than from conventional agricultural practice.
This principle has been criticised with regard to its underlying assumptions and its narrow framework (Barret & Abergel 2000). For instance, the decision thresholds for the extrapolation of safety that are supposed to ensure that adverse effects do not exceed those of the non-GM counterpart will vary significantly, depending on the nature of their subject, i.e. organic versus chemical-intensive agriculture. Furthermore, the argument of analogy to the safety of conventional agriculture is not a valid comparison and cannot be extrapolated to GM crops, because no similar conventional crops have been commercialised. Conventional breeding involves using natural plant reproductive methods which is only possible between closely related species, or breeding methods that introduce new traits into plants via chemical or radiation mutagenesis of the plant's genome. GE, on the other hand, involves the exchange of genes from both distantly related and non-related species, which in many cases would never breed with each other, by using gene guns or microinjections in order to transfer the genes.

To assess the safety of GM food, the concept of 'Substantial Equivalence' was introduced by the OECD in 1993 and later affirmed by the FAO (OECD 1993b; FAO 1996; 2000). Substantial Equivalence is considered by some as a guiding principle for risk assessment with the intention to consider whether a GM food product is as safe as its traditionally bred counterpart. For example, in the US, GM food and GM products that are considered substantially equivalent, i.e. as safe as their non-GM counterparts, are being commercialised without labelling requirements and post-market monitoring (see Chapters 32 and 33).

The Expert Panel of the Royal Society of Canada (2001) identified two different uses of the concept of Substantial Equivalence: the decision threshold interpretation and a safety standard interpretation. The panel accepted the validity of the safety standard, but expressed that its validity as a decision threshold interpretation was restricted. The safety interpretation requires rigorous scientific analyses with the purpose of identifying all changes being introduced to the organism. At the same time, the panel raised the question of how to define 'rigorous demonstration' and suggested that an integrated approach is needed to consider changes in the GMO (The Expert Panel of the Royal Society of Canada 2001).

Inevitably, it has been argued that the application of Substantial Equivalence does not ascertain the problem that needs to be solved, and that the adequate assessment of ecological effects requires a broader basis. The narrow focus on risk has caused an extensive debate among regulators and scientists, leading to both support (Gasson & Burke 2001) and criticism (The Expert Panel of Royal Society of Canada 2001; Myhr & Traavik 2003). The issue of novelty of GE has been central in these discussions. It has been argued that there does not seem to be any reason to expect different impacts from genetically modified organisms than from traditional agricultural products.

On the other hand, as has been argued in Chapters 4, 8 and 9, the present methods for genetic modification entail a lack of precision and control over insert integration. The Codex Alimentarius Commission has suggested that risk assessments of GM foods need to be broadened in order to encompass not only health related effects of the food, but also to include unintended effects (Haslberger 2003). For instance, there is growing awareness that unintended effects in GMOs might arise as a result of gene or base pair/gene fragment insertion. The expression level of a gene rather than the amino acid sequence of the protein product can determine phenotypes that will contribute to natural varieties which can be influenced both by climatic and environmental conditions. Consequently, the significance of the genetic modification process needs to be elaborated at several levels: see Chapters 3, 4, 8, and 9 in this book.

Contrary to the use of the Familiarity Principle and the concept of Substantial Equivalence, the employment of the Precautionary Principle may initiate debate concerning the quality of risk-related scientific advice and the identification of areas where scientific understanding and knowledge is lacking, and perhaps most importantly increase recognition of the extent of ignorance (i.e. accept that we do not know that we do not know). A precautionary approach might, therefore, be seen as more scientific since it depends on broader judgements and involves initiation of basic research that either concedes or rules out risks of harm to human and animal health or the environment.

4. The Need for Proactive Measures

The level of precaution to be implemented will depend on the probability of harm, the level of uncertainty, the seriousness/irreversibility of the potential harm, and the availability of alternatives. Within GMO use and release, precautionary action might vary from restricted use (based on required monitoring of impacts) to labelling of the products, to a banning of a GM product or moratorium on action. Implementation of precautionary measures entails more science, since it depends on broader judgements and involves initiation of basic research that either concedes or rules out risks of health and environmental harm. The determination of a country's chosen level of protection needs to be a political decision, where 'consistency' and 'non-discrimination' have, for instance, been relevant guidelines for employment of the Precautionary Principle in the EU (see Table 29.1).

Table 29.1. Guidelines for implementation of the Precautionary Principle (EU 2000).

Proportionality	'measures … must not be disproportionate to the desired level of protection and must not aim at zero risk'
Non-discrimination	'comparable situations should be treated differently and … different situations should not be treated in the same way'
Consistency	'measures … should be comparable in nature and scope with measures already taken in equivalent areas'
Scientific research	'The measures must be of a provisional nature pending the availability of more reliable scientific data … scientific research shall be continued with a view to obtaining more complete data'
Demonstrated benefit	'examination should include an economic cost/benefit analysis when this is appropriate and feasible'

The types of precautionary measures that are considered acceptable by the international community under some multilateral agreements such as the World Trade Organization are (so far) unclear. For instance, the Biosafety Protocol may set a new precedent with regard to the relationship between environmental protection and the international trade regime. Other international treaties involving the Precautionary Principle focus on environmental problems and the conflicts have centred on the significance of scientific understanding and the uncertainty involved. The Biosafety Protocol is concerned with both environmental impacts and food safety, where trade issues may be a reason for conflicts.

Accordingly, countries may face the threat of a WTO complaint such as the one that the USA, Canada and Argentina have submitted to the WTO over the EU's alleged failure to apply its authorisation system for GMOs. According to WTO rules, an importing country needs to prove scientifically that a particular product is unsafe in order to implement a legal ban on the import of that food (although in the case of insufficient scientific evidence, temporary precautionary measures may be applied). Hence, the demands of the WTO may come into conflict with the degree of scientific evidence necessary to trigger action under the application of the Precautionary Principle in the Cartagena Protocol on Biosafety (Helmuth 2000).

5. The Precautionary Principle and the burden of proof

Within the general use of technology it has been those who claim an existence of yet unproven effects who have had the burden of demonstrating that the activity in question is causing harm to health or the environment. With employment of the Precautionary Principle, the burden of proof is shifted to the proponent (notifier or exporter) which now needs to demonstrate that the activity is necessary and that it will not harm health or the environment. This is reflected in the Cartagena Protocol on Biosafety and in the EU and Norwegian regulatory frameworks.

The proponent has the responsibility to demonstrate that the GMO in question is reasonably safe. Most countries have therefore implemented a case-by-case and step-by-step approach. The case-by-case procedure entails a mandatory scientific evaluation of every notification of a GMO. The step-by-step procedure facilitates a progressive line of development of GMOs by evaluating the environmental impacts of releases in decreasing steps of physical/biological containment (from greenhouse experiments, to small-scale and large field tests to market approval). The purpose of the case-by-case and step-by-step procedures is also to establish a learning practice that enables the authorities and the notifiers to collect information. In addition, in the EU, the proponents have also to submit a well-designed monitoring programme for how environmental monitoring is to be

carried out after commercialisation. It has also been suggested that assigning liability or financial bonds together with conditional approval and broad-scale testing might be means to ensure the GMO developers' responsibility.

6. The Precautionary Principle and the influence of normative standards

Risk assessment and management strategies are developed within particular regulatory frameworks, including normative standards and preferences regarding our relation to the natural environment and the preservation/promotion of human health. For instance, in the EU Directive 2001/18/EC it is stated that an environmental risk assessment needs to consider direct and indirect effects, immediate and delayed effects, as well as potential cumulative and long-term effects due to interaction with other GMOs and the environment. Article 1 of the Cartagena Protocol specifies that the entire objective of the document is to protect and conserve biodiversity according to a precautionary approach. One of the purposes of the Norwegian Gene Technology Act is that use of GMOs shall be in accordance with the principle of sustainable development (see Chapter 24). Normative standards may affect the scope of risk management of GMO use and release, and affect legal interpretations about the acceptable risks, thereby function as guidance for when and how to apply the Precautionary Principle.

Conclusions

The challenge of implementing the Precautionary Principle in proper ways involves both taking into account scientific and value uncertainty. A change to more integrative risk assessment and management, where the Precautionary Principle has an important role in situations of scientific and moral uncertainty may make science more accountable to public concerns. The ultimate objective is to find the right balance between too little and too much precaution.

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Chapter 30

The Precautionary Principle and the Cartagena Protocol on Biosafety: Development of a Concept

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1. Introduction

The Precautionary Principle and the Cartagena Protocol on Biosafety (CPB) are considered as so intimately linked that a closer reflection on the evolution of this linkage seems to be superfluous. A review of the literature dealing with the CPB reveals that numerous publications that analyse and evaluate the CPB exist (e.g. Cosbey & Burgiel 2000; Eggers & Mackenzie 2000; Hardstaff 2000; Meyer; 2000, Newell & Mackenzie 2000; Hutchison 2001). Publications of authors, who themselves have observed or conducted the negotiations, reflect on the work leading towards the negotiations (1992–1995) and on the flow of the negotiations (1996–2000) (e.g. Leskien 1996; Eckelkamp et al. 1998; Gupta 2000; Swenarchuk 2000; Bail et al. 2002; Latorre et al. 2003; Mayr & Soto 2003). During this time, the 'If' and 'How' of the inclusion of the Precautionary Principle was highly controversial, and the opposition by some governments and stakeholders remained fundamental.

This chapter starts with a description of the development and contextualization of the concept in the negotiations of the documents of the Rio Summit in 1992. It then shows how the concept of the Precautionary Principle was developed in the negotiations of the Cartagena Protocol of Biosafety. The chapter is mainly based on the UN documents concerning these negotiations and the experience of the author, who followed the negotiations from 1997 until 2000 as an NGO observer. In the final part, the article describes how the WTO dispute settlement mechanism has decided in prominent cases, testing government import restrictions imposed in situations of scientific uncertainty. Until now, no decisions under the Cartagena Protocol based on the application of the Precautionary Principle have been taken that could be analysed. The chapter does not deal with the WTO case dealing with the EU GMO *de facto* moratorium and national GMO bans.

2. Genetic engineering and the Precautionary Principle at the Earth Summit in Rio 1992

Many stakeholders intended to use the UN Conference on Environment and Development in June 1992 in Rio de Janeiro as the crucial event to overcome the critical discussion on genetic engineering in the US and the EU, which has accompanied the development of the new technology. These actors wanted the results of the conference to support a fast and smooth adoption of genetically engineered (GE) plants worldwide. To this aim, Chapter 16 of Agenda 21 presented future benefits of genetic engineering especially for the developing countries and called for international support for developing the technology, but favoured a restriction on possible regulations concerning potential risks, at the national level.

2.1 Biotechnology and Rio: Leitmotif and camouflage

It was 1992 in Rio when the still controversial topics of 'biological diversity' and 'genetic engineering' were coupled for strategic reasons. Agenda 21 propagates the use of biotechnologies as particularly useful to protect and sustainably utilize biological diversity. The discussions at, and the outcome of, the Rio Summit laid the foundation for the debate of the following decade,

presenting the use of genetic engineering as a core technology for a 'greener' and more sustainable food production. At the level of text, the Rio documents speak of biotechnology in general, while at the level of substance, the application of gene technologies, only one of the many biotechnologies, was almost exclusively discussed.

This semantic and, finally also, legal vagueness was a result of:

- the influence of the US governmental positions reflecting its approach to GMO regulation
- the support of this position through a broad range of stakeholders from science and corporations
- the negligence of the topic, but also support of the US position, by European delegations.

2.2 The political conflict around the Precautionary Principle

With respect to risks of genetic engineering and application of the Precautionary Principle, the Rio documents reflect different positions.

2.2.1 Rio-Declaration: Reference to the precautionary approach

The Rio Declaration in its Principle 15 speaks of the 'precautionary approach'.[1] Principle 15 links precautionary activities with cost-benefit considerations. This linkage is a reflection of the changes in the environmental policy of the US under the Reagan administration in the 1980s. In order to give more protection to industrial activities and investments, the requirements for governmental interference were increased, and restrictions were increasingly required to be based on scientific evidence showing risks and proving damages. The importance of risk assessments and cost-benefit analyses and thus the role of scientists in the field of political decision-making was strengthened considerably.

2.2.2 Agenda 21: Silent on the Precautionary Principle and the precautionary approach

By analogy to the US regulations, Chapter 16 'Environmentally sound management of biotechnology' of Agenda 21 takes up the principle of familiarity as one guiding principle in GMO risk assessments.[2] Neither the Precautionary Principle nor the precautionary approach is mentioned in this chapter. Only a few paragraphs of Chapter 16 are dedicated to the aspects of risks and international cooperation in risk assessment and management – similarly sparse is the mention of international financial support for biosafety activities. Future biosafety agreements should be negotiated bilaterally or laid down in voluntary guidelines.

2.2.3 Convention on Biological Diversity: Reflecting the Precautionary Principle

Sensing the disproportion between the elements referring to genetic engineering and biosafety in the emerging Agenda 21 and anticipating its restrictive effect on possible future biosafety activities in the international framework, some governments from developing countries and Northern Europe as well as some civil society observers proposed a more effective consideration of risk aspects in the Rio documents (Nijar 1996; Mayr & Soto 2003: 13). The only text that

[1]Rio-Declaration, Principle 15: 'In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.' http://www.unep.org/Documents.multilingual/Default.asp?DocumentID=78&ArticleID=1163 (accessed July 2006)

[2]Agenda 21, Chapter 16.29: 'Several fundamental principles could underlie many of these safety procedures, including primary consideration of the organism, building on the principle of familiarity, applied in a flexible framework ...'http://www.unep.org/Documents.multilingual/Default.asp?DocumentID=52&ArticleID=64&l=en (accessed July 2006)

could still be influenced for this purpose was the draft Convention on Biological Diversity (CBD). The negotiators of the CBD were able to bring a more stringent version of the Precautionary Principle into the text, but only in the preamble.[3] The CBD does not link precautionary activities with cost-benefit analyses and states that governments may act without having full scientific certainty. Article 19 'Handling of Biotechnology and Distribution of its Benefits' was amended by a paragraph that allowed the Member States to consider the necessity and content of an international agreement on GMO risk assessment.

3. UNEP Technical Guidelines for Safety in Biotechnology

The first international document which, based on the results of Rio, dealt with biosafety were the UNEP Technical Guidelines for Safety in Biotechnology (UNEP 1995). These guidelines were written to implement Chapter 16 of Agenda 21 and thus served as instrument for the introduction of GMOs in developing countries. The provisions were designed mainly by representatives of EU governments. In contrast, within the context of CBD Article 19.3, those delegations that were sceptical of the UNEP Guidelines worked towards the development of a more comprehensive international biosafety framework. In November 1995, at the second Conference of the Parties to the CBD, a working group was established, despite strong resistance from the EU and the US, to start the negotiations on the Biosafety Protocol. These negotiations began in 1996 and were finalized in January 2000 after six meetings of the Biosafety Working Group (BSWG), two sessions of the Extraordinary Conference of the Parties (ExCOP) in Cartagena and Montreal, and the intersessional informal meeting in Vienna. On 11 September 2003, the Cartagena Protocol on Biosafety entered into force.

4. The Precautionary Principle as an element of the Biosafety Protocol – An overview

In four paragraphs, the Biosafety Protocol reflects precautionary decision making: in the preamble, in Article 1 (Objective), in Articles 10 (Decision procedure) and 11 (Procedure for living modified organisms intended for direct use as food, feed, or for processing), and in Annex III paragraph 4 (Risk assessment) (UNEP 2000). The Protocol does not mention 'Precautionary Principle' but – quoting the Rio Declaration – uses 'precautionary approach'. The use of the wording 'Precautionary Principle' was blocked by the US, Australia and some other governments. The dispute is based on the legal point of view that the Rio Declaration itself contains the latter expression and that the Precautionary *Principle* is – still – not an internationally recognized principle of law. The US and supporting governments did not want the Biosafety Protocol negotiations to set a precedent and recognize the Precautionary Principle as a principle. The EU, represented by the European Commission, initially supported the inclusion of the Precautionary Principle in the preamble and the scope of the Protocol. The implementation of the principle by including it in the operational paragraphs on decision making was only supported by European negotiators in the final negotiation round. It was the African Group that, in the course of the negotiations, seized the historic moment and demanded the inclusion of the Precautionary Principle in the operational paragraphs of the Protocol. The African Group – which had represented like-minded developing countries since February 1999 – was able to keep the language in the text against the wishes of a strong group of industrialized countries until January 2000, at which point the EU was ready to support the African position on this issue.

[3]CBD preamble tiret 9: 'Noting also that where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat'http://www.biodiv.org/convention/articles.shtml?lg=0&a=cbd-00 (accessed July 2006)

4.1 The genesis of Cartagena Protocol provisions reflecting the Precautionary Principle

4.1.1 Biosafety Working Group 2 – May 1997

At the beginning of the biosafety negotiations, the Precautionary Principle had been introduced by the African Group, the EU and Canada in the preambular text (Table 30.1).

Table 30.1 The Precautionary Principle – start of the negotiations at BSWG-2

African Group	Canada	EU
Noting that, in accordance with the *precautionary principle*, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize risk where such a risk is posed by living modified organisms resulting from biotechnology …	Canada suggests that the Protocol may benefit from a 'Principles' section. One possible inclusion could be reference to the *precautionary principle* as defined in the Convention.	Noting that the provisions of the Protocol should contribute to protection in the field of biosafety, based on scientific risk assessment and the *precautionary principle* …

Source: UNEP (1997a): African Region p. 1; Canada p. 1; European Union p. 2 (emphasis added by the author)

4.1.2 Biosafety Working Group 3 – November 1997

When the negotiations ended in November 1997, the report contained the inputs of the African Group and the EU (UNEP 1997b). Canada's more specific suggestion for a 'principles' section was not taken up, but there was a third option calling for deletion of the reference to the Precautionary Principle.

4.1.3 Biosafety Working Group 4 – February 1998

The fourth round resulted in text that reflected the diversity of governmental positions in its numerous options for the individual articles and many square brackets, indicating non-consensus (UNEP 1998a). With regard to the preamble, the language of the African Group was generally accepted. During this session, for the first time a reference to the Precautionary Principle was introduced into the operational part of the draft protocol, serving as a basis for the later, final version of the treaty. Thus, the draft version of Article 6 presented the basic text on the application of the Precautionary Principle in government decision making under scientific uncertainty. Meanwhile, Annex II for the first time in the biosafety negotiations quoted the wording of the Rio Declaration – precautionary approach – instead of using the term 'principle'.

4.1.4 Biosafety Working Group 5 – August 1998

Apart from the African Group and the EU, several other countries also called for the inclusion of the Precautionary Principle in the text. (UNEP 1998b). Three developing countries and a country in transition (Peru, Thailand, Venezuela, Slovenia) demanded a reference to the principle in the draft article on decision making in the operational part of the Protocol. Norway and Thailand referred to the text of BSWG-4 and supported the wording 'precautionary approach' in Annex II. The participants of the fifth negotiation round expected this meeting to lead to a breakthrough, producing a final text with only controversy on some crucial matters. The Conference of the Parties of the CBD had called for a finalization of the biosafety negotiations in early 1999. These expectations could not be fulfilled, however, as the three negotiating blocks – Miami Group,[4] EU and the majority of the developing countries – could not work out compromises on the contentious issues (UNEP 1998c). In relation to the Precautionary Principle, BSWG-5 was actually a step backwards: all parts of the text that referred to the principle were bracketed, and no

[4]The Miami Group was formed by the USA, Canada, Argentina, Australia, Chile, and Uruguay: in 2000 the first three states harboured 99% of all commercial GE crop planting. Australia is a leading export country for agricultural products, Chile and Uruguay had been brought into the group to maintain a balanced North-South representation.

solution was in sight. In retrospect, the text of the draft article on decision making carried the core wording paving the way for the final Protocol text. The earlier phrase 'the State of import has the right to prohibit import of the LMO in question' was replaced by 'Decisions taken by the Party of import shall be based upon ...' The text did not explicitly state any longer that a Party has a right to ban GMO imports.

4.1.5 Biosafety Working Group 6 & Extraordinary Conference of the Parties – February 1999
The sixth and supposedly last negotiation round in February 1999 in Cartagena reached a compromise with regard to three of the four references to the Precautionary Principle. However, following extremely intense negotiations, BSWG-6 ended with a devastating result: In the early morning of 22 February 1999, two hours before the Extraordinary Conference of the Parties (ExCOP) was scheduled to adopt the Protocol text, 63 countries expressed their discontentment with the final 'Draft Text of the Chair' (UNEP 1999). During the next two days, nothing could break the deadlock. On 24 February at 6 a.m. the delegates were sent home for a 'break'. One of the crucial problems was the article on 'Decision procedure under the AIA', which was trying to define the conditions for the application of the Precautionary Principle.

BSWG-6 solved the struggle about the choice of words when it decided to replace 'Precautionary Principle' in the preamble and in Article 1 with 'precautionary approach'. This change may have substantial consequences when the Protocol is implemented nationally. The controversy about paragraph 4 in Annex II was solved when delegates agreed to give up using the words 'Precautionary Principle' and instead developed a definition of what they had in mind when arguing to subject the interpretation of the results of scientific risk assessments to the concept of the Precautionary Principle. This solution in the end led to a much better text because it did not simply name an approach, but defined it.

Only the article on decision making – at that time numbered as 8.7 – remained in brackets. The strategy applied to the risk assessment annex – abandonment of the emotive word but definition of the underlying approach – did not work in this case. The Miami Group could not agree to Article 8.7. Furthermore, the wording 'shall not prevent the Party of import from prohibiting the import' had been reintroduced into the text. On the final night of the negotiations, the European Commission presented a 'package' containing eight suggestions on the contentious matters, including the offer to delete article 8.7. It hoped to get the Miami Group on board with this renewed concession.

Observers were convinced that the Miami Group was only interested in diluting the Protocol text until it actually became meaningless. Some argued that if decision making under the Cartagena Protocol, and consequently decision making under national regulation implementing the Protocol, were not based on the Precautionary Principle, the power to define what is possible and what is not would be exclusively left to the WTO. In such a case, the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) with its article 5.7[5] would potentially offer more room in the struggle to apply and defend the Precautionary Principle than the Biosafety Protocol.
However, the WTO SPS Agreement is not an environmental agreement; its objective is not to protect the environment or biodiversity but to reduce trade barriers and to eliminate

[5]SPS 5.7: 'In cases where relevant scientific evidence is insufficient, a Member may provisionally adopt sanitary or phytosanitary measures on the basis of available pertinent information, including that from the relevant international organizations as well as from sanitary or phytosanitary measures applied by other Members. In such circumstances, Members shall seek to obtain the additional information necessary for a more objective assessment of risk and review the sanitary or phytosanitary measure accordingly within a reasonable period of time.'http://www.wto.org/English/docs_e/legal_e/15sps_01_e.htm (accessed July 2006)

discriminatory treatment in international trade (GATT 1947). In the context of a free trade agreement, countries have the right – if they do not violate the objective – to take measures 'necessary to protect human, animal or plant life or health'. Contrary to the Cartagena Protocol, the SPS Agreement does not recognize the ecosystem or any other holistic approach, and it refers to the health of living organisms as isolated individuals. In addition, the SPS definitions of possible risks would not fully cover the general concerns with regard to ecological risks of GMOs. The SPS Agreement speaks of risks 'arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms' or 'arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs'. From the perspective of ecological sciences it appears difficult to discuss possible disturbances of ecosystems or the further extinction of the soil-borne seed banks in agricultural soils through the application of highly efficient herbicides such as glyphosate, under the SPS Agreement.
Those delegates and observers who worked towards a 'strong' Biosafety Protocol meanwhile judged the rejection of the EU offer by the US as a stroke of luck. A coalition between the Miami Group and the EU would have driven the developing countries – which meanwhile formed the 'like-minded group' – into a complicated situation. They either would have had to agree to a Protocol text that no longer contained their main demands, or they would have had had to explain why they refused to accept this compromise.

4.1.6 Extraordinary Conference of the Parties – January 2000

In January 2000, the ExCOP session was reconvened. Just two months before, in November 1999 in Seattle (USA), at the ministerial meeting of the WTO, the direction of the international biosafety process, and thus the operationalization of the Precautionary Principle, was on a knife edge. During that meeting, a working group was meant to have been launched to incorporate the issues of biotechnology into the WTO work.[6] This venture, prominently supported by the European Commissioner for Trade, failed due to the determined counteractivities of the Ministers for environment from Denmark, France, Belgium, and Italy (Williams & de Jonquières 1999; Dawkins 2000) who, in an informal declaration, rejected the plans of their colleagues representing trade interests.[7] To strengthen the biosafety process, ten EU Ministers for environment and the European Commissioner for Environment took part at the final Montreal session in January 2000. On the other side of the table, the US and Canada were merely represented by higher administrative officials. This unequal balance of power led observers to speculate that the

[6]EC, Hungary, Japan, Korea, Switzerland, Turkey. 1999. Common Working Paper of the EC, Hungary, Japan, Korea, Switzerland and Turkey to the Seattle Ministerial Declaration of November 29, 1999: 16:'Immediate Decision at Seattle – Biotechnology-related issues: We agree to establish a working party with a fact-finding mandate on the relationship between trade, development, health, consumer and environmental issues in the area of modern biotechnology. The work of the group shall proceed in two phases. First, the group shall complete its identification and examination phase by the fourth session of the Ministerial conference, drawing on relevant work under way in the WTO and in other multilateral fora, including the codex, IPPC, the OECD as well as the bio-diversity convention. Second, using the results of this work, the group shall then present recommendations to the TNC with a view to clarifying these issues.'http://www.lex.unict.it/cde/documenti/rel_ester/98_99/jap01_12_99.htm (accessed July 2006)

[7]'Seattle WTO MinisterialProposal to establish a Working Group on BiotechnologyMeeting informally in Seattle, Environment Ministers from Denmark, United Kingdom, France, Belgium and Italy expressed opposition to the establishment of a WTO Working Group on Biotechnology within the structure of the new Round (as proposed by the US and Canada) for the following reasons:–The proper forum for deciding a multilateral approach to biotechnology issues is the ongoing process to agree a Biosafety Protocol to the Convention on Biological Diversity. This process would be undermined by the establishment of a WTO Working Group.–One of the EU's main priorities for the negotiation on the trade and environment relationship is to clarify the interface between Multilateral Environment Agreements and WTO rules. A WTO Biotechnology Working Group would run directly counter to this key objective by potentially subordinating the Biosafety Protocol negotiations to discussions in the Round, thereby setting a precedent for the WTO's relationship with other MEAs.–Biotechnology issues will arise naturally in some areas of the negotiations; there is, therefore, no need for a specific Working Group.'

struggle on the Precautionary Principle was going to be decided by the EU – now also representing the position of the developing countries.

4.1.6.1 Influence of civil society on the final negotiations

Although the biosafety negotiations had taken place in Montreal since 1997, only this last meeting in 2000 led to a significant engagement of Canadian civil society and the Canadian media. The failure of the WTO conference in Seattle and the crisis of the biosafety process in Cartagena – which in the eyes of many Canadian observers was partly caused by the activities of their own government – triggered broad public interest in the biosafety negotiations. Canadian NGOs organized a demonstration in bitterly cold weather, and meetings were held in the Universities. The Canadian Environment Minister was forced to appear at the negotiations after 'wanted' posters were distributed widely, urging for the defence of national environmental standards at the biosafety negotiations. Ironically, it had also been Canada who in 1997 suggested the inclusion of the Precautionary Principle in the Biosafety Protocol. Canadian NGOs erected a tent on the pavement outside the negotiation venue, which served as meeting place for activists and delegates, as an information centre and as public place for cheerful or critical words for passing delegates depending on their role in the current negotiations. This tent was the location in which on 30 January at 6 a.m. the young agreement was welcomed.

5. Decisions of the WTO on risk assessments, the Precautionary Principle and decision-making under scientific uncertainty

It is stated frequently that the application of the Precautionary Principle in GMO decisions will be incompatible with the provisions of the WTO, which only allow 'science-based' decisions. As already explained, the SPS Agreement of the WTO does allow temporary precautionary action in situations of scientific uncertainty. Import restrictions concerning GM crops using the SPS logic have already been implemented – but in a legal setting in which the SPS Agreement does not apply. For example, the Australian state Tasmania had adopted a moratorium on the planting of herbicide-tolerant GM rapeseed in 1999; in 2003 this moratorium was prolonged until 2008. Tasmania regards this rapeseed amongst others, as a potential weed (Government of Tasmania 2003). Tasmania and many other Australian States also claim that socio-economic risks accompany the introduction of GM crops, especially of GM rapeseed through the contamination of seeds and harvests.

Salient sources that help analyse trade-relevant decisions of WTO members regarding GM crops are the decisions of the WTO Appellate Body (AB) on the cases 'European Communities – Measures Concerning Meat and Meat Products (Hormones)' (WTO 1998a) and 'Australia – Measures Affecting Importation of Salmon' (WTO 1998b).

5.1 Risk assessments in the context of protecting human and animal health

In the 'hormone case', the AB defined the injected hormones as 'contaminants', in the sense of the SPS Agreement. It has yet to be seen how, in the light of the SPS Agreement, transgenes and their new proteins and properties would be defined. In this decision the AB has laid down essential criteria regarding the extent of certainty in scientific and economic risk assessments to make them suitable as a basis for a SPS decision. It explained that with respect to the SPS provision in Annex A 4 to evaluate 'the potential for adverse effects', a quantification of the risk or a development of thresholds is not obligatory. The risk assessment as a basis for an import restriction in order to protect animal or human health does not have to present a calculation of the risk, but has to show a potential for adverse effects on a scientific basis. Furthermore, the AB points out that 'theoretical uncertainty is not the kind of risk which, under Article 5.1, is to be

assessed'. With respect to existing or future import restrictions for GMOs, the WTO seems to have set certain minimal standards for a health-related risk assessment: it has to present an analysis that describes the potentials of risks in a scientifically plausible manner.

5.2 Risk assessments in the context of protection of the environment against introduced pests

It is clear that the SPS Agreement with regard to the protection of the environment against introduced pests sets significantly higher standards: Annex A 4 demands 'the evaluation of the likelihood of entry, establishment or spread of a pest or disease [...] and of the associated potential biological and economic consequences'. A decision under the Cartagena Protocol to restrict the import of a GMO to protect biodiversity is likely to fall under this SPS category. In paragraphs 120–124 of the 'salmon case' it is explained that a risk assessment cannot simply show potentials of adverse effects to justify an import restriction according to the SPS Agreement, but that it has to present at least a qualitative judgement of the probability of the risks within the context of possible plant protection measures. The AB confirms in paragraph 130 of its decision that in every case of import restriction a scientific risk assessment according to the provisions of the SPS Agreement has to be presented. However, the AB explicitly differentiates between the assessment of risks and the determination of the level of protection by the government. The AB states in paragraph 125 of its decision that nothing in the SPS Agreement prevents a member from taking a 'zero risk' decision.

5.3 Application of the Precautionary Principle

The aforementioned decisions of the AB have, however, nothing to do with the invocation of the Precautionary Principle in its strict sense – taking a decision under scientific uncertainty that is more favourable for the protection of health and environment than for the advancement of free trade. The governments that have restricted the trade in certain commodities have never based their decisions on SPS Article 5.7, which allows them to use the Precautionary Principle. They have always presented risk assessments that in their point of view were elaborated enough to scientifically justify an import ban. The decision in the 'hormone case' discusses but does not clarify the meaning of Article 5.7. The AB underlines that the decision of the European Commission to forbid the import of hormone-treated beef was not based on a risk assessment in conformity with the SPS Agreement, thus violating Articles 5.1 and 5.2. The report states 'that the European Communities has explicitly stated in this case that it is not invoking Article 5.7'. The Commission had never claimed to act in a situation of scientific uncertainty; from that point of view Article 5.7 cannot be applied. In paragraphs 124 and 125 of the 'salmon case' the AB presents an explanation of the relationship between the Precautionary Principle and the SPS Agreement, reiterating that precautionary decisions can be in accordance with the SPS Agreement. However, the text also states that the SPS Agreement does not name the Precautionary Principle and that this Principle, in contrast to the judgement of the European Commission, is not a 'general customary rule of international law or at least a general principle of law'. Consequently, there is no justification to make SPS-relevant decisions against the provisions of the SPS Agreement, especially in not abiding to the minimal standards of risk assessments.

It is unclear until today, what 'threshold' the SPS Agreement establishes to determine the critical amount of scientific uncertainty that would justify a decision based on Article 5.7.

6. Perspectives

The development of the provisions in the Cartagena Protocol on Biosafety is characterized by negotiation tactics and compromises. The Protocol defines the circumstances in which governmental decisions regarding GMOs can be based on the Precautionary Principle – without naming it. In other paragraphs it mentions the precautionary approach – which has its own distinct definition. On the one hand, the Protocol may cause disagreement amongst legislators and other societal groups which strive to implement it nationally. On the other hand, the Protocol is the first international, legally-binding instrument that provides a far-reaching definition of the application of the Precautionary Principle. Since the adoption of the Protocol, some government decisions concerning GMO import restrictions have used its provisions to justify their activities. It is highly likely that the interpretation of the CPB Articles 10.6 and 11.8 and of the SPS Article 5.7 will play a major role in the legal, scientific and public discussion about the relationship of multilateral environmental agreements and the WTO agreements. From the perspective of those experts and groups who are supportive of the Precautionary Principle, it will be important to defeat the argument that the WTO would forbid the application of the Precautionary Principle. The complex discussion within the WTO and the hitherto unresolved questions around SPS Article 5.7 have to be carried into the discussions about environmental policies and strategies.

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Chapter 31

Liability and redress for damage arising from genetically modified organisms: Law and policy options for developing countries

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1. Introduction

1.1. Background

The Cartagena Protocol on Biosafety was finally concluded in the year 2000 after a failed first attempt. The negotiations were intense and rancorous. The advanced developed countries led by the United States – together with a very small number of developing countries – stood in stark opposition to the rest of the developing world. Amidst these negotiations it was not possible to complete the negotiations on two highly contentious and divisive issues: one was the documentation to accompany exports of genetically modified (GM) commodities intended for direct use for food, feed and processing;[1] the other was liability and redress if genetically modified organisms (GMOs) or living modified organisms (LMOs) moved across boundaries were to cause damage to the environment or to human health in the receiving country.

The Protocol, by its Article 27, provides for a process to be initiated for the elaboration of international rules and procedures for liability and redress. The first Meeting of the Parties established this process in February 2004 in Kuala Lumpur by Decision BS-1/8. An Open-ended Ad Hoc Working Group of Legal and Technical Experts was set up with clear Terms of Reference. The Working Group is to complete its work in 2007. Work has started in earnest and many – often conflicting proposals – have been collected under useful headings as a basis for negotiating the rules and procedures of a potential international liability and redress regime.

This chapter looks at the considerations developing countries may wish to take into account in deciding upon the nature of the regime, its key features and elements.

1.2. Overall approach

Law quintessentially captures policy that decision makers consider important. Behind each theory for adopting a position on an issue there is always a policy choice. The policy is based upon, and derived from, values and interests that a particular society wishes to advance: to protect certain activities, to afford justice to victims, or a trade-off of interests. These are then articulated into a specific law. It follows, then, that the first task of a decision maker is to identify the policy that the law should encapsulate.

What then is the policy in relation to a liability and redress regime for GMOs in the context of the Cartagena Protocol on Biosafety? First, the overall goal of the Protocol and the interest it advances must be reflected in the regime. Secondly, the purpose of the regime in relation to the specific activity must be clearly identified. What happens when there is damage which can be linked causally to a GMO or activity in relation to it? How is liability to be established? Who is to be held liable? What kind of damage is recoverable and how is that damage to be assessed? What defences are acceptable?

These questions must be addressed in the context of the nature of the harm and damage that accompanies the advancement of science and technology – in this case modern biotechnology and

[1]The issue on documentation was finally resolved by a decision taken at the recently concluded 3rd Meeting of the Parties of the Protocol in February 2006 in Brazil.

its products. All this has to be placed within the overall goal of a justice system: *ubi jus ibi remedium – where there is a grievance there is a remedy.* What is the consequence of leaving a victim without a remedy? Can the victim prove his or her claim – given the complexities involved and the existing legal modes of proof, both substantive and procedural?

2. Overall goal of the Cartagena Protocol on Biosafety

2.1. Balancing competing interests

A crucial consideration in setting up a liability regime, and that underlies much of the ongoing negotiations, is the need to maintain a balance between, on the one hand, the protection of the public and the environment, and, on the other hand, the public and industry interest not to stifle innovation or drive away investors in biotechnology, or trade in products of biotechnology.

These interests are reflected in the Convention on Biological Diversity (CBD), the parent convention of the Cartagena Protocol on Biosafety. Article 16(1) emphasises making available biotechnology that will enhance the conservation and sustainable use of biodiversity – an acknowledgment of the potential of biotechnology to promote human well-being, particularly in meeting critical needs for food, agriculture and health care.[2] The biosafety aspects are made clear by two articles in the CBD:

- Article 8(g) – which requires Parties to take national measures to ensure safety in respect of harm by LMOs to the environment that could adversely affect the conservation and sustainable use of biodiversity as well as human health
- Article 19(3) – which requires Parties to put in place an internationally binding instrument for biosafety.

It was this latter provision that set the stage for the final promulgation of the Cartagena Protocol. The Protocol, as finalized and adopted, is seen as a significant step forward in providing '*an international regulatory framework to reconcile the respective needs of trade and environmental protection*' with respect to the biotechnology industry.[3] This means that research, development and trade in biotechnological products must be undertaken in conditions that do not compromise the safety of the environment and human health. The preamble to the Protocol makes this clear: '*Recognizing that modern biotechnology has great potential for human well-being* if *developed and used with adequate safety measures for the environment and human health.*'

2.2. Precautionary approach

The central paradigm of the Protocol is the precautionary approach in addressing safety issues. The precautionary approach allows States to take regulatory measures even if there is no certainty of the harm occurring. This is made necessary by the nature of the technology, which is relatively new. There is still considerable uncertainty surrounding these issues as well as the timeline for harm to manifest. Leading insurance companies attest to this fact and are reluctant – if not refusing – to provide cover. Part of the reason is that the potential for harm to be caused by GMOs *may* be great. A single remote incident may cause harm of an immense magnitude.

2.3. Liability and redress only applies when damage from GMOs results

In working out a liability and redress regime, it is as important to keep the objective clear. The issue of liability will only arise when there has been damage caused by GMOs. It must be established – in fact and in law – that the harm is directly attributed to the GMO (in particular its

[2]Secretariat of the CBD, Cartagena Protocol on Biosafety to the Convention on Biological Diversity: Text and Annexes, Montreal, 2000, Introduction, p. 1. Also CPB, preamble paragraph 6.
[3]See footnote ii.

properties, their reproduction or modification) or the activity in relation to it. Further, it must be established that there is a person who can be identified as being responsible. Only then will the issue of compensating for the harm done arise.

2.4. Scenarios of harm

Harm from GMOs could result from any one of the situations described in the following. The harm could be to the environment, biodiversity, the ecosystem, and to species of flora and fauna, or it could be to human health. The harm could also be physical and/or socio-economic. The following is a non-exhaustive list of the potential harm that may be caused by a GMO:

1. GMOs from one field could cross over to the fields of other farmers and contaminate their non-GM crops. As a result, the farmers either would not be able to sell their crops or suffer a loss in income because of a reduced demand. This would be essentially economic loss.

2. GM bacteria in the soil may cause soil infertility. This could cause widespread damage to farmers and result in loss of livelihood.

3. GMOs can potentially affect the environment adversely through effects on non-target organisms, ecosystems and biodiversity. There may be displacement of conventional crops by a small number of GM crops or contamination of native or wild relatives. This could threaten biodiversity. It could also be particularly damaging to crops in centres of origin.[4]

4. Biodiversity could be destroyed if there is an impact on the ecosystem through the introduction of GMOs. For example, if vast tracts of natural forest are interspersed with non-flowering insect-resistant GM trees, animal life could be adversely affected. The richness and abundance of insects may also be destroyed.[5]

5. Human health may be impaired through the consumption of a GMO food product which causes allergic or toxic reactions.[6]

6. There may be long-term negative impacts on human health from small amounts of DNA in GM foods surviving in the gastrointestinal tract.

7. GM virus-resistant crops may create new, more virulent or widely spread viruses.[7]

8. GMOs using antibiotic resistant marker genes could cause antibiotic resistance in human gut bacteria or soil bacteria.

[4]Simonetta Zarrilli, 'International Trade in GMOs and Multilateral Negotiations: A new dilemma for developing countries', in Francesco Francioni (ed), Environment, Human Rights and International Trade, 2001, p. 43.
[5]Based on Monsanto's plans in New Zealand to create a plantation of these trees to harvest wood: Nathan Batalion, 50 harmful Effects of GMO Foods, www.satori-5.co.uk/word_articles/misc/50_harmful effects_gm_foods.html
[6]WHO has identified potential impacts of transgenic crops to human health: WHO Report, Modern Food Biotechnology, Human Health and Development: an evidence-based study, 23 June 2005, at www.who.int/foodsafety/publications/biotech/biotech_en.pdf
[7]Union of Concerned Scientists, Risks of Genetic Engineering, www.ucsusa.org.food_and-environment/biotechnology/page.cfm?pageID=346-

3. Considering the key elements

3.1. Establishing liability: What standard to adopt?

In this part I consider how liability is established when harm results. This section presents the factors taken into account – in law and fact.

How liability is established depends on the standard of liability imposed. There are two possible standards: fault-based liability and strict liability.

3.1.1. Fault-based liability

This means that liability will only exist if fault is established. In common law jurisdictions, one has to prove three elements to establish fault:

I. duty of care – that is, one has to identify a wrongdoer who owes the victim a duty of care
II. breach of that duty
III. damage resulting from the breach.

a. Proving the elements

i. The duty of care

This requires the identification of a wrongdoer. Sometimes it may be quite easy to identify the person responsible for the damage. This is not always the case, especially if the harm could be attributable to a large number of players. The identification of a person to be liable under the common law turns on a number of factors, such as

- the foreseeability of the harm
- the proximity of the relationship between the parties
- considerations of fairness and reasonableness[8]
- policy considerations to deny or limit liability.[9]

How easily is the duty of care established when we apply the fault-based standard of liability? It may often be difficult to identify a particular entity or person as responsible when applying these factors in a case of harm from a GMO. This is especially so in cases where GMOs spread beyond the intended receiving environment. For example, if a farmer buys and grows GMO seed, and this contaminates the fields of a neighbour, is he the wrongdoer?[10] In the Canadian Supreme Court decision in *Monsanto Canada v Percy Schmeiser*, a farmer who contended that his field was contaminated by GMOs was held liable for patent infringement. Would he be liable if fields in the vicinity were then contaminated by the GMOs from his field? If a liability regime were to hold him responsible, would this discourage farmers from buying and growing GM seeds and plants? In any case, how is proximity determined? What if there is an indeterminate class of people who are harmed by the activity? In the Australian High Court decision of *Perre v Apand Pty Ltd.*,[11] owners and growers of potatoes were held to be owed a duty of care by a farmer who had introduced bacterial wilt to other farms. This case did not involve GM crops. However, it suggests that a duty of care may be imposed with regard to an indeterminate class of people who can show harm. In this case, there was a law that banned the import of potatoes grown within 20 km of land affected by bacterial wilt, and hence the plaintiffs could not sell their potatoes. In the

[8]Caparo Industreis Plc v Dickman [1990] 2 AC 605, 628 (HL).
[9]Anns v London Borough of Merton [1978] AC 728, 751 (HL); South Pacific Manufacturing Co Ltd v NZ Security Consultants Ltd [1992] 2 NZLR 282, 294 (CA).
[10][2001] FCT 256, High Court; [2003] 2 FC 165, Supreme Court
[11]There were actually seven judgments: [1999] HCA 36; (1999) 165 ALR 606 at http://www.austlii.edu.au/au/cases/cth/high_ct/1999/36.html

absence of such a law, it is uncertain whether the court would have reached the same decision. Will courts elsewhere arrive at this decision?

Because of the application of multifarious factors in establishing who owes a duty of care, and to whom, the outcome is unpredictable. This means that it may not always be easy to establish this first element in a fault-based system of liability.

ii. Breach of the duty of care

The plaintiff must then prove that the defendant failed to exercise reasonable care. The standard of care is judged objectively – that of a reasonable person. The breach could be in relation to the creation of the GMO construct (in product liability parlance – the design defect), the testing of the GMO, the commercial manufacturing of the GMO (the manufacturing defect) or the marketing of the GMO (the marketing defect). The conduct of the defendant will be scrutinized. It is based on the factual circumstances of each case.

There are also other established bases on which courts in common law jurisdictions have acted in deciding a breach of the duty of care. These include: the probability of the risk, gravity of the danger, social utility of the activity, and the burden or difficulty in taking preventive measures. There are thus various bases on which a duty of care will be held not to have been breached. So, even if there is damage, a balancing of all these factors may mean that there is no liability. Compliance with statutory requirements may also be an important factor to suggest that the standard of care has not been violated. Additionally, the standard required of a producer of the GMO may be based on the state of the scientific and technical knowledge then existing. Standards under the law of negligence are also expected to require reasonable, and not absolute, safety. Some level of damage is thus accepted. In design and manufacturing of products, the manufacturer/producer is not an insurer in the sense that he is liable for all damage caused by the product. Thus, liability for negligence may be avoided for the making of low-quality products. If this can be extrapolated to the actual reproduction techniques involved in the production of GMOs, then again liability can be avoided.

iii. Damage

The damage that is caused must have been reasonably foreseeable. Otherwise it is not recoverable and said to be too remote. Again, even if damage is caused by the GMO, the plaintiff is not compensated for his loss or injury if the defendant can prove that he did not foresee the damage. Neither the precise extent nor the precise manner of the infliction of the damage needs to be foreseeable.

b. Burden of proof in fault-based liability

In a fault-based liability system, the burden is on the person harmed to provide the evidence of the facts that will prove each element. Only then will negligence be established. The burden of proving that the damage from a GMO is the result of the breach of the defendant's duty may be onerous, given the complex and technical nature of the subject. The task is made more difficult and expensive if a relatively small plaintiff is pitted against a large GMO producer.

To establish his case, the victim must know quite completely the whole process in the production of the GMO, the circumstances of its creation, its testing, and distribution – matters which may be exclusively within the knowledge of the producer. Some parts of the process may even be the subject of trade secrets. There are procedural rules in most common law jurisdictions to assist access to some of this information. All the same, the task is formidable, may involve complex procedural manoeuvres and could involve huge costs.

c. Difficulties posed by long time lag

The difficulty of establishing liability is increased if there is a long time lag between the introduction of the GMO and the damage; this is an expected scenario. The number of people involved in handling, or using the product may also exacerbate the difficulty. It may make identification of the wrongdoer difficult too. If there is a time limitation for bringing the action, by the time the correct person is found, it may simply be too late. Alternatively, the correct party may have disappeared from the scene. Sometimes, if there could plausibly be more than one cause of the injury, the plaintiff may have to eliminate the role of the other causes.[12]
Critics of the fault-based system – especially for new and complex technologies – often claim that:

- there is arbitrariness and uncertainty of the end result – victims of the damage may go uncompensated
- the system is not cost effective[13]
- there is delay in obtaining compensation.

3.1.2. Strict liability
The alternative to fault-based liability is strict liability. Under such a regime, there is no need to show fault in order for liability to be imposed. To succeed, the victim needs only to prove the damage (the defect in the GMO, if any), and the causal link between the damage and the GMO. The conduct of the wrongdoer is irrelevant – unlike in the fault-based system. The crucial feature is the GMO and the activity in relation to it.

a. Strict liability not exclusively for dangerous goods
A strict liability standard is more commonly applied to dangerous or hazardous goods or activities, though not exclusively. For example, most jurisdictions – initiated originally by the United States – impose strict liability for any damage arising from defective consumer products. For instance, while the product itself – for example, a baby teat, or the activity in relation to it – a baby sucking on it – is not dangerous, strict liability can be imposed for a number of reasons.

b. Policy choice and practical considerations: Better consumer protection, profiteer to bear loss, raising safety standards, easier for victims to obtain remedy
Such a liability standard is said to overcome the problems inherent in contractual and negligence remedies – that have been highlighted earlier – and therefore gives better protection to consumers.[14] It is a matter of policy choice and other practical considerations. The United Kingdom's Pearson Commission justified the imposition of strict liability on the basis that the producer who profits from the product should accept its losses; that he was best placed to arrange for insurance and redistribute the loss; that strict liability would raise safety standards; and that all consumers should have the same protection as a consumer-purchaser.[15] In the United Kingdom, the introduction of strict liability was justified by cogent policy reasons and supported by considerable practical considerations – the key one of which was to make it easier for victims of harm caused by defective products to prove their cases as they no longer had to prove fault by the manufacturer.

[12]Evans v Triplex Safety Glass Co [1936] 1 All ER 283.
[13]For the UK: The Pearson Commission Report stated that in its estimation the cost of administering the tort system was roughly double the benefits of the compensation: para 83. The Civil Justice Review made a similar finding.
[14]UK: The Law Commission and the Scottish Law Commission, Liability for Defective Products, HMSO Cmnd 6831. The Royal Commission Report on Civil Liability and Compensation for Personal Injury (1978) Vol. 1, HMSO Cmnd 7054. The former Commissions dealt with defective products and compensation for personal injury, damage to property or any other loss. The Royal Commission was not confined to defective products but limited to compensation for personal injury. It was chaired by Lord Pearson and its report is usually referred to as the Pearson Report.
[15]The Pearson Report, paras 1227–1236; and the Law Commissions Report, paras 38–42.

Even if liability is to be restricted to dangerous products or activities, these are judged on the basis of the incidence or the probability of occurrence and the magnitude of the harm. Hence, if the incidence is remote but the magnitude of the harm great – the Chernobyl disaster is a typical example – this would still constitute a dangerous activity for which, generally, the strict liability standard is imposed. The Swiss Reinsurance firm Swiss Re states that the issue is not whether genetic engineering is in fact dangerous, but how dangerous it is actually considered to be.[16] It noted that, as a new technology, 'there are no means for comparison, hopes and fears are boundless and potential uses and supposed damages are initially unquantifiable'. It also noted that the lack of knowledge of the probability of the risk rather than the size of such risk made for difficulty in obtaining insurance for GMOs.[17]

c. Precautionary Principle and strict liability

It has also been suggested that, on the basis of the Precautionary Principle – the overarching and operating principle in the Cartagena Protocol on Biosafety – strict liability should be the standard because of the current uncertainties as to the magnitude of the potential damages and the extent to which they may occur over a long timeline.[18]

The precautionary approach or principle allows regulatory measures to be taken even where there is scientific uncertainty of the potential risks associated with particular uses of biotechnology. Indeed, the very necessity of adopting the Protocol stemmed precisely from the need for Parties to take precautionary measures.[19] The inclusion of the precautionary approach in the preamble and the objective (Article 1) of the Protocol suggests that the Protocol is itself an embodiment of the principle, aimed at '*ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs*'. The precondition to triggering the implementation of precautionary measures is 'potential adverse effects' as stated in Articles 10(6) and 11(8) of the Protocol. There are no objective and qualitative thresholds, which means that each Party can determine what threshold level it deems appropriate.

How are 'potential adverse effects' established so that a decision based on the Precautionary Principle can be taken? This requires a decision to be taken as follows.[20]

First, identify the potentially negative effects of the LMO. This requires scientific research.
Second, carry out a risk assessment. This is based on existing knowledge and available information providing the views of scientists on: reliability of the assessment, remaining uncertainties, and topics for further discussion. Where it is not possible to complete a comprehensive assessment of risk, there should be an evaluation of available scientific information.

Scientific uncertainty results usually from five characteristics of the scientific method:
the variable chosen

[16]http://www.swissre.com/INTERNET/pwswpspr.nsf
[17]Thomas Epprecht, 'Biotechnology Risk Perception in Liability Insurance', accessible at http://www.cid.harvard.edu/cidbiotech/comments/comments86.htm. See also Duncan Currie,' Liability for Damage for Genetic Modification: the scope and limit of common law remedies in the GM context', 2004, at pp. 31-32.
[18]See for example, Philippe Cullet, 'Liability and Redress in Biotechnology: towards a development of rules at the national and international levels', COP/MOP 1 Biosafety Protocol, Background Paper, Feb 2004, International Environmental Law Research Centre, Geneva, http://www.ielrc.org/content/w0401.pdf
[19]Laurence Graff, 'The Precautionary Principle', in The Cartagena Protocol on Biosafety: Reconciling Trade in Biotechnology with Environment and Development? Bail C, Falkner R and Marquand H (eds), Earthscan, 2002, p. 410 at p. 412.
[20]Based on Markus Gehring and Marie-Claire Segger, 'Precaution in Trade Law: The Precautionary Principle and its Implications for the WTO', Research Paper.

- the measurements made
- the samples drawn
- the models used
- the causal relationship employed.

It may also arise from controversy concerning existing data or lack of some data, and may relate to quantitative or qualitative elements of the analysis.

Scientific evaluators take these into account in their research methods. Further, risk managers are aware of these uncertainty factors when they adopt measures to manage risk. However, in some situations, for example where there is insufficient, inconclusive or imprecise data, it is not possible to apply these cautionary aspects in practice. It becomes impossible to determine the risk in question with sufficient certainty. It is clear, then, that the Precautionary Principle is not directly triggered by the existence of potential harm, but specifically addresses situations where this harm is scientifically uncertain. Not just any degree of uncertainty prompts the application of the principle. It is only triggered when the degree of harm is approximately proportionate based on a rough balancing of different considerations.

In these situations, then, the decision maker is entitled to take a decision he deems necessary to deal with the LMO. The absence of scientific consensus is no basis for inaction. Even a minority scientific view – if credible and reputable – can be the basis of the action, as made clear by Item 4 of Annex III to the Protocol.[21]

Fault-based liability – as discussed earlier – requires proof, not only of damage resulting from the product but also that the damage was caused by the manufacturer failing in his duty to take reasonable care. In contrast, the focus for strict liability is on the actual performance and condition of the product, not on the manufacturer's care. The application of the Precautionary Principle also dispenses with the need for establishing the duty of care of the manufacturer. Hence, the Precautionary Principle and strict liability go hand in hand.

d. Shifting the burden of proof – mechanism for implementing the precautionary approach
Reversing the burden of proof facilitates the proof of liability, as discussed earlier. When the burden of proof to establish safety is reversed, the manufacturer must show that the product is safe. This is, in fact, a mechanism for the implementation of the precautionary approach. The mandatory requirement of prior approval before certain products can be put on the market – drugs, pesticides, food additives – is a clear reflection of countries applying the precautionary approach. The risk assessment provisions in the Protocol require the manufacturer or developer of the LMO to show that his product is safe for releasing into the environment. The producer bears the burden of showing that the product is safe. Both these facets taken together suggest that the strict – not the fault-based – liability standard is appropriate where the Precautionary Principle is the basis for decision making.

In the *EC-Asbestos Dispute*, the WTO's Appellate Body applied the two facets of the Precautionary Principle without referring to it directly. First, it was applied by confirming the right of Members to determine the level of health they deemed appropriate. By this, the burden of proof shifts to the proponent of the potentially harmful activity. The Appellate Body also held that a risk may be evaluated either in quantitative or qualitative terms, and that countries could take into account minority scientific opinion that is qualified and respected. In its words:

[21]Supported by the WTO Appellate Body in The EC- Asbestos Dispute: For a contrary view, see Laurence Graff, cited earlier, at p. 418.

(A) Member is not obliged, in setting health policy, automatically to follow what, at a given time, may constitute a majority scientific opinion.[22]

4. Countries adopting strict liability in relation to GMOs

4.1. National regimes

4.1.1. Norway: Gene Technology Act, Act 38 of 2 April 1993
The person responsible for an activity pursuant to the Act has liability for damages regardless of any fault on his part when the activity causes damage, inconvenience or loss by deliberate release or emission of GMOs into the environment: Section 23.

4.1.2. Switzerland: Law on Genetic Engineering, 2003
Anyone who is responsible for obtaining authorization and labelling and who deals with GMOs under contained conditions or releases such organisms for experimental purposes or illegally places them on the market, is liable for any damage due to the modification of the genotype arising out of these dealings: Article 30(1).
Also, if damage is caused by any other legally marketed GMO due to the modification of the genotype, the person responsible for obtaining authorization shall be liable if the organism is defective: Article 30(4).

4.1.3. Austria: Gene Technology Act (510 of 1994), amended in 1998 and 2002
The Act imposes strict liability on any party releasing GMOs for harm to health, property or the environment.

4.1.4. Germany: the Gene Technology Act 1990[23]
Liability is strict.[24]

4.1.5. New Zealand: Hazardous Substances and New Organisms Act 1996[25]
In 2003 New Zealand introduced legislation to establish a strict liability regime for victims of harm caused by activities in breach of the regulatory regime for new organisms, including GMOs.[26]

4.2. Supra-national liability regimes

4.2.1. Council of Europe Convention on Civil Liability for Damage Resulting from Activities Dangerous to the Environment – the Lugano Convention
This Convention is commonly referred to as the Lugano Convention. It is a pan-European convention and is not yet in force. The Convention provides for strict liability of operators of activities dangerous to the environment.[27] It covers the production, culturing, handling, storage, use, destruction, disposal, release, or any other operation dealing with one or more GMOs which as a result of the properties of the organism, the genetic modification or the conditions under

[22]At p. 64.
[23]It came into effect on 20 June 1990. It has been altered by eight Acts, the last one on 22 June 2004.
[24]Anja Gerdung, 'Germany's Liability Law for GMO Cultivation', June 2006, Sustainability Council of NZ, at pp. 3-4.
[25]Amendments made to the Act: sections – 124A – 124I, www.legislation.govt.nz.
[26]Submission of NZ to the Working Group of Legal and Technical Experts on Liability and Redress in the context f the Biosafety Protocol on Biosafety, 2nd Meeting, Montreal, 20-24 Feb 2006, UNEP/CBD/BS/WG-L&R/2/INF/2*, 12 Jan 2006, at p. 4.
[27]Article 6.

which the operation is exercised, present a significant risk for man, the environment or property.[28] Liability lies for damage caused by the activity as a result of incidents.[29] The term refers to sudden, continuous or a series of occurrences having the same origin. It must cause damage or create a grave and imminent threat of causing damage.[30] An emission as well as the dispersal of GMOs constitutes an incident.

4.2.2. Biosafety legislation of the European Union (EU)

The EU has a comprehensive regulatory system governing the release and marketing of GMOs. Directive 2001/18[31] on the Deliberate Release into the Environment of GMOs sets out the law relating to the placing on the market of GMOs as or in products as well as other releases.

The Directive aims to address the issue of coexistence of, on the one hand, conventional and organic farming, and on the other, GM farming. It does this by setting out the appropriate risk assessment and authorization procedures for the marketing of GMOs for cultivation. Nonetheless, it recognizes the possible occurrence of gene flow. Accordingly, in 2003, the EC amended the Directive to enable member states to 'take appropriate measures to prevent the adventitious presence of GMOs in other products'.[32] The EC was asked to develop Guidelines to allow for the coexistence of GM and non-GM farming. The Guidelines focus on such matters as separation distances and coordination between neighbouring farmers. The Guidelines make a passing reference to liability, advising member states to ascertain whether their existing national civil liability laws offer sufficient and equal opportunities to ensure coexistence. They also say that farmers, seed suppliers and other operators should be fully informed of the liability criteria that apply in their country in the case of damage caused by admixture.[33] This does not preclude, but paves the way for, the adoption of strict liability regimes.

4.2.3. EU Liability Directives: Directive 85/374 as amended by Directive 1993/34

This Directive deals with product liability, which includes agricultural products. Product liability provides for strict liability of producers for defective products, that is, products that do not provide the safety that can be expected of them. This excludes recovery of contamination damage by coexistence farming, as such damage will usually be caused by cultivating GMOs that are fit for their purpose. Such damage may be avoided by cautious handling of the GMOs. Under product liability, a producer may be strictly liable for lack or insufficient instructions for handling. However, the instructions for handling are usually included in the authorization procedures. So again, coexistence damage is not covered. Additionally, liability under the Directive for property damage excludes property used commercially. This will effectively prevent recovery in virtually all cases.

4.2.4. Countries opting for strict liability – based on the submissions to the Legal and Technical Experts Working Group on liability and redress[34]

The following countries have suggested strict liability in a liability and redress regime: Brazil, Egypt, the EU (with a limited number of defences and combined with a fault-based liability scheme), India, Liberia, Norway (possible combination with fault-based liability needs further

[28]Articles 2(1)(b) and 6(1)(b).
[29]Article 6(1).
[30]Article 2(11).
[31]Of 12 March 2001. For placing on the market, the Directive is pre-empted by sectoral regulation, in particular placing on the market of transgenic food and feed: Regulation 1829/2003 and 1830/2003. Seeds must be authorized under both the Directive and the relevant sectoral directives and implementing national seed laws.
[32]By Article 43 of Regulation 1829/2003, introducing a new article 26a.
[33]Guidelines of 23 July 2003, Commission Recommendation 2003/556, recommendation point 2.1.9.
[34]Montreal, 18-20 October 2004, UNEP/CBD/BS/TEG – L&R/1/INF/1, 20 Sept 2004.

discussion), Palau, Slovenia, Sri Lanka, Switzerland, and Uganda. This represents 10 out of the 22 countries that responded in English. The EU represents a further 25 countries.
Further, in the ongoing negotiations on liability and redress, strict liability was the preference for the African Union – representing some 33 countries – as well as a large number of the 17 developing countries with the largest areas of diversity and known as the 'Group of Megadiverse Countries'.

5. Defences to strict liability

Views vary on how a strict liability regime is to be implemented. Some provide for no defences or exemptions at all – more properly described then as an absolute liability regime; some allow limited defences; and some allow defences, which, critics say, undermine the reason for introducing strict liability.

The rationale for the defences is to strike a fair balance between the interest of the producer and that of the user. It is the quid pro quo – a sort of trade-off – for imposing strict liability.[35]

5.1. Defence: 'Development risk' and 'state of the art'

5.1.1. Differentiating

The most controversial defence is the development risk – sometimes also referred to as the 'state-of-the-art' -- defence. There is, however, a clear difference between the two expressions. 'State of the art' connotes that the product was safe when judged against the prevailing safety standard at the time it was put into circulation. 'Development risk' describes situations in which the product is defective when put into circulation but the producer can seek to avoid liability relying on the defence that the defect was not reasonably discoverable given the then existing knowledge.[36]

5.1.2. Arguments for and against including the 'development risk' defence

Policy makers will have to decide on whether to include this as a defence to strict liability. The prime reason will be for the one who profits to bear the responsibility and to ensure that the victim is not left uncompensated. Excluding this defence in the product liability laws of industrialized countries, such as the US and several European countries, in respect of pharmaceutical products has not stifled product innovation.[37] Indeed, it may even spur these companies to invest more actively in safety research.[38] Those who argue in favour of this defence say that it would discourage innovation and stifle research – especially in high technology development areas where there are likely to be unknown hazards and always subject to technological improvements – where producers have carried out reasonable research, testing, literature review, monitoring, and warning about their product.[39] The defendant bears the burden of proof to establish the defence. This may not necessarily tilt the balance in the victim's favour. All the defendant needs to do is give some evidence of the lack of requisite knowledge. The victim must then disprove the assertion.The risk must be *absolutely* undiscoverable, that is, that the particular risk was absolutely unknown and undiscoverable at a given time. The issue in strict liability is whether the defect in the product could be scientifically and technically discoverable.

[35]This was the explanation for the EC Directive on Product Liability: Dept of Trade and Industry, Implementation of EC Directive on Product Liability: an explanatory and consultative note, 1985.
[36]Clark, A, Product Liability, 1989, p. 151.
[37]Bradgate & Savage, 'The Consumer Protection Act (1987) NLJ 1049.
[38]US: Beshada v Johns-Manville Products Corp 90 NJ 191 447 A 2d 539 (1982) 206, 548.
[39]Hodges C, Product Liability: European Laws and Practice, 1993, 82-83.

5.1.3. The defence and the precautionary approach
A modified form of the defence to take into account the precautionary approach may be that the defendant needs to prove that he adopted the precautionary approach in considering the possible adverse effects of an LMO product.[40]

5.2. Defence: Compliance with legal requirements
This allows a defendant to plead in his defence that the defect is due to compliance of the product with mandatory regulations issued by the authorities. The defect must be the inevitable result of compliance. That is, that the product could not have been produced in accordance with the regulations without causing the product to be defective. The decisive factor is that the defendant had no choice because he had a legal obligation to comply. There must be a direct causal link between the defect and the compliance. For example, if the law requires that the product be made of a material resistant to, for example, fire, then the defence will only be available if the harm is directly and inevitably related to making it fire resistant. The defence may not be available if other harm is caused or if other factors contribute to the harm. Any such defence must take into account any discretion given to the producer in complying with the law, for example, if a minimum specification standard of compliance is imposed. If it is still impossible to produce a non-defective product, only then is the defence available. If it is clear that by using even the minimum standard the product would be rendered defective, then it could be argued that the producer should not go ahead to market the product. He would then, in any event, be liable in negligence even if he keeps within the legal limits of the statutory requirement.[41]

5.3. Defence: Limitation

5.3.1. For bringing an action
It is quite common to impose time limits for initiating legal proceedings. Time runs from the date on which the cause of action accrued, usually the date the victim has knowledge of the facts:

- about the damage as would lead a reasonable person to consider it sufficiently serious to justify instituting an action for damages
- that the damage was wholly or partly attributable to the facts and circumstances alleged to constitute the defect
- the identity of the defendant.[42]

5.3.2. Cut-off date
Some laws include, in addition, a cut-off date – referred to as a repose period – for bringing an action.

5.4. Other defences
Examples of other usual defences include the following: force majeure; intentional intervention by a third party; act of God; war and hostilities; compliance with a compulsory measure by a public authority.[43]

[40]Kate Cook, 'Liability: No Liability, No Protocol', in Bail et al. at p. 384. also see White Paper etc.
[41]Albery and Budden v BP Oil Ltd and Anor The Times May 9 1980.
[42]Limitation Act 1980, s. 14(1A) enacted pursuant to Schedule 1 para 3, Consumer Protection Act (UK).
[43]The Lugano Convention makes an additional requirement: where the damage was caused 'by the pollution at tolerable levels under local relevant circumstances': Article 8.

6. Causation: establishing the causal link

6.1 Problems

Under fault-based liability laws, a causal link must be established between the breach of duty and the harm. This is usually the most onerous task in a negligence claim. The chain of causation may be disrupted even partially. In the drugs liability situation there may be multifarious causes for the harm caused, such as environmental or biological causes. Much the same problems will apply to harm caused by GMOs. The damage may take a long time to manifest, sometimes even decades. The source of the damage may also have travelled over long distances, even from outside the territorial jurisdiction of a country. Establishing the link may involve highly complicated and competing expert opinions. In common law countries, causation can only be established by proving that the act caused or made a material contribution to the damage. In a situation where there is uncertainty amongst scientific/professional opinion of the cause of the harm then it must be shown that it is the alleged cause and not any of the other causes that – more probably – caused the harm.[44] That is, the product made a material contribution to the damage.

In product liability cases, the problem can be complicated by the often lengthy chain of distribution and assembled products containing component parts manufactured by others. There may also be a large number of GM growers in the vicinity – any one or more of whose acts may have contaminated the non-GM farmer's fields. Alternatively, the seeds causing the contamination may have travelled from afar. Intervening acts of others, however, only break the chain if the subsequent conduct or knowledge of the danger is held to be the sole cause of the harm;[45] otherwise liability will be joint or concurrent amongst the parties at fault,[46] although the victim may have to sue many parties and end up paying the costs of those exonerated from the fault.

There have been attempts to overcome these problems. The first attempt is by relieving the affected consumer from having to prove fault. He need only prove that the *product* caused the harm, not the *producer*. Also, intermediate examination does not excuse liability of the manufacturer for defects existing at the time the product is put into circulation. Further, a large range of potential defendants who can be sued are identified[47] – saving the difficulty of having to identify and, perhaps needlessly, suing all potential wrongdoers. Also, the remedy is in addition to that obtainable under the common law.

These options may be considered by developing countries for inclusion in a liability and redress regime to overcome the difficulties associated with establishing causation under the common law.

6.2. National laws

6.2.1. Austria: Law on Genetic Engineering

The Austrian law reverses the burden of proof. There is a presumption that the damage is caused by the characteristics of the LMO resulting from the genetic modification. The presumption is rebuttable by showing a likelihood that the damage is not due to the characteristics of the LMO resulting from the genetic modification (or in combination with other hazardous activities) of the

[44]Wilsher v Essex Area Health Authority [1988] 1 All ER 871 (UK HL) explaining McGhee v National Coal Board [1973] 1 WLR 1 (UK HL) – which allowed a claim on the grounds that the defendant's negligence materially increased the risk of the plaintiff developing the injury on the state of the existing knowledge.
[45]Evans v Triplex Safety Glass Ltd [1936] 1 All ER 283; Taylor v Rover Co Ltd [1966] 2 All ER 181.
[46]Griffiths v Arch Engineering Co Ltd [1968] 3 All ER 217.
[47]See text: section 8.

LMO. This is to overcome the difficulty of establishing proof because of the complexities of the interaction of the LMO with the receiving environment and the possible timescales involved.

6.2.2. Germany: Gene Technology Act[48]

The Act provides for strict joint and several liability for both alternative and cumulative causation of damage by GMOs. If the GM material cannot be traced back to a particular farmer, all neighbours that *appear* to have (potentially) caused the transfer of GMO features are jointly and severally liable.[49] This eases the burden of proof if it is beyond doubt that a farmer in the given area is growing the type of GMO that has caused the harm.[50] There is criticism that there is no need to establish the chain of causation to establish fault, and that the claimant farmer can place the blame on any farmer in his region growing the offending type of GMO. Also the biotechnology industry and the German Research Foundation have criticized the fact that a GMO farmer can be held liable even if he has complied with good practice as outlined in the Act.[51] They propose that a GMO farmer be held liable only if he disregards the good practice. In all other cases the victim is to be compensated by a fund.[52]

7. Damage recoverable

7.1. Scope

The damage recoverable can be defined narrowly or broadly. There have been various proposals to the Working Group on Liability and Redress in the ongoing negotiations under the Cartagena Protocol on Biosafety. Industry's proposal confines the damage to the conservation and sustainable use of biodiversity. This is interpreted to mean an adverse and significant change resulting in the decrease in the variability among living organisms. Damage may be determined by reference to the causative event: directly attributable to the properties of the GMO, their reproduction or modification, and the transfer of genetic material from these organisms: see the Austrian law (Section 6.2.1).

Canada also suggests that under Article 27 of the Protocol, damage should be confined to damage to biological diversity; and human health damage should be that which arises from adverse effects on biological diversity.[53]

The EU categorizes the damage as:

- damage to the conservation and sustainable use of biodiversity
- traditional damage
- damage to human health.

Damage covered by the first bullet is as to the variability among living organisms from all sources – including diversity within species, between species and of ecosystems. The EU proposes that thresholds of damage be described either in non-determined qualitative adjectives, such as: 'significant'; or in quantitative terms. If the former, it will then be for a court to decide what constitutes 'significant' damage. If the latter, these should be based on baselines and

[48]As amended: First Act Reforming Genetic Engineering Law – passed on 21 Dec 2004 and in force from 1 Feb 2005: Anja Gerdung, Germany's Liability Law, Sustainability Council of NZ, June 2006, at p. 8.

[49]If the separate and independent acts of two or more persons or corporations combine naturally and directly to produce a single indivisible injury, then the actors are joint tortfeasors, jointly and severally liable for the full amount of the plaintiff's damages: Restatement of Torts, US. A defendant held liable to pay the whole damage to a plaintiff may seek recourse against the other defendant(s) for the extent to which that other was liable.

[50]Anja Gerdung, at p. 10.

[51]Section 16b. Anja Gerdung, at p. 13.

[52]Anja Gerdung, at p. 13.

[53]At p. 11.

identified criteria.[54] Reinstatement should be an important remedy. It should be primary restoration, to the condition that existed before or the nearest equivalent (complementary remediation).[55]

The Lugano Convention states the following damage that has to be compensated for: personal injury, loss or damage to property, and, loss or damage by impairment of the environment including loss of profits from such impairment resulting from the properties of GMOs. Also recoverable: cost of preventive measures that have been taken after an incident has occurred to prevent or minimize loss or damage.[56] In the case of pure environmental damage, the costs of measures of reinstatement are recoverable. 'Reinstatement' refers to reasonable measures to restore or reinstate damaged or destroyed components of the environment, or to introduce, where reasonable, the equivalent of these components into the environment. A member country's internal law is to determine who is entitled to take these measures.

8. Determining the wrongdoer

8.1. Who is liable?

One way out of the difficulties in identifying the wrongdoer in fault-based systems is to specify in the law the person to be held liable; or prescribe criteria to ascertain such a person or entity.

Many jurisdictions opt to hold the person/entity, that either created the harm or who is in operational control, liable. Determining this would depend on the facts. If damage results from the inherent quality of the modification of the GMO, then it would be the person who produces or develops the GMO. It depends on the nature of the activity which causes the damage or the measures that need to be taken.[57]

Some also suggest as the wrongdoer, the person who obtains the approval for export or import of the GMO. In most cases, it will be the patent holder as commercialized GMOs are almost always protected by patents and approval is sought by the holder. It is suggested that this will solve the problem where GMOs spread beyond the specific environment into which they have been introduced, for example, as in the case of a farmer who buys and grows GMO seed which contaminates the fields of a neighbour.

8.1.1. Person who causes the harm?

It is clear that it is generally accepted that the defendant should be the person who causes the harm. Yet who is such a person? The person or the entity can be identified by reference to its role or the activity that has a clear connection with the harm, such as for example, 'producer', 'notifier', 'transporter', 'patent holder'.

8.1.2. Where more than one person is liable: channelling liability, joint and several liability

What if there is more than one person who has caused the harm? For example, where the GMO itself, as well as the lack of instructions as to its use, or improper use cause the harm. Then it should be possible to channel liability to a chain of multiple persons. The concept of joint and several liability can be a useful solution especially where more than one person is potentially

[54]At pp. 22-23.

[55]This could be for replacement of the loss by other components of the biodiversity at the same location or for the same use, or remedial action in relation to the same or other components at another location or for other type of use.

[56]Articles 2(7), (9), and 6(1).

[57]The EU proposes any one (or more) of the following: the developer, the producer, the notifier, the exporter, the importer, the carrier, and the supplier: UNEP/CBD/BS/WG-L&r/2/INF/1, and INF/2, 12 Jan 2006, second meeting, Montreal, 20-24 February 2006.

liable, and when it is unclear which person contributed to the damage and to what extent. The concept allows for an action to proceed against any one or more persons who caused or contributed to the damage – and recovery is sought against any one or more of them. The total pecuniary liability remains the same.

8.1.3. Additional tiers of liability
What if the damage cannot be entirely, or partially, redressed by the person to whom primary liability is channelled? Situations like this may arise in the following cases:

- The primary liable person cannot be identified
- The primary person escapes liability because of a defence
- Expiry of a time limit for bringing the action
- A financial limit has been reached
- The financial securities of the primary liable person are insufficient to cover liabilities
- The provision of interim relief is desired.

Then it may be desirable to consider providing for other parties who are involved to assume liability. In such a situation there could even be considered residual liability of the State.

8.2. National laws

8.2.1. Switzerland: Gene Technology Law
Switzerland's Section 30(2) provides:
The person subject to authorization is solely liable for damage that occurs to agricultural or forestry enterprises or to consumers of products of these enterprises through the permitted marketing of genetically modified organisms, that is the result of the modification of the genetic material.

8.2.2. Norway: Gene Technology Act
Under this Act, the liability is of the person responsible for the activity.[58] The activities that the Act covers are: contained use, and deliberate release – defined as any production and use of GMOs other than contained use. The 'person responsible for the activity' is defined as the person who produces or uses GMOs within the meaning of the Act. This could be a physical or legal person who operates the activity ('operator') from which the GMOs are discharged. In general, the person with the duty to provide information or to obtain approval under the Act may be subject to orders under the Act. This is said to be in line with the 'polluter pays' principle.

9. The form of the regime to be developed

Article 27 of the Cartagena Protocol on Biosafety does not prescribe the form of the regime to be adopted for the liability and redress regime. There are three possible forms that could be considered:

- A transnational regime
- A civil liability regime
- An international arbitral regime.
- The relative merits and demerits of each of these are considered.

9.1. An international arbitral regime
By using an arbitral regime, States can submit a dispute to an international arbitration body. The parties are States, not private actors. Parties can either establish a complete negotiated claims procedure which is detailed; or leave it simple and let most of the key issues and features be

[58]Section 23, The Gene Technology Act (Act no. 38 of 2 April 1993).

established by the ad hoc tribunal to which the dispute is referred. The key procedural issues and features to be dealt with in respect of an international arbitral regime include:

- On jurisdiction: procedural rules for determining jurisdiction
- On applicable law or choice of law: may provide procedural rules for choice of law, as well as concrete legal standards to determine liability for all disputes
- On recognition and enforcement of judgments: may include provisions for the same, as well as require parties to ratify – if they are not already parties – the UN Convention on the Recognition and Enforcement of Foreign Arbitral Awards.[59]

Two multilateral agreements use this arbitral regime for dispute settlement: the 1982 UN Convention on the Law of the Sea, and the 1972 Convention on International Liability for Damage caused by Space Objects. Issues of liability and redress are then referred to these tribunals.[60]

International arbitral bodies include: the Permanent Court of Arbitration, the International Court of Environmental Arbitration and Conciliation, and possibly the International Court of Justice.

9.2. A transnational regime

A transnational regime will facilitate private parties to bring claims to national courts. It will establish the process for parties to do so. It will rely on pre-existing national, and generally accepted international rules on private international law to instruct parties and courts in determining jurisdiction, choice of law and the recognition and enforcement of foreign judgments. There will be common procedures but no internationally recognized standards for determining jurisdictions; and no internationally accepted procedures to instruct courts on how to choose the applicable law. The court will do this by applying its own laws and procedures.

The difficulty is that different states and regions have differing and conflicting rules and principles. Some states may not even have these rules. Resolving such conflicts of law may be too onerous a task in any dispute.

9.3. A civil liability regime

A civil liability regime will – unlike the aforementioned two regimes – establish rules and substantive standards for the adjudication of disputes. Cases will still be brought to national courts. However, the national and the international legal standards for liability and redress will be harmonized. Thus, there will be established clear rules to determine jurisdiction, and there will be set internationally recognized legal standards on the applicable law. It will provide for the recognition and enforcement of judgments, as well as include provisions on access to justice and non-discrimination. Most multilateral environmental agreements that have addressed liability have opted for civil regimes. Examples include: the Paris and Vienna Conventions on nuclear liability; the 1992 Protocol amending the International Convention on Civil Liability for Oil Pollution Damage; the 1977 Convention on Civil Liability for Oil Pollution Damage resulting from Exploration for and Exploitation of Seabed Mineral Resources; the 1989 Convention on Civil Liability for Damage Caused by Road, Rail and Inland Navigation Vessels; the 1999 Basle Convention; and the 1996 International Convention on Liability and Compensation for Damage in connection with the Carriage of Hazardous and Noxious Substances by Sea.

[59]Awards issued by arbitration bodies, subject to very limited exceptions, can be enforced easily in the courts of countries that have ratified the Convention. To date 137 countries have ratified it.

[60]The Space Objects Convention provides standards for the potential parties to a claim, the standard of liability, damage, compensation, applicable law, time limits, possible interim measures for large-scale danger and final binding agreement or recommendatory award.

10. Conclusion

The various options that may be considered by developing countries in designing their liability and redress regime have been discussed. The adoption of any particular option will have to balance the competing domestic interests. In some countries these interests have crystallized around the proponents of biotechnology (usually the ministries of trade and innovation) and those concerned with the environment and human health (ministries of natural resources and the environment and health). Ultimately, however, a liability and redress regime must serve the wider interest of justice, and assure a remedy for any damage caused by GMOs.

Chapter 32

Post-Commercialization Testing and Monitoring (or Post-Release Monitoring) for the Effects of Transgenic Plants

SUSAN BARDOCZ AND ARPAD PUSZTAI

Background

It is recognized that the organisms created by recombinant DNA technology are basically different from those naturally present in nature, and may present special risks. Therefore, all GMOs/LMOs should be monitored for their health and environmental effects. Monitoring is essential to reassure that the original risk assessment was correct and the released GMO/LMOs are safe. Monitoring also identifies unanticipated effects.

Observing and recording the health and environmental effects of a GMO/LMO after its release is called 'post-release', 'post-commercialization', or 'post-market' monitoring. This activity is a must, independent of the costs and the resources required, and we should insist that it is done in the interests of present and future generations. The records of the monitoring activity should be kept for generations to come. However, *before* releasing any GMO/LMO, we should consider and decide how the post-release monitoring is to be carried out, what should be monitored and where, what are the best methods to use, for how long this activity should continue, and who will pay for it. It also has to be decided *in advance* where, and for how long, the records should be stored, and who is responsible for keeping and releasing the information. We have to bear in mind that monitoring should be carried out *independently, transparently and inclusively, and that the records should be made available for everyone*.

How should we start monitoring?

It is essential to start monitoring *before* the release of any of the GMOs/LMOs, otherwise it would be impossible to establish a baseline. Therefore, monitoring should start with an inventory of *all our natural resources*, cataloguing the local fauna, flora, and the health status of humans and their animals. It is important to pay due attention to all sites and locations where GMOs/LMOs are being produced, stored or transported. Without this information no data can be interpreted later.

Why should we monitor for the effects of a GMO/LMO?

There are compelling theoretical and practical reasons to carry out this expensive task (Box 32.1). Generally, monitoring of past and present status, or trend of a resource is essential for decision making. For example, storekeepers' record sales, stocks, consumer behaviour, etc. The records are used for forecasting business, and for making decisions about the stocks. Similar reasons apply for monitoring a GMO/LMO.

Box 32.1. Reasons for monitoring for the effects of a GMO/LMO.

Theoretical:

Pre-commercialization risk analysis has several weaknesses
Small-scale experiments only detect large effects
Low probability, low magnitude effects are unnoticed in test-experiments
Small, less frequent risks become evident only in the long term
Evidence collected over a long time confirms the accuracy of pre-release protocols

The public wants it
Learning process
Practical:
Essential for decision making
Part of quality control
Validation of risk assessment
Needed to forecast future trends

Theoretical justifications (BANR 2002) are firstly, that pre-commercialization risk analysis has several weaknesses (small-scale experiments are only capable of detecting large effects, order of magnitude differences). Secondly, all low probability and magnitude effects would likely escape detection in test experiments or field trials. To observe smaller and less frequent health or ecological risks, a longer time-scale is needed. Evidence collected over time can confirm the accuracy of pre-release protocols and risk assessments. Social factors provide additional rationale for monitoring: the public wants it, rigorous monitoring reassures them, and in a democracy to ignore public concerns is irresponsible.

From a practical point of view, monitoring is needed, since general characterization of a GMO/LMO may not pick up all the environmental effects. With post-release monitoring, there is an opportunity for multi-year testing of a GMO/LMO, and to see if the pre-commercialization testing protocols assessed the risks adequately. This is called validation. As Kareive and co-workers (UK GM Science Review Panel; July 2003) wrote 'we have so little faith in models and short-term experiments regarding prediction about invasion, that we advocate extensive monitoring of any introduced (GM-plant) with any ecologically relevant traits (such as disease resistance, herbivore tolerance, and so forth)'. Since GMOs/LMOs are different by nature and in their characteristics, no single rule can be applied for monitoring them. However, it should be kept in mind that our priority must always be monitoring for environmental and health effects, as well as socio-economic impacts. Post-release monitoring and testing of a GMO/LMO is a new endeavour, and at present it is not being done.

Who should carry out the monitoring of a GMO/LMO, and who should pay for this?
According to the EU directive 2001/18/EC, monitoring is the notifier's responsibility. However, if the producers of GMOs/LMOs are in charge of monitoring, it cannot be assured that this is carried out independently and transparently. Since the responsibility for the health of the citizens and their animals, and for the environment, lies with the national governments, monitoring should also be their responsibility, in spite of the high costs involved.

Long-term grants are needed for the monitoring projects, since any effect of a GMO/LMO might take a long time to develop and be noticed. As for who should bear these costs, it has been recommended (BANR 2002) that the cost should be covered by individuals (as tax payers), the private sector (the companies selling and distributing them), and by the local and state governments (as the regulators). However, it would be more just if the companies cover these costs (see the EU directive 2001/18/EC). Our recommendation is also, that the biotechnology companies, who profit from the sale and distribution of GMOs/LMOs, should cover the full costs of monitoring. One idea is to force companies to pay a levy of 0.1% of all the profits from the sales of their GMOs/LMOs, which would go towards covering the monitoring costs.

It is the duty of national governments and the local authorities to assure that the post-release monitoring of a GMO/LMO is properly carried out, preferably by independent scientists.

The authorities provide the costs and resources needed to monitor all the essential resources, such as water, soil, air, or public and animal health. It should thus also be their duty to provide the cost of monitoring for the effects of GMOs/LMOs, despite the manpower and large sums of money needed. It should be their task to devise means for recovering the expenses.

Environmental effects – what needs to be monitored?
GMOs are produced by novel techniques, and as a result, they represent unique risks (Box 32.2). Therefore, GMOs/LMOs require greater scrutiny than organisms produced by traditional techniques of breeding (Snow et al. 2005).

Box 32.2. Unique environmental risks of GMOs/LMOs.

* Little or no prior experience with the trait and host combination
* GMOs may proliferate and persist without human intervention
* Genetic exchange possible between a transformed organism and non-domesticated organisms
* Trait confers an advantage to the GMO over native species in a given environment (Snow et al. 2005)

The most important aims of environmental monitoring are either to prevent the development and spread of any undesirable effects, or, if such a risk has already occurred, to implement preventive strategies to impose immediate restrictions on commercialization. This can be done by instructing the producers to modify the conditions of production and release, or by any other means.

Based on our present understanding, some of the major risks associated with transgenic plants persist because of fundamental flaws in the risk assessment legislation. According to this, the pre-release risk assessment only considers the effects of a GMO/LMO, but ignores the risks associated with the gene-construct and the transgenic technology itself, which is declared to be neutral. However, these risks should be taken into consideration, and should form part of monitoring the impact of a GMO/LMO on the environment (Box 32.3). Monitoring should observe the result of gene escape and of the GMOs, the impact on pests, on agricultural practices, and on the evolution of resistance to their traits (Wolfenbarger & Phifer 2000; Lovei et al., see chapter 10). Transgenes are inherited and have the potential to disperse between individuals of the same species, or to wild relatives. Therefore, monitoring of the transgene movement is essential. In the case of some transgenic plants, fitness of the transgenes conferring resistance has an effect on plant population dynamics.

Box 32.3. Possible risks of GMOs/LMOs.

Persistence/invasiveness
- In the fields (GMO)
- Outside fields (GMOs)
- Transgenes

Gene transfer
- Vertical
- Horizontal

Target effects
- Resistance developing in insects
- Resistance developing in weeds

Non-target effects

Appearance/dominance of secondary pests
Creating new, and more vigorous pests and pathogens, or exacerbating the effects of existing pests
Harm to non-target species
Disruption of biotic communities, including agro-ecosystems
Irreparable loss or changes in species diversity or genetic diversity

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Horizontal gene transfer
Movement of a transgene via horizontal gene transfer (Box 32.4) must be monitored (see Chapter 13). Unfortunately, in the pre-release risk assessments submitted to the regulators, the probability of horizontal gene transfer is calculated to be near zero. Nonetheless, the risks associated with horizontal gene transfer can be significant, thus monitoring is essential.

All testing should be conducted at spatial scales appropriate to evaluate the environmental changes in both the agricultural and natural ecosystems. Ecosystems are complex and sensitive. Therefore, GM plants with some environmentally sensitive traits require closer scrutiny.

Box 32.4. Horizontal gene transfer.
1–20% of the DNA of an organism derives from foreign DNA (Ochman et al.
2000, Koonin et al. 2001)
Major source of microbial evolution
Depends on population density
Less frequent between distantly related taxa
Most likely to occur, and has been detected, in microbial communities
Gene flow

Special risks relating to herbicide-tolerant crops
Contamination of the soil, surface water and groundwater, and the herbicide residue in the GM crops should be monitored. The Roundup Ready gene, conferring glyphosate resistance, is the most often used transgene worldwide. It is recognized that its use may not be sustainable if weed shifts occur to favour glyphosate-tolerant weeds, or if weeds develop tolerance to glyphosate. The basis of the present popularity of glyphosate is based on the assumption that it breaks down quickly in the soil, and is more 'environmentally friendly' than many other herbicides. Unfortunately, this is not true. There is evidence suggesting that it persists in the environment and accumulates in the groundwater. Moreover, it harms mammals, including humans (see Chapter 14).

Special risks associated with Bt-transgenic plants
Special risks associated with Bt crops are the accumulation of the active toxin in the seeds and the green parts of GM plants, as well as in the soil. We also should monitor for the development of pest resistance in the target organisms.

A variety of Bt crops are grown worldwide. They are popular, since they are considered to be environmentally friendly by reducing the use of pesticides. However, growing them may not be sustainable if secondary pests become more of a problem and/or if target pests evolve resistance to Bt.

Problems with disease-resistant transgenic crops

Only a few crops with transgenic disease resistance have been released to date (such as virus-resistant squash, papaya and potatoes). With the virus-resistant crops, the main hazard is the occurrence of a new virus – transgene recombination, resulting in formation of new viruses, increased virulence of the virus, alterations in host-specificity, or the change of its transmission characteristics with transcapsidation (encapsidation of viral RNA of one virus by the coat protein of another). Synergistic interaction between viruses might also occur in mixed infections.

Human and animal health effects – what needs to be monitored?

When monitoring for the health effect of a GMO/LMO, we have to know when, what, and how much of a GMO/LMO was eaten, and for how long. In the case of foodstuffs, this means exact labelling of all GMO/LMO components. However, labelling of GM food or feed is not compulsory in many countries.

When monitoring for the effects of GM crops, we have to take into consideration that the pre-release risk assessment is mostly based on assumptions. One of these assumptions is that all DNA is degraded by the saliva and in the gut. However, in the case of edible DNA vaccines, sufficient amounts of the DNA must survive to be able to evoke an immune response. Therefore, it is important to determine the extent of DNA breakdown by using an in vivo system, and measure whether any foreign proteins and DNA survive passage through the stomach and small intestine.

Animal health monitoring

Short- and long-term monitoring of a GMO/LMO effect should be based on observing all changes in animal behaviour, physiology and metabolism, as well as observing alterations in the immune- and hormone-responses (Pusztai & Bardocz 2005, Pusztai & Bardocz Chapter 14 in this book). It is essential to monitor for any change observed in growth rate, organ development, life span, and reproductive function. Changes in disease susceptibility, of immune status, pathogeneicity, or infectiousness of an organism can also be important indicators. The aforementioned parameters should be monitored and recorded over at least four generations.

Monitoring of human health

In the case of humans, several non-invasive techniques can help to monitor the effects of a GMO/LMO. The easiest is to follow changes in immune responsiveness by taking consecutive blood samples. Hormone assays can be carried out with the same samples. It is easy to assess the changes in bacterial status from regularly collected faecal samples. With the help of invasive techniques, such as collecting gastric- and colon biopsies, one can monitor the primary effects of GMOs/LMOs in the alimentary tract, and in its bacterial flora.

Tissue samples from tumours collected for histological/pathological evaluation can be assessed for cancer effects, and also to establish the presence of foreign DNA, or of the vector/construct.

In the longer term, the science of epidemiology can help post-release monitoring. In particular, case-controlled epidemiological studies can give vital clues as to the effects of a GMO/LMO. However, in order to establish human health effects conclusively, one would need to carry out human volunteer studies. When these are performed, one should look out for new microbes (viruses, bacteria) containing GM vector elements, and bacteria with antibiotic-resistance, and other transgene- or vector elements. We should also monitor for immunological differences as well as changes in susceptibility to diseases.

A few years ago in the UK, plans were made to monitor for the effects of GMO-containing foods on humans. The idea was to use consumer loyalty cards of supermarkets, in combination with individual health records. Nothing came of these ideas, since several problems are connected with

the scheme. Firstly, cardholders do not shop for one person, and not all GMO/LMO containing-food is labelled. With the use of the cards there is no way to keep records on everybody's food consumption (consumers shop around, consume food outside their home, and eat out during trips and on holiday, etc.). There is also the problem of matching consumption with the individual's health records, which are confidential.

For the authorities, data collection is possible through regular health checks, medical reports, and using epidemiological studies.

Where should we monitor for the effects of a GMO/LMO?
Obviously, monitoring should be carried out on and around the sites where the GMO/LMO has been released, and also in the wild. The area should be dependent on the type of organisms released. It should include monitoring of all the natural resources, in particular, water, air and soil.

When monitoring for a local effect, we also have to consider: pollen transfer, local contamination by excreta, microbial spread, migratory populations, the food web, etc.

One of our target-sites should be the *soil*. However, there is a problem with this: only a small proportion of soil organisms are known. Their effects and the interactions between them and with other organisms are not understood at all, since we do not know 99% of the soil microorganisms. At present, soil is monitored for its nutrient content, structure, contamination by heavy metals, chemicals, etc. Monitoring for the effects of a GMO/LMO is still possible, based on differences between soil DNA extracts taken before and after the release of a GMO/LMO, and, with repeated measurements the differences can be interpreted.

Another target site should be the *air*. Pollen, for some, can be a major allergen, and air is continuously monitored for its pollen content in developed countries. Using the same samples, one could also monitor for GM pollen, and when it is detected outside the GM crop field, one should take immediate action. When pollen escape is a serious risk, the government could ask the growers of GM plants to prevent this, for example by building tall plastic/glass walls around GM production sites, or around the GM field trial sites. This would not stop all birds and insects from carrying the pollen around, but would somewhat decrease the chances of cross- pollination.

Water quality, and contamination by pesticides/herbicides and their residues are monitored regularly. Sea- and fresh-water organisms are monitored for stocks and contaminants (such as heavy metals, etc). When collecting the samples for monitoring these aspects, the same samples can be used for testing for foreign DNA, their effects or products. Changes in an organism's physiology/pathology should be monitored for at least four generations.

When monitoring for changes in the environment, we should look out for new microbes (viruses, bacteria) containing GM vector elements, and for bacteria with antibiotic-resistance genes. We should observe if invasion by a GMO/LMO of a neighbouring ecosystem has occurred, or if crops, weeds and other plants with resistance traits have appeared. Shifts in insect and predator populations and their feeding habits should also be monitored for any change.

Who should monitor for the effects of GMOs/LMOs, and for how long?

Monitoring should be carried out using every possible means. Everybody should be involved, from government employees and officials to farmers, civil societies, NGOs, interested individuals, and even schoolchildren through specific projects.

The time span of post-release monitoring should last for at least four generation-times, as a minimum. Generation-times for microorganisms vary between a few minutes to a few hours, and for humans it takes about one hundred years. This length of time is needed to detect long-term effects, and to observe the influence of a GMO/LMO on the reproductive function. Environmental influences, lifestyle, and even the amounts of food consumed by grandparents may have an influence on their offspring for generations. Therefore, as a minimum, a four generation-timescale may be required to observe the true effects of a GMO/LMO. This means that if we want to match up a late effect of any GMO/LMO, we must keep the records for 120–150 years, at least. Storing the data and making them available to anyone for consultation is a major task for the local and national authorities. However, it should be done and, if at all possible, it would be useful to keep the records for even longer.

A two-part approach should be used for monitoring: first, trained observers should monitor immediate post-release changes in the environment, since they are the ones who are able to differentiate between temporary and spatial effects of a GMO/LMO. Secondly, everyone should report any changes observed in connection with a GMO/LMO to the local and national authorities. These observations should also then be validated by trained personnel.

The present status of monitoring health and environmental effects in the EU and worldwide
In the EU, a Directive (Directive 2001/18/EC) was passed to regulate the post market-, or post-release monitoring of all GMOs/LMOs, but it leaves the question of how it should be carried out open for the individual countries. Nations should create their own laws on post-release monitoring systems, and provide the finances and trained personnel to carry out these tasks. The EU Directive sets out guidelines also for the design of a monitoring plan (Box 32.5), which should form part of the dossier presented by the notifiers (e.g. the company) to the regulatory authorities. According to the Directive, the request for releasing a GMO/LMO should contain plans for monitoring. The Directive also makes the notifiers directly responsible for paying and carrying out the monitoring. Therefore, it is *essential* that the notification contains a plan for monitoring, including a proposal for the period (Directive 2001/18/EC 2001; Bardocz & Pusztai 2004). The Directive also introduces an obligation for notifiers to implement monitoring plans in order to trace, and identify any direct or indirect, immediate, delayed, or unforeseen effects on human health or the environment of GMOs after they have been placed on the market, including obligations to report to the Commission and competent authorities. In addition, to ensure transparency ‘the results of monitoring should also be made publicly available’. According to the Directive, monitoring should be developed on a case-by-case basis. It also gives guidelines for working out a monitoring strategy (Box 32.6).

Box 32.5. The monitoring strategy.
Risk assessment, before release and background information
- Approach: case-specific monitoring, general surveillance
- Baselines
  - Status of the environment and changes therein
  - Causes of such changes
  - Expected development of the environment
- Time period
- Assigning responsibilities
  - Notifiers
  - Third parties
- Existing systems of monitoring

The regulators may use the monitoring plan set out in the Dossiers, or can work on other plans.

Box 32.6. Design of monitoring plan.

–Should contain guidelines for:
–The monitoring methodology
–Monitoring parameters/elements
  –Areas/samples
  –Inspection
  –Sampling and analysis
  –Collection and collation of data
–Analysis, reporting, review
  –Evaluation
  –Review and adaptation

In the USA, no official monitoring programme exists. We know very little about monitoring systems in other parts of the world.

In reality, only a few countries have an inventory of the various biological resources, and the health status of the population, which can be used as a baseline. In many countries, GMOs/LMOs have already been released into the environment, and most of their populations have already been exposed to foods prepared from GM crops. Based on data in the scientific literature, very little is being done at present to monitor the effects of any released GMO/LMO. It is crucial that public programmes of biological risk assessment and management be expanded substantially.
We must conclude that at present not a single country has developed an efficient post-release monitoring system, although several countries are producing GMOs/LMOs on a large scale.

Cost versus benefits analysis of post-market/post-release monitoring for the effects of GMOs/LMOs

At present, the cost of monitoring, health care and cleaning up the environment is the responsibility of the national governments, through the taxes the citizens pay for the expenses of monitoring, data collection and storage. At the same time, the citizens are the ones who are exposed to most of the risks of GMOs/LMOs.

The most surprising fact in connection with a GMO/LMO is that, in the absence of international rules on liability and redress, which are only now being negotiated under the Cartagena Protocol on Biosafety, it is extremely difficult to hold a GMO/LMO producer, especially if a foreign entity, legally responsible for its product. This means that if anything goes wrong with a GMO/LMO, the company may be free to walk away and leave the national authorities to deal with the problem and force the citizens to pay for the clean up.

In summary, monitoring should be carried out *independently, transparently and inclusively*. It should start with an inventory of *all* GMOs/LMOs, and the sites/locations where they are being produced, stored and released. Without this knowledge, no data can be interpreted later. The inventory should be *kept for a minimum of four generations*. When considering deliberate release of any GMOs/LMOs into the environment, we should think first, and not forget that governments have the power to legislate, but the citizens – who are also the consumers – have a vote, and can vote also with their money. The national governments and the regulators have the right to ask the

producers to carry the costs of all extra tests relevant to the special conditions of a country before a GMO/LMO is released there, and also the costs of monitoring, after the GMOs/LMOs are released.

Emergency planning

Even during the very short time-period since the first GMO was released into our environment and food chain, we have already seen escapes of genes and contamination of our food supplies. For instance, there has been the StarLink disaster, or the controversy and the problems with Prodigene, growing pharmaceuticals in GM plants, not to mention the presence of additional, unapproved cry-proteins in some Bt crop varieties. Therefore, before we release any GMO/LMO, we must have emergency plans in place. We have to keep in mind that we have no techniques to 'take back' or recall any of the escaped genes or organisms. Furthermore, it is very difficult to contain or control the spread of an already escaped GMO/LMO. If contamination has already happened, we are more or less sure that it will happen again. We also have to have some ideas in advance, of how we are going to clean up any contamination in case something goes wrong.

We must have different emergency plans and be prepared for emergencies and have an action plan to be able to act to control the situation. In addition to having emergency procedures in place for all kinds of scenarios (Box 32.7), we must have the capacity and personnel to deal with the problems.

Box 32.7. Steps in emergency planning to deal with a GMO/LMO-related accident.

SCENARIO:

A. Plans for accidents that occur in containment
B. During transit
C. Food/feed production, food chain contamination
D. Deliberate release into the environment of
   –organisms unable to self-replicate
   –self-replicating organisms

STEPS:

1 establish facts – verify source, collect the material to prevent its spreading
2 assess damage
3 clean up – beware of 'dumping'
4 follow-up (health/environmental checks)

Coexistence

The EU and several other countries have chosen to regulate GMOs/LMOs on a case-by-case basis, and thus they do not exclude the growing of GMOs/LMOs, however unfair it is to those who want to remain GM-free. Therefore, the national authorities have to regulate the conditions in law to allow the coexistence of agricultural practices for growing organic- (bio), traditional-, and GM-crops. However, the national governments also have the right to regulate GMO/LMO production by restrictions, setting special requirements or conditions of production (walls around sites, separate irrigation systems, etc.). National governments can also, by legislation, force labelling and monitoring, and make the producer liable for damage caused by their product(s).

When it comes to cost-benefit analysis, one must consider all alternatives (sustainable, low chemical input/organic, local produce using local seeds), and weigh up the costs. We also have to see if there is a real chance for the coexistence of the different production systems. It is clear that

GMOs/LMOs make organic production systems impossible in the neighbourhood. In contrast, organic production endangers neither traditional agricultural methods, nor the growing of GM crops, even if organic crops cross-pollinate them.

According to German law, the production system that was in place first, has the priority over the newer methods and technologies of crop production. Accordingly, in Germany, the farmer or producer who contaminates the lands or products of another pays compensation. It is worth noting, that the responsibility is not assigned to the GMO/LMO producer, such as the biotechnology companies, although liability can be eventually channelled to them by the GMO/LMO farmer. This, however, means that they may not be held legally responsible for their products and the damage they inflict.

Identity preservation systems

In March 2006, the Third Meeting of the Parties to the Cartagena Protocol on Biosafety agreed new documentation requirements for shipments of GMOs/LMOs that are intended for direct use as food, or feed, or for processing. At issue is the need to know exactly which GMOs/LMOs are entering a country. This international minimum standard will help encourage a global system of identity preservation, segregation and traceability for GMOs/LMOs, The idea of bio-tagging has also been considered separately. Bio-tagging means that every biotechnology company should have a 'company sequence' inserted to the genome of all of their GMO/LMO products, although the risks associated with such insertions should also be assessed.

The reason for the efforts to ensure identity preservation is intimately linked with monitoring. It is important to be able to track and trace the GMOs/LMOs that are entering a country for monitoring requirements, risk management and reviews of decisions in the light of new scientific information. In case something goes wrong, such a system is also critical to be able to ensure product recall and to take emergency measures. It is also important to have a clear system of traceability, to be able to identify what caused the damage and to identify the producer, so that liability can be assigned and redress obtained. This would be important in light of the future development of an international liability and redress regime for GMOs/LMOs under the Cartagena Protocol. Although all manufacturers are prosecuted for selling dangerous articles, or shops and restaurants closed down and taken to court for selling dangerous products or bad/infected foods, at the present there are no international liability and redress laws for GMOs/LMOs.

All previously developed and established technologies are fully controllable. Electricity, and even nuclear power, can be turned off. Production and distribution of GMOs/LMOs is a new endeavour. This is a technology with a difference. GMOs/LMOs are self-replicating, they cannot be recalled, their genes cannot be turned off, and we have no method to take a released GMO/LMO or their genes out of the environment once it is released. *This is the first irreversible technology in human history*, therefore it requires more scientific scrutiny, legal control and monitoring, not less.

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Chapter 33
Monitoring GMOs Released into the Environment and the Food Production System

JOHN FAGAN
GENETIC ID

Introduction

In order to systematically assess the impact of any genetically modified organism (GMO) on health or the environment, one must be able to answer the questions, 'Is the GMO present in the material of interest?' and 'How much of it is present?' This is the first step in assessing whether the presence of a given GMO is correlated with specific effects either on the environment or on health. The ability to track GMOs in the environment and food chain is, therefore, an essential capacity required for biosafety assessment.

The vast majority of countries that have implemented, or are in the process of implementing a biosafety framework recognize the need to track GMOs released into the environment or the food production system. The only notable exceptions are Canada and the US. The latter's system of authorization for environmental release for food purposes is permissive in many ways. The environmental assessments by the US Department of Agriculture and the Environmental Protection Agency are weak at best, and further, the US Food and Drug Administration does not impose mandatory food safety assessment of GMOs before release.

Biosafety frameworks generally identify the following purposes for establishing systems for post-release tracking of GMOs:

- To enable the efficient and timely withdrawal of products, where unforeseen adverse effects on human or animal health or the environment are established
- To facilitate the targeting of monitoring programs to examine potential harmful effects on health or the environment
- To support the implementation of risk management measures in accordance with the Precautionary Principle
- To facilitate accurate labeling of genetically modified (GM) products:
- To ensure that accurate information is available to the food industry and consumers to enable them to exercise freedom of choice
- To enable control and verification of labeling claims
- To verify that GMOs, and the mode of their release into the environment, are in compliance with international accords, such as the Cartagena Protocol on Biosafety to the Convention on Biological Diversity
- To verify that GMOs, and the mode of their release into the environment, are in compliance with national regulations

More broadly, to monitor the movement of released GMOs in the environment and food chain. Analytical methods, aimed at the identification and quantification of specific GMOs, can be integrated with document-based traceability and labeling systems to efficiently, economically, and reliably track the movement of GMOs in the environment and the food chain. This integrated approach is of great benefit, especially to operators within the food chain and to regulators, since it both reduces the need for time-consuming and costly testing, and actually increases the effectiveness of monitoring efforts.

This chapter will begin with an overview that considers document-based traceability and labeling systems, as well as testing, in the context of biosafety assessment of GMOs released into the environment and food chain. The chapter will then discuss GMO testing methods more deeply.

Tools for Tracking GMOs Released into the Environment

Testing—Positive identification, based on empirical evidence, such as test results, is the foundation for the traceability chain of every product. In principle, the traceability data for a given lot of product should document a chain of custody that traces the product and its precursors all the way back to the initial transformation event that generated the specific GMO contained in the product. However, in most cases, the starting point is a test result that identifies and/or quantifies the specific GMO present in the product or in a precursor of that product.
Once the genetic status of a specific lot or consignment of food has been established through testing, documentation systems and labeling can be used to track the movement of that product through the food chain.

Testing continues to play an important role at later stages in the chain, however. Testing and representative sampling are a necessary part of the quality control systems, used by industry to verify that traceability and labeling procedures are operating effectively in the transport, storage, and processing chain. Sampling and testing are also of importance to government regulators charged with operating surveillance programs designed to confirm that suitable traceability or labeling is being maintained for approved GMOs, and to verify that only approved GMOs are being introduced by importers and domestic operators into the environment and the food production system of the nation.

Document-based Traceability Systems—Two different models are used for traceability systems. The most rigorous approach is where a centralized documentation system tracks, handler-by-handler, the chain of custody of a specific lot of product through each step in the journey from the farmer's field to the consumer's dinner plate. At any point in time, the whole chain of custody is fully and immediately available. This is the traceability model that is used in organic certification and a few other applications.

The second, more common, traceability system is the 'one-forward, one-back' system. In this system, each participant in the chain is required to maintain the following four pieces of information, for each specific lot of product that they handle: (a) from whom they received that lot of product, (b) the date on which they received it, (c) to whom they released that lot of product, or lots of product derived therefrom, and (d) the date of release. This system does not provide a chain of custody document for a given lot of product, but imbeds in the supply chain sufficient information to assure that it should be possible to trace any given lot of product back to its source ingredients, if needed.

This second form of traceability has been required since 2005 for every food product and food ingredient sold in the European Union under regulation EC 178/2002. This system is also used in a modified form for traceability of GMOs in the European Union, as outlined in regulation EC 1830/2003 (European Commission 2003). This regulation requires that, in addition to retaining information on the immediate supplier and immediate buyer, the operator must retain, and supply to the buyer, information on the specific GMO contained in the product, if it is a GM product, or, if it is a product derived from GMOs, an explicit declaration that the product 'contains GMOs'. A system similar to that specified in EC 178/202, the 'trace-back system', is under development in the US.

One-forward, one-back traceability is designed to enable regulators, who identify a health hazard associated with a specific package of product, to trace that product back to each of its component ingredients, and thereby locate the source of the contamination. Although this approach to traceability is more economical, insufficient evidence has been gathered to date to demonstrate its consistent effectiveness in practical application.

Labeling–Traceability systems can also make use of labeling, bar-codes, radio frequency (RF) tags, and a diversity of other physical devices. These are useful in maintaining traceability of packaged goods or other strictly defined units, the integrity of which is not compromised as the product changes hands in the chain. Examples would be a package of breakfast cereal, a sealed tank of lecithin, and a living farm animal, such as a cow. Traceability and the validity of the labeling are destroyed as soon as the seal on the lecithin tank is broken, or as soon as the animal is rendered into separate meat products, unless documentation is created that traces the next steps of the production process.

Segregation–Segregation measures are distinct from traceability. Segregation maintains the physical integrity of a given lot of product as it passes through the chain. For instance, a consignment of grain can be traced from the farm, to a centralized storage facility, to a barge, to an export terminal storage bin, to the hold of a boat, and finally to an import storage bin owned by a manufacturer who converts the grain into consumer products. Records can be created accurately documenting each of these steps—this is traceability. However, this documentation does not assure the integrity and purity of the product that the final buyer incorporates into the consumer product. At each step in the transport, storage and processing of the product, contamination can occur; a storage bin may not have been cleaned out properly and may contain residual grain from a previous use. A ship's hold may be loaded with multiple products, creating significant risk of cross-contamination. The manufacturing facility may be operating multiple production lines simultaneously, and inputs or work in progress may spill from one line to another, contaminating the product of that line. Even at the farmer level, contamination can occur due to cross-pollination from a neighboring field.

Segregation measures are procedures designed to preserve the integrity of the product by preventing cross-contamination of the kinds described. The stringency of segregation measures determines the purity and degree of physical integrity of the final product. For instance, non-GMO soy is sold in multiple grades. The highest grade is guaranteed to contain less than 0.1% GM soy, and is used in many countries by operators who want to make claims that their products are 'non-GMO'. The next grade is guaranteed to contain less than 0.9% GM soy, and is often used in the EU by operators who wish to produce products that are exempt, according to Regulation EC 1830/2003, from being labeled as 'genetically modified'.

Identity Preservation—Segregation together with traceability documentation comprise identity preservation. To credibly preserve the identity of a lot of product, it is necessary to both segregate that lot from other lots, and to maintain adequate traceability documentation for that lot.
When used properly, the aforementioned components—testing, traceability, labeling, segregation, and identity preservation—function together to assure that a specific lot of product, whose genetic status is known, can be tracked efficiently, economically, and reliably through the food production chain.

Technically speaking, the most challenging of these components is testing, and this is also the most critical component for assuring the initial identity of the GMO and verifying the accuracy of the traceability system at intermediate points in the chain of custody. The following sections of

this chapter discuss the technical aspects of GMO testing, and how to maximize accuracy and reliability of this critical element of traceability systems.

Basic Rationale of GMO Testing

Gene modification (also called recombinant DNA methods or gene splicing techniques) introduces new genetic information, new DNA sequences, into the genome of an organism. Once introduced into the genome, the transgenic (also called genetically modified) DNA reprograms the cells of the recipient organism to produce new mRNA species and new proteins. The transgenic proteins confer new characteristics or functions upon the organism. GMO detection methods could, in principle, measure transgenic DNA, mRNA, or proteins, or even the novel biosynthetic products or biological functions conferred by the new genes. However, in practice, analytical methods have focused almost exclusively on detection of transgenic DNA and protein. I will consider both of these analytical approaches in some detail.

Immunological Analysis of GMOs

Immunological tests for GMOs detect the transgenic proteins encoded by recombinant genes. These tests employ both the ELISA (enzyme-linked immuno-sorbent assay) and the lateral flow test formats (Lipton et al. 2000, Lipp et al. 2000, Stave 1999).

Although there are many different configurations for ELISA tests, the basic design is illustrated in Figure 33.1. First, antibodies specific for the analyte of interest are immobilized to the wells of the ELISA assay plate. When exposed to a solution containing the analyte of interest, the immobilized antibodies capture the analyte. This immobilized complex is then exposed to a solution containing a second antibody that also recognizes the analyte, and which is also linked to an enzyme. This second antibody becomes immobilized to the complex, as well, where the enzyme catalyzes the conversion of a compound present in the reaction vessel into a second compound that can be quantified colorimetrically or fluorimetrically. Thus, ELISA technology is in essence a method for linking the antibody-analyte recognition reaction to a reaction that generates a colored material that can be detected and quantified.

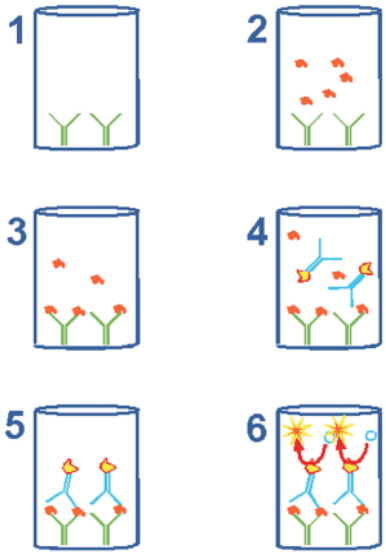

Figure 33.1. ELISA immuno-detection process
The figure shows the basic principles of enzyme-linked immuno-sorbent analysis (ELISA), which is used to detect transgenic proteins for GMO analysis. Step 1, antibodies are bound to the surface of the reaction well. Step 2, analyte (antigen) solution is added to well. Step 3, analyte binds to antibodies. Step 4, a second antibody, with conjugated enzyme is added to the well. Step 5, second antibody binds to the complex between the analyte and first antibody, which is bound to the surface of the well, thereby immobilizing the second antibody to that surface. Step 6, the enzyme conjugated to the second antibody converts colourless substrate (blue circle) to bright coloured or fluorescent reaction product, which can be quantified colorimetrically or fluorimetrically.

The lateral flow test makes use of the same basic immunochemistry but is configured to allow convenient field analysis with visual assessment of results. On the biochemical level, the main difference between ELISA and lateral flow strip tests is that the enzyme-linked second antibody, used in ELISA assays, is replaced in strip tests with antibodies conjugated with colloidal gold. Because immuno-tests require minimal processing of the sample, they can be completed quite quickly (Lipp et al. 2000, Stave 1999). Moreover, in the lateral flow format, immuno-tests are very convenient and easy to carry out, do not require sophisticated equipment, and are inexpensive on a test-by-test basis. This format is particularly useful for field GMO tests, where they can be used to rapidly screen truckloads of soy or maize at the grain handling facility for a single GM trait.

The speed and convenience of immunological tests offer substantial utility. However, the limitations of this method should be recognized in order to assure appropriate application. One crucial limitation of immunology-based tests is in the area of quantification (Stave 2002, Fagan 2001). Although ELISA tests can be configured to function quantitatively, in the context of GMO testing, the capacity for quantification cannot be used advantageously. This is because it is difficult, if not impossible, to translate mass of transgenic protein, measured in the sample extract, into percent GMO.

Percent GMO is the quantitative basis for most national regulations on genetically modified foods, such as in the EU regulation EC 1830/2003 (European Commission 2003). Percent GMO refers to the weight percentage of food derived from genetically modified materials. For example, a truckload of 20% genetically modified maize might contain 5 metric tons of transgenic maize and 20 metric tons of conventional maize.

If one were to conduct a quantitative ELISA analysis of a representative sample of that maize, the analysis would provide information, with reasonably good accuracy and reproducibility, on the mass (nanograms) of a specific transgenic protein, such as Cry1Ab, extracted from a given number of grams of maize. The difficulty arises in accurately extrapolating from this value to percent GMO. This is due to the fact that there is no constant relationship between these two parameters (mass of transgenic protein extracted and mass of maize grain or grain derivatives). Several factors contribute to this.

First, the level of expression of the transgenic protein is not constant, i.e., the ng of transgenic protein expressed per gram of transgenic maize is not constant. If it were, then one could compare the result of this analysis to a series of standards containing known amounts of transgenic maize, to estimate percent GMO. However, expression is not constant. It is influenced by weather, soil, and other cultivation conditions. For example, Roundup Ready soy has been found to express transgenic EPSPS (5-enolpyruvyl shikimate 3-phosphate synthase) at levels ranging from 0.179 to 0.395 ng/mg (Monsanto 1994). This is more than a two-fold range in variation.

In virtually every instance, the standards used for calibrating the analysis will be derived from a different lot of soy cultivated under conditions different from those under which the soy present in the sample were cultivated. Therefore the level of expression in the sample will differ from the reference materials, and it will not be valid to estimate the GMO content of the sample by comparison with those reference materials. A sample judged to contain 1% GM soy based on such a comparison could contain as little as 0.5% or as much as 2%.

A second contributor to variability in expression of transgenic proteins is the fact that different transgenic events are engineered to express the same recombinant proteins at widely varying levels. For example, Bt176, Bt11, and Mon 810 all express transgenic Cry1Ab proteins, but at very different levels. Cry1Ab is present at 0.09 μg/mg, in E176 maize, while the levels in Mon 810 and Bt 11 maize are 0.31 and 4.767 μg/mg, respectively (Ciba Geigy 1995, Monsanto 1996, Northrup King 1995). Thus, if an ELISA test indicated that the Cry1Ab content of a truckload of maize was 0.09 μg/mg, this could indicate that the truck contained 100% E 176 maize, 29% Mon 810 maize, or 1.9% Bt 11 maize, or any combination of the three.

In the real world, the analyst will not know whether a sample is comprised of a single event or of a mixture, nor will the relative proportions of the events that may be present be known. Thus, it is virtually impossible, in practice, to determine percent GMO for maize using ELISA. This problem does not arise at this time for soy, because there is only one transgenic soy event, Roundup Ready, in open, commercial production.

Another factor that influences quantification by ELISA is efficiency of extraction. If the sample and standard reference materials are not ground to the same mesh size and extracted for the same length of time, the transgenic proteins will be extracted with different efficiencies from the reference materials and the sample, making it impossible to make a valid comparison of the two. In summary, due to several confounding factors, the amount of a transgenic protein present in a grain or food is variable and cannot be used as a measure of the proportion of that food which is transgenic. Thus, percent GMO cannot be determined accurately by immunological methods, such as ELISA or lateral flow strip tests.

A similar limitation is apparent in considering processed foods. Proteins, including transgenic marker proteins, are easily denatured during food processing. This either destroys the ability to recognize these proteins with immunological reagents or reduces sensitivity to detection (Lipp et al. 2000, Hubner et al. 1999). Thus, detectability is variable and is process dependent, again compromising the utility of immunological quantification methods. As stated by others (Lipp et al. 2000, Stave 2002), matrix-matched reference materials would be required for valid quantification. Not only would it be necessary to process the standard reference material under conditions identical to those of the sample, but also the proportions of different genetically modified events comprising the standard would have to necessarily match that of the sample. These are conditions that can be fulfilled in only a small fraction of the circumstances where it is necessary to quantify GMO content.

A third limitation of immuno-assays is that the transgenic proteins expressed in some GM crops are not detectable by immuno-analysis. For example, the glyphosate-resistant maize variety GA21 expresses a transgenic EPSPS protein that differs from the native maize EPSPS by only two or three amino acids (Monsanto 1997). The structures of the transgenic and native EPSPS proteins are so similar that all attempts to develop antibodies capable of differentiating the two have been unsuccessful. Thus, to date, no immuno-test exists that is capable of detecting this transgenic event.

Despite limitations, immunological tests serve a useful role. Their application at early stages of the chain is well accepted at this time, especially at points where rapid field tests are needed. For instance, they are often used in checking trucks before they unload their cargoes at grain-handling facilities. The initial results from these tests prevent the introduction of truckloads of maize or soybeans that contain high levels of GM material into silos designated for non-GM products. ELISA is also being used for quantification in situations where economy and convenience are considered more critical than accuracy or where it can be known with confidence that only one event exists that can produce the transgenic marker protein of interest.

Genetic Analysis of GMOs by Using the Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is widely used in genetics-based analysis of GMOs. PCR uses biochemical processes to scan through a sample of DNA and to locate one or more specific DNA sequences, called target sequences. This target sequence is then amplified billions of times, making it possible to detect that target sequence with high sensitivity and also to quantify the proportion of DNA molecules in the sample that contain that target. See Fagan (2003) for a full description of the PCR mechanism.

Because of the powerful amplification that occurs during PCR, this method is highly sensitive. Because the interactions between the primer and target DNA molecules are highly selective, the PCR process is highly specific. A third advantage is that PCR is capable of detecting all GMOs. This is because, even if the transgenic protein is not expressed in the food part of the plant or even if the transgenic protein is indistinguishable from the native protein by immuno-analysis, the transgenic DNA will still be present and can be detected by PCR. A final advantage is that DNA is less subject to denaturation and degradation during food processing than are most transgenic proteins. Thus, even when transgenic proteins have been degraded to the point where immuno-tests are ineffective, PCR analysis can, in most cases, still successfully detect the presence of GM material (Hubner et al. 1999, Jankiewicz et al. 1999).

The robust and versatile nature of this method makes it possible to use PCR to test for the presence of GM material at almost all points in the food chain, from the farmer's field to the consumer's dinner plate. PCR can also be used to quantify GMO content in most food products, including many highly processed foods. The only exceptions are the most highly modified food ingredients, such as certain chemically modified starches, the most highly refined grades of vegetable oil, and highly fermented products, such as soy sauce.

One of the most significant advantages of PCR-based GMO analysis lies in the area of quantification (Hubner et al. 1999, Vaitilingom et al. 1999). The DNA extracted from a sample contains not only the transgene, but also all of the other genes naturally present in the organism. The copy number of each transgene should be invariant in any GMO. Also, the vast majority of endogenous genes of all organisms will be invariant in copy number. The PCR signal derived from a transgene can be used as a measure of the number of GM genomes in the sample. Similarly, the PCR signal derived from a selected endogenous gene (a species-specific reference gene) can be used as a measure of the number of total genomes present in the sample for the species of interest. The ratio of these two signals can be used to accurately calculate the proportion of transgenic genomes—the percent GMO—present in the sample as shown in the following formula:

$$GMO\% = \left(\frac{\text{(Concentration of GMO target sequence)}}{\text{(Concentration of species specific reference gene)}} \right) \times 100$$

This provides a quantitative determination of the percent of GM material present in the sample. In essence, the naturally occurring gene serves as an internal reference point that allows consistent quantification. Immuno-analysis does not make use of such an internal reference and thus fails to provide definitive quantification. Thus, although both immuno-methods and PCR methods can be used effectively to screen for GMOs, PCR is the preferred method when quantification is required.

Because of these advantages, PCR is recognized as the gold standard for GMO testing in Europe and Asia.

Overview of PCR Analysis of GMOs

PCR analysis of GMOs involves five steps: sample preparation, DNA purification, target amplification, detection of reaction products, and interpretation of results.
Sample preparation—For an analytical result to provide meaningful information regarding the original consignment of food, the field sample, drawn from that consignment, must be representative of the consignment as a whole, and the analytical sample, derived from the field sample, must be representative of the field sample.

The first key step is that the field sample must be obtained in a manner that ensures representation from all parts of the lot. Statistical methods are used to define a sampling plan that yields a representative sample. The field sample also must contain a sufficient number of units to ensure that the analysis will be statistically robust at the limits of detection and quantification relevant to the assay. If the sample size is too small, the full power of PCR cannot be exploited.

More specifically, the limit of detection (LOD) for PCR is typically 0.01% or lower. To gain full advantage of an LOD of 0.01%, or 1 part in 10,000 requires that the sample be quite large. For instance, if the true GMO content of a consignment of rice is 0.01%, one must take a sample of 30,000 seeds in order to have 95% confidence that the sample will contain at least one GM rice grain. The probability of picking up at least one GM rice kernel in a sample of, for instance, 1000 seeds would only be 9.5% and the probability for picking up one GM kernel in a sample of 10,000 seeds would only be 63%. For rice, a small seed grain, a sample of 30,000 kernels is not prohibitive, consisting of only 900 g. However, for soy beans, 30,000 seeds would weight *c.*10 kg, and for maize, *c.*12 kg. Thus, sample sizes in this range are on the far outer limit of practicality for most routine applications, except for small grains and for powdered or ground materials, such as soy meal or maize flour.

These examples make it clear that in many cases, the factor limiting the overall sensitivity of GMO detection is not the PCR method, but practical limitations of field sample size.
Sample processing, and the size of the sample taken from the processed and homogenized field sample for DNA extraction and purification (the analytical sample) are also very important in determining whether final analytical results are representative of the original consignment of food. The sample should be finely ground and homogenized to assure that any suitably-sized sub-sample taken from the analytical sample for DNA extraction will be representative of the whole. It is a common error to take sub-samples that are too small to be representative. Typically, samples of 50 mg to 150 mg are used, because this makes it possible to conveniently carry out the

whole DNA extraction procedure in micro-centrifuge tubes. However, empirical studies have demonstrated that samples in this size range fail to yield representative and reproducible results. Only when sample size exceeds 0.5 g to 1.0 g do replicates begin to show acceptable consistency. For routine purposes, samples of at least 2.0 g should be used for DNA extraction of most materials.

DNA Extraction and Purification—To gain reliable and informative results, purification procedures must produce DNA that is free from PCR inhibitors, minimize DNA degradation, and also achieve good yields. Because food products vary tremendously in their physical and chemical compositions, it is essential to customize DNA extraction methods to function optimally for each food matrix. DNA extraction kits purchased from a scientific supply house are unlikely to perform adequately for all sample types. Figure 33.2 compares the performance of a customized system of DNA purification methods, Fast ID, with four kits available in the marketplace today, and with a public domain method, the CTAB method (Murray & Thompson 1980, Scott & Benedich 1988). In this study, DNA was extracted from soybeans and from three soy products. In each case, the kits and methods were used exactly as recommended by their developers. The quality of the DNA prepared using these six methods was then assessed by quantitative real-time PCR. The matrix-specific Fast ID system performed better with all food matrices, but the greatest difference in performance was observed with complex, multi-ingredient products. These were virtually un-analyzable using many of the other methods, while with Fast ID reasonable results were obtained. Similar findings were obtained, as shown in Figures 33.3 and 33.4, where the effectiveness of Fast ID and one commercially available kit were compared for the analysis of other food matrices.

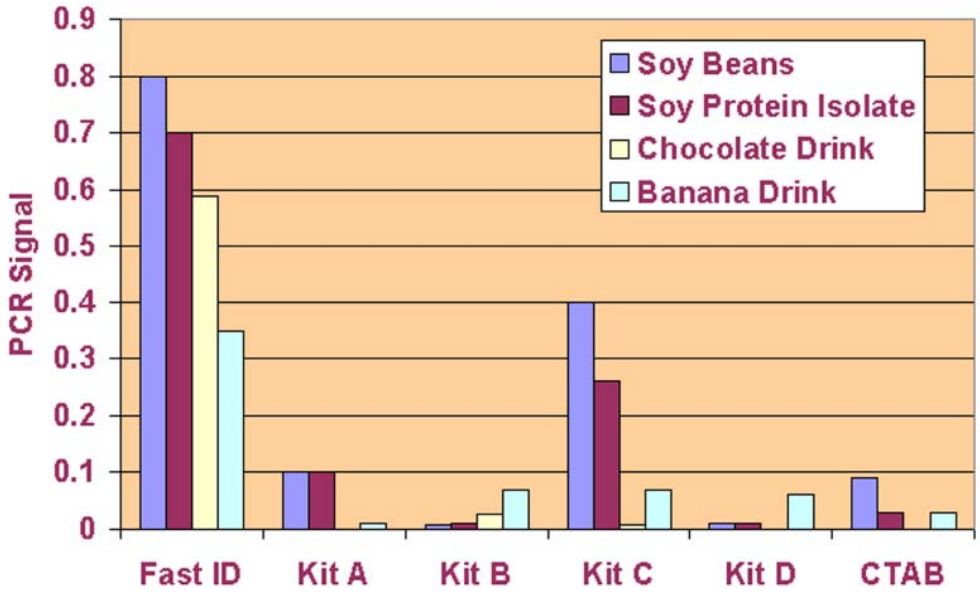

Figure 33.2. Comparison of fast ID DNA extraction with commercial kits and CTAB – real-time quantitative PCR analysis

DNA was prepared from four food samples using the Fast ID method, four DNA extraction kits that are commonly used for analysis of genetically modified foods and agricultural products, and the public domain CTAB method. The quality of the DNA was assessed by real-time PCR. A standard amount of DNA (50 ng, quantified by absorbance at 260 nm) was introduced into each PCR reaction. PCR signals are reported relative to signals obtained with a standard of highly purified soy DNA. Somewhat reduced signals for chocolate and banana drinks for Fast ID are not due to the presence of inhibitors, but to the presence of DNA from other species, derived from other ingredients in these samples.

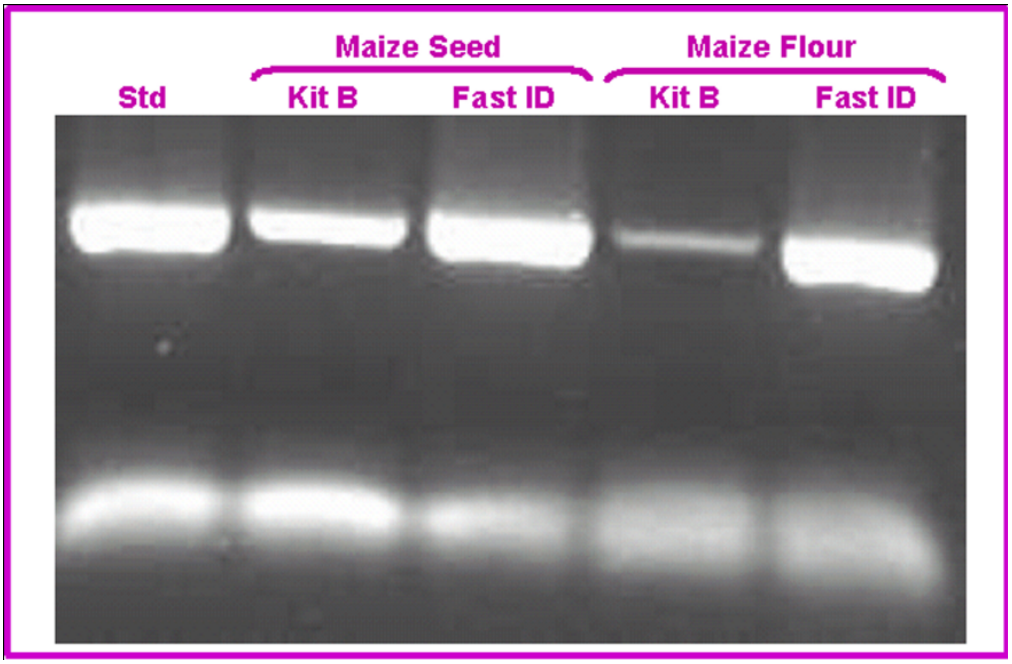

Figure 33.3. Comparison of Fast ID DNA extraction with a commercial kit – analysis by conventional PCR

DNA was prepared from food samples using the Fast ID method and another commonly used DNA extraction kit. The quality of the DNA was assessed by conventional PCR. A standard amount of DNA (50 ng, quantified by absorbance at 260 nm) was introduced into each PCR reaction.

Figure 33.4. Comparison of Fast ID DNA extraction with a commercial kit – analysis by conventional PCR DNA was prepared from food samples using the Fast ID method and another commonly used DNA extraction kit. The quality of the DNA was assessed by conventional PCR. A standard amount of DNA (50 ng, quantified by absorbance at 260 nm) was introduced into each PCR reaction.

PCR Amplification—The ability to amplify a target sequence billions of times is the basis of the sensitivity of the PCR method. PCR is an exponential amplification process. Each cycle doubles the number of target molecules. If one starts with one target molecule, completion of one PCR amplification cycle yields two molecules. In two cycles, 4 molecules, and in 3 cycles, 8 molecules are generated. Ten cycles will generate more than 1,000 copies, and 20 cycles, more than 1,000,000 copies, and so on. If the quality of the DNA preparation is good, between thirty and forty cycles of PCR amplification are more than adequate to yield signals that are easily observed even if the original target sequence is present in only one or a few copies. With this method it is possible to routinely detect the presence of GM material at concentrations well below 0.01%.

Sample size is equally important when taking samples of the DNA extract for PCR analysis. For instance, if the true GMO content of a lot of maize is 0.01%, and proper sampling had been done at both the field sample and analytical sample levels, one should have a DNA preparation whose GMO content is very close to 0.01%. When one takes a sample of 200 ng from this DNA preparation, it will contain approximately 77,000 copies of the haploid maize genome. Using the normal approximation to the binomial distribution, the probability is only 87% that such a sample will contain ± 50% of the true value. That is, the probability is only 87% that the actual GMO content of the sample will be between 0.005% and 0.015%. The limit of detection (LOD) is defined as that concentration of analyte that can be detected with 95% confidence. In the present case, the probability of detection is only 87%, thus, 0.01% is below the LOD. We can calculate that the actual LOD for this sample size would be 0.02%. Thus, despite the fact that the PCR amplification process is fully capable of detecting 0.01%, the limited number of genome copies in the DNA sample subjected to PCR analysis significantly reduces the LOD of the over-all analytical process. In order to achieve a LOD of 0.01%, a sample of 400 ng maize DNA would be required, which is very high, in fact, inappropriately high, except for the most pure DNA preparations.

It is clear from the literature, that most methods employ DNA sample sizes in the range of 50 to 150 ng of maize DNA. For samples in this size range, sample size, not the inherent properties of

PCR amplification, is the limiting factor in determining the LOD of the over-all analytical method. Fortunately, the maize genome is exceptionally large. For a grain such as rice, which has a genome only 17% the size of the maize genome, a sample of 200 ng is quite adequate to achieve detection at the 0.01% level.

Detection of PCR Reaction Products—The products of the PCR amplification process can be detected by several different methods. One of the most common is electrophoretic analysis, where the amplified DNA molecules are resolved into, and appear visually as, distinct bands on an agarose or acrylamide gel, when stained with a fluorescent dye. The other common method is fluorimetric analysis, where the PCR process is modified to generate fluorescent products which are detected in proportion to the number of amplification events that take place. This method is the basis of real-time quantitative PCR technology. Figure 33.2 illustrates the kind of quantitative data that are obtained when fluorimetric analysis is used as part of real-time quantitative PCR. Figures 33.3 and 33.4 illustrate the results of electrophoretic analyses of conventional PCR products.

Applying PCR to the Analysis of Food Samples for GMO Content

For GMO detection, PCR can operate either (a) qualitatively, or (b) quantitatively; and can either (c) target many different varieties (events) of GMOs, or (d) selectively target a single transgenic event. Table 33.1 summarizes the specific analytical questions addressed by each of these four categories of methods.

Table 33.1. Classification of GMO testing methods.

	Qualitative Method	**Quantitative Method**
Broad Spectrum Primers	Broad-spectrum Screening: Is any genetically modified material present in the sample?	Rough Quantification: Approximately how much genetically modified material is present in the sample?
Event-Specific Primers	Event-/Variety-Specific Detection: Specifically which GMO(s) is (are) present in the sample?	Precise Quantification: How much of a particular GMO is present? (one primer set) How much total GMO is present? (total measurements for all GMOs in the sample)

Regardless of whether a GMO analytic system is qualitative, quantitative, broad-spectrum, or event-specific, the basis of all such systems is a core set of PCR reactions that employ primer sets specific for the GMO(s) of interest. There are several additional design elements, controls, and reference reactions that are common to all analytical systems and are designed to achieve the following objectives:

Detection of inhibitors that reduce or block the PCR process.

- Assessment of the degree to which the sample DNA is degraded.
- Verification that the PCR reagents and equipment are functioning properly and that PCR amplification actually occurs.

- Determination of the limit of detection and/or limit of quantification of the PCR method, and confirmation that the PCR process is operating at a consistent level of sensitivity from PCR run to PCR run.
- Confirmation of positive and negative results.

In addition, a practical analytical method that includes all of these design elements must be an integral part of a comprehensive quality assurance/quality control (QA/QC) system in order to achieve the degree of reliability and consistency that is necessary for the analytical system to be of practical utility for industry or government regulatory bodies.
The following sections discuss the four classes of PCR systems used in GMO analysis, and the required assay design features for each, as well as how they can be used within a QA/QC system to assure reliable GMO analysis.

Qualitative PCR and Illustration of Basic Controls Required for All PCR Analyses

A diagrammatic example of electrophoretic analysis of PCR reaction products generated from two food samples is presented in Figure 33.5. Six separate PCR reactions were run for each sample, duplicate reactions with each of three distinct primer sets. These are presented in lanes 1 through 12 of the figure. Lanes 13 through 26 present PCR reactions run with reference DNA samples (lanes 13, 14, and 19 through 26) and no DNA (lanes 15 through 18).

The first primer set recognizes an internal control DNA preparation. These reactions are used as an indicator as to whether or not PCR inhibitors may be present in the sample DNA preparation. If inhibitors are not present the intensity of the electrophoresis bands from the control reactions (lanes 1 & 2 and 7 & 8) will match the intensity of the internal standard control (lanes 13 &14). The intensity of the bands in lanes 1 & 2, and 7 & 8 indicate that no inhibitors are present in either DNA sample #1 or #2.

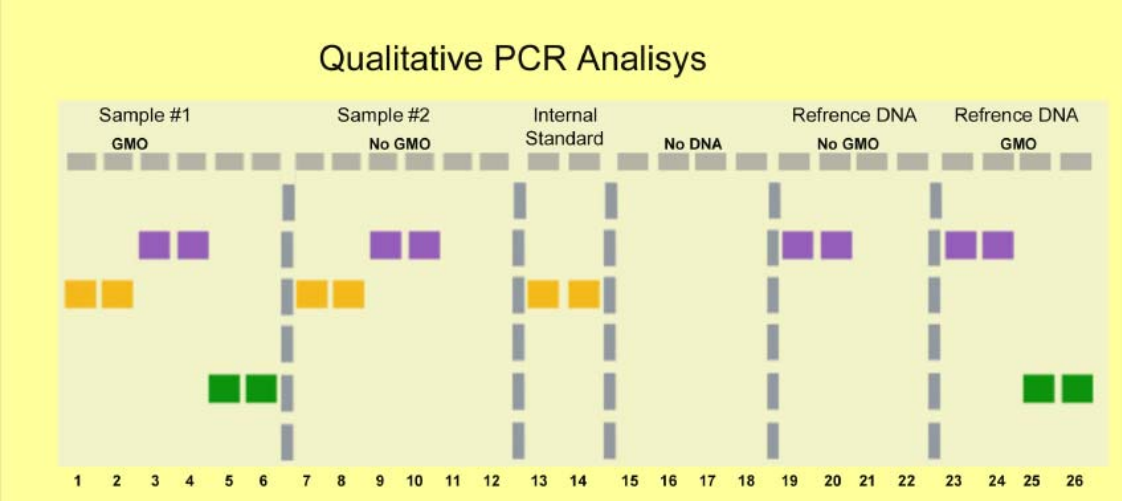

Figure 33.5. Typical configuration for GMO analysis by PCR
Reactions 1 & 2 and 7 & 8 contain sample DNA, internal standard DNA, and primers for internal standard DNA. The products of these reactions are compared to reactions containing internal standard DNA and primers for internal standard DNA only (lanes 13 & 14). If the intensity of bands is comparable, then it implies that the sample DNA does not contain compounds that inhibit the PCR process.
Reactions 3 & 4 and 9 & 10 contain sample DNA and primers for a gene common to all varieties of the genetically modified crop of interest (e.g. soy). The intensity of the bands produced in these reactions is compared to the corresponding standards (lanes 19 & 20 and 23 & 24). Weak or absent bands would imply

poor recovery of sample DNA or degradation of that DNA. Absence of bands could also imply that the PCR system was not functioning properly due to inhibitors or faulty reagents or equipment. These alternatives can be sorted out by comparison of these results with those for Reactions 1 & 2 and 7 & 8.
Reactions 5 & 6 and 11 & 12 contain sample DNA and primers specific for a genetically modified sequence present in the GMO(s) of interest. The intensity of the bands for this primer set is compared to reactions containing non-GMO and GMO DNA, run with the same primer set (lanes 21 & 22 and 25 & 26). Absence of signal in lanes 21 & 22 is expected, implying that the primer set does not interact with sequences in the non-GM DNA, and is, therefore, specific for the GM sequence of interest. Presence of signal in lanes 25 & 26 is expected, implying that the primer set is effectively detecting the GM sequence of interest in the GMO DNA. The results indicate that Sample #1 contains the GMO of interest, while Sample #2 does not.
Duplicates – All analyses are carried out in duplicate, beginning with duplicate sub-samples of the ground and homogenized food. Two independent DNA preparations are made from these food samples and are carried through PCR independently. It is not sufficient to run duplicate PCR reactions from a single DNA preparation.

The principle behind the internal standard is illustrated in more detail in Figure 33.6. In Figure 33.6, no inhibitor is present in the first reaction, but is present in the second (indicated in red). The intensities of the bands corresponding to the first reaction are equal to those obtained when the internal standard is run alone (far right), while the intensities of the bands corresponding to the second reaction are much less than those of the internal standard when run alone. This is due to the effects of the inhibitory molecules (red) present in the second reaction.

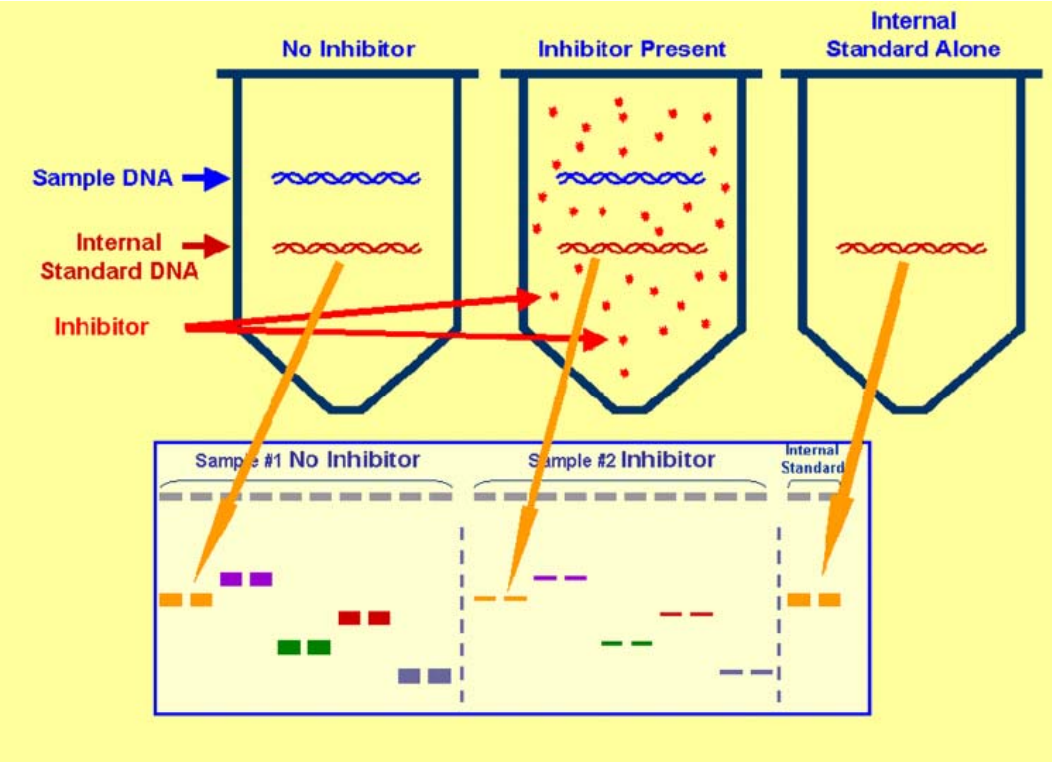

Figure 33.6. The internal standard – A control for the presence of PCR inhibitors
A defined concentration of a known target DNA molecule (internal standard template) is added to all three reaction tubes (red DNA molecule). When this template is amplified in the presence of a DNA sample that is free from inhibitors (left-hand tube) the resultant signal is equal to that obtained when the internal standard template is amplified alone (right-hand tube). In contrast, amplification of this template in the presence of a sample that contains inhibitors (centre tube) results in reduced signal intensity.

The second primer set used in the analysis presented in Figure 33.5 is designed to determine whether the DNA sample is intact and free from degradation. However, results with this primer set must be considered in light of the results obtained with the first primer set, since inhibitors will influence the reactions with the second primer set, as well as those with the first. This primer set is specific for a reference gene relevant to the species of interest. This is termed the species-specific reference gene primer set. For example, to test for GM soy, a primer set would be selected that targets a gene known to be present in all soy, whether transgenic or conventional. Many laboratories use a primer set specific for the soy lectin gene for this purpose. The reaction products generated with samples #1 and #2 using this primer set (lanes 3 & 4, and 9 & 10) are compared to those in lanes 19 & 20, and 23 & 24. If the sample DNA is free from degradation, then the intensity of the bands generated from those DNA preparations (lanes 3 & 4, and 9 & 10) will correspond to the intensity of the bands generated in reactions containing reference DNA preparations (lanes 19 & 20, and 23 & 24).

The principle behind the species-specific reference gene control is illustrated in more detail in Figure 33.7, where it is shown that partly fragmented DNA (right-hand reaction tube) results in weaker PCR bands than intact DNA (left-hand reaction tube).

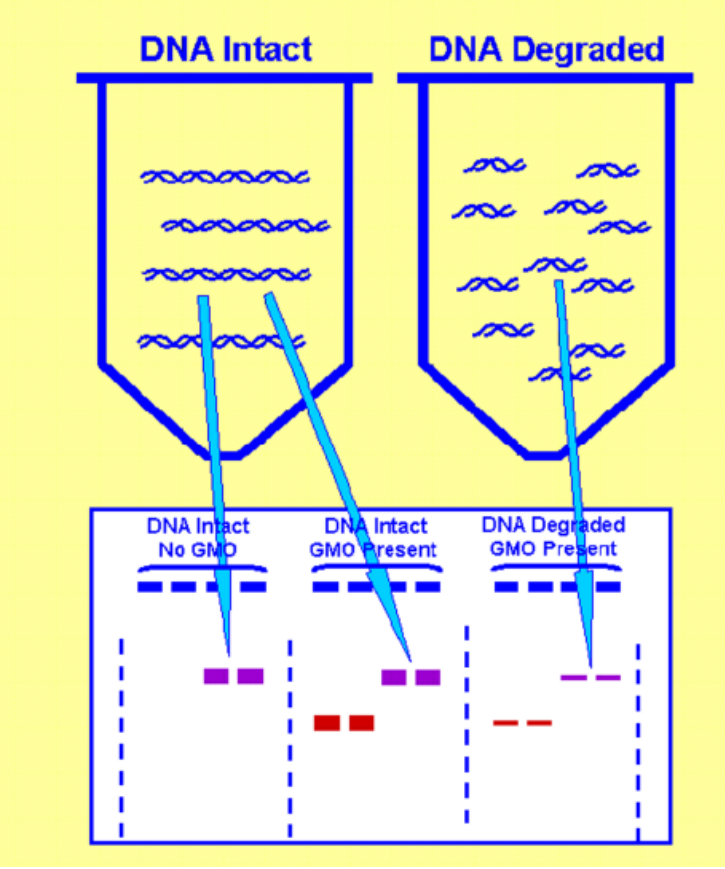

Figure 33.7. Positive control using primers specific for a species-specific reference gene – A control for DNA degradation and reduced DNA recovery
A primer set targeting a gene common to all varieties (conventional or transgenic) of the crop species of interest can be used to assess the integrity of the sample DNA. Both DNA degradation and presence of PCR inhibitors will reduce the signal generated from this primer set. By using the test described in Figure 33.5 to detect inhibitors, the contribution of DNA degradation can be understood.

The final primer set used in Figure 33.5 detects a specific transgenic sequence. The intensities of the signals generated for samples #1 and #2, using this primer set (lanes 5 & 6, and 11 & 12), can be compared to the respective PCR reactions with external standard DNA (lanes 21 & 22, and 25 & 26). This comparison indicates that sample #1 contains GM material, while sample #2 does not.

A successful PCR analysis requires that all duplicates agree, that the no-DNA controls display no bands (lanes 15 through 18), that the controls with both GM and non-GM reference DNA preparations be consistent with the expected characteristics of the primers (lanes 21 & 22 no signal, all other lanes positive), and the internal control reactions (lanes 1 & 2, 5 & 6, and 13 & 14) must all be positive and of roughly equal intensity.

This example illustrates that 20 data points must be considered in determining the GMO content of any given sample: a total of 6 PCR reactions are carried out with DNA derived from the sample, and 14 additional reactions are carried out with reference DNA preparations.

Safeguards to Ensure Reliable PCR Results

Incorporated into the procedure illustrated in Figure 33.5, are eight elements designed to assure the accuracy and consistency of results. These include the following:

1. An internal standard primer set and template are incorporated into the assay to test for the presence of PCR inhibitors in the DNA preparation.
2. A positive control primer set that recognizes a species-specific reference gene is used to assess whether the DNA is intact and free from degradation (also influenced by inhibitors).
3. Each sample is analyzed in duplicate from start to finish. These duplicates do not originate at the PCR stage of the analysis. Instead, duplicate analytical samples are taken from the homogenized field sample and processed in parallel throughout the whole analytical procedure.
4. A set of external reference DNA preparations is employed to verify the sensitivity of the method and to provide evidence that the PCR system is operating properly.
   A number of other measures that are not apparent from this example are also essential to reliable PCR analysis:
5. The PCR reaction conditions and the primer sets used must be optimized for sensitivity and specificity.
6. DNA purification procedures must be optimized for each food matrix to ensure freedom from inhibitors and to minimize degradation.
7. To reduce the risk of cross-contamination, the laboratory must be organized such that the steps of analysis are physically and operationally isolated.
8. Stringent quality control measures must be implemented for all analytical procedures, all reagent preparation, and for data analysis and reporting of results.

When these or equivalent measures are employed, highly accurate and consistent results can be obtained. For instance, Table 33.2 summarizes the results from a ring trial conducted by the Joint Research Centre of the European Commission in 1998 (Scott & Benedich 1988). In this part of the study, the frequency of false positives was 2.1%. The frequency of false negatives was 5.1% for samples that contained 0.1% GM material, but no incorrect results were reported for samples containing 0.5% or 2% GM material. For both false positives and false negatives, incorrect results were reported by only 2 of the 25 laboratories. Thus, the vast majority of laboratories performed perfectly on all analyses.

Table 33.2. Reliability of GMO analysis by PCR.

Actual GMO Content	0.00%	0.10%	0.50%	2.00%
Samples Reported Negative	94	5	0	0
Samples Reported Positive	2	93	105	101
% Samples Reported Correctly	97.9	94.9	100	100
% False Positives	2.1			
% False Negatives		5.1	0	0
Labs Making Errors	2	2	0	0
Labs Performing Without Error	23	23	25	25

1998 Ring Trial Conducted by Joint Research Centre, European Commission

Assay Design Features to Confirm Results

When putatively positive results are obtained in PCR analysis of GMOs, it is necessary to carry out confirmatory analysis before regulatory or other action is taken. The following two approaches are recommended by Swiss and German law (Schweizerisches Lebensmittelbuch 1998, German-Federal-Foodstuffs Act 1998), (a) Southern hybridization of PCR products to a probe known to be homologous to the bona fide amplicon of interest, or (b) cleavage of the PCR products into fragments of expected size using restriction endonucleases.

Real-time PCR analysis, using TaqMan, minor groove-binding, Molecular Beacon, and FRET (fluorescence resonance energy transfer) probes, has built into it a third option for confirmation that is equivalent to Southern hybridization. The probes used in these real-time methods hybridize to the sequences within the amplicon, and a signal will not be generated unless both the primers and the probe are homologous to the target. Thus, hybridization of the probe confirms that the amplicon amplified possesses the sequence of the bona fide target. The requirement that the probe must hybridize to the target sequence provides an additional level of confirmation equal in specificity and stringency to the requirement that amplicons hybridize to a Southern blot or be cleaved by a restriction enzyme into fragments of predicted size. Thus, generation of a real-time signal inherently and automatically confirms the identity of the amplicon.

Although these methods verify the identity of amplicons, they do not differentiate between (a) amplification of the bona fide target sequence present in the genome of the sample and (b) amplification of amplicons from another PCR reaction that might have contaminated the sample. This possibility is very real, because such amplicon contamination is the most common form of contamination in the PCR laboratory. To gain additional confidence that the putative positive result is not due to contamination of the sample with amplicons, multiple primer sets are advantageous as a routine part of analysis. With this approach, results obtained with one primer set are confirmed when amplification is also observed with a second primer set that targets a second, independent site that will be present if and only if the site targeted by the first PCR amplification is actually present. This is a stronger method of confirmation than simply confirming the sequence of amplicons. It constitutes a true, independent confirmation that the sequence of interest is actually present in the DNA of the sample.

Simultaneously running PCR analyses with two primer sets, both of which independently recognize separate domains within the sequence of interest, can be used as a strategy for accelerating delivery of final analytical results. In this case, confirmation is achieved simultaneously with the initial observation of positive results, instead of carrying out amplification with one primer set, and then carrying out a second series of reactions with a

second confirmatory primer set. In addition, the use of multiple primer sets provides greater certainty in avoiding false negatives as well as false positives.

Real Time Quantitative PCR

The existence of thresholds for the GMO content of products, whether mandated by government regulation or by contractual agreement between buyer and seller, necessitates methods for quantifying GMO content of foods and agricultural products. This need has triggered a move to methods that offer increasingly more robust quantification. Real-time quantitative PCR methods are currently the methods of choice (Hubner et al. 1999, Vaitilingom et al. 2000).
While conventional PCR measures the products of the PCR reaction at a single point in the reaction profile, real-time PCR methods generate fluorescent reaction products that can be monitored continuously to follow the time course of several PCR reactions simultaneously (See Fagan (2003) for fully a referenced discussion of real-time PCR methodology). The basis of this approach is the linkage of PCR amplification to the generation of one fluorescent reporter molecule for every amplicon that is generated during PCR. For Taqman technology, this occurs through the use of a fluorescently labeled probe that anneals between primer recognition sites. Taq polymerase has an exonuclease function in addition to its DNA polymerase activity, and during strand elongation, the fluorescence-labeled probe is cleaved from the oligonucleotide allowing it to produce a fluorescent signal that is proportional to the number of amplicons generated during the reaction. With this method, the complete reaction profiles for as many as 96 samples can be determined simultaneously.

A typical series of real-time PCR reaction profiles is presented in Figure 33.8. By comparing the profiles of a sample of unknown GMO content with those of a series of standards of known GMO content, it is possible to quantify with reasonable accuracy the GMO content of the unknown. This is illustrated in Figure 33.9, which shows that, when the log of GMO content of a series of standards of known GMO concentration is plotted against the number of PCR cycles required to generate a certain threshold of fluorescent products (indicated by the orange line in Figure 33.8), the GMO content of a sample of unknown GMO content can be deduced based upon the number of cycles required to generate that same level of fluorescent products from a series of samples of known GMO content (assuming uniform DNA input in all reactions). An alternative, but less rigorous, approach to quantification by real-time PCR is to run only one concentration of the GMO reference DNA, and generate the standard curve using this one point and assuming an ideal slope of -3.33.

Real-time PCR provides reasonably good quantification over four to five orders of magnitude, and for a sample containing 1% GM DNA precision of analysis should generally be in the range of ± 20%.

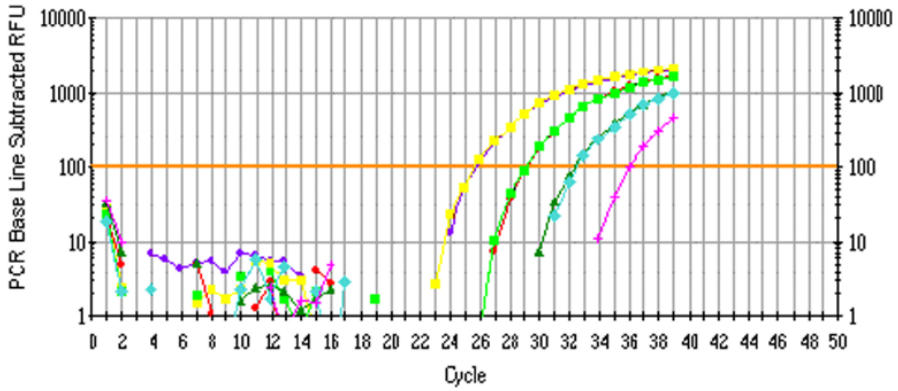

Figure 33.8. Real-time quantitative PCR analysis
Examples of fluorescence profiles generated during real-time PCR, plotted as log fluorescence signal (arbitrary units), versus PCR cycle number. The number of cycles required to generate the threshold level of fluorescence indicated by the orange line is proportional to the log of the initial concentration of the target sequence in the sample. In principle, the threshold specified by this line can be set at any point within the logarithmic portion of the fluorescence profile.

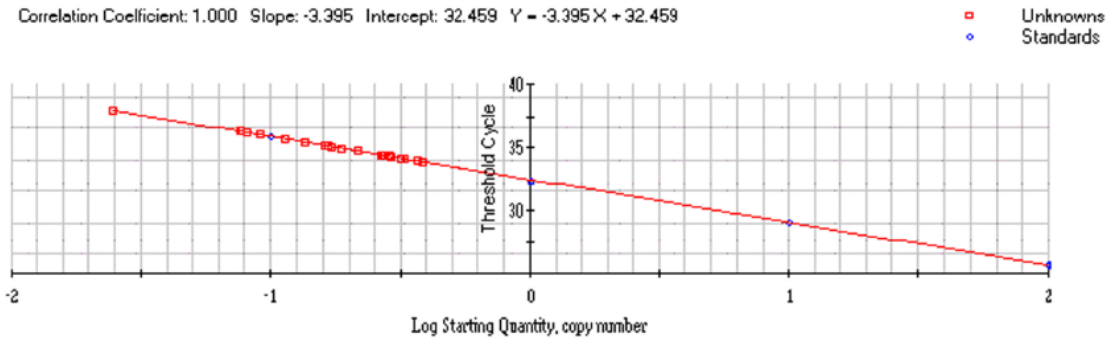

Figure 33.9. Quantification by real-time PCR
When the number of PCR cycles required to generate a fluorescence signal corresponding to the threshold value specified by the orange line in Figure 33.8 is plotted against log of the initial concentration of GMO target sequences in a series of standards (blue circles), a line with slope close to -3.33 is obtained. The GMO concentration in samples of unknown concentration (red squares) can be determined from this plot, based on the number of PCR cycles required for the sample to achieve the fluorescence threshold corresponding to the orange line in Figure 33.8.

The critical design features of a real-time PCR assay for GMOs are similar to those for qualitative PCR in many respects. These include an internal control reaction series, a species-specific reference gene reaction series, and one or more GM-specific reactions series. Some laboratories replace the internal control reaction series, designed to assess the presence/absence of PCR inhibitors, by another approach that assesses the presence of inhibitors through a series of PCR reactions that contain successive dilutions of sample DNA. The cycle number required to reach threshold is compared for PCR reactions containing (a) undiluted sample DNA, (b) sample DNA diluted 1:2, and (c) sample DNA diluted 1:4. If no inhibitors are present, each 1:2 dilution should increase by one the number of cycles required to achieve the threshold. If inhibitors are present, little increase in cycle number, or a non-integral increase will be observed. This dilution approach only provides a qualitative measure of inhibition and, therefore, is not considered as rigorous as

the use of an internal control reaction series, using which, the extent of inhibition can actually be quantified.

Inhibition is generally not a large problem due to the development of efficient DNA purification procedures that remove inhibitors from virtually all sample types, including highly complex multi-ingredient products. The greater limitation to reliable quantification is recovery of sufficient PCR-active DNA to enable quantification in cases where sample DNA is partially degraded. Scaling up purification may improve recovery by 5- to 20-fold; however, some sample types have undergone such extensive processing that it is impractical to recover sufficient intact DNA for a full quantitative analysis. Recoveries of DNA from highly processed materials are highly dependent on the batch of material and cannot be predicted before analysis is carried out. In cases where recoveries are not sufficient for full quantitative analysis, results must be reported in qualitative format only.

In addition to controls for inhibitors and DNA degradation, one must also include controls verifying that the PCR system is operating properly, as well as controls defining and verifying the limit of detection of the method. Analyses must also be run in replicate to verify that analytical results are repeatable and meet pre-established criteria for precision of analysis.

Screening versus Event-specific PCR Analysis

Primer sets that are complementary to DNA sequences unique to a single GMO make it possible to detect specific transgenic crop varieties or events. Such event-specific, or Varietal ID^{sm}, methods can specifically and unambiguously identify each transgenic soy, maize, potato, rice, cotton, etc. variety commercialized to date.

Generally, the importation of GM food or feed is contingent upon approval of these products for specific uses. National and regional differences in approval status for a given GM crop can create substantial challenges for import. Table 33.3 illustrates this situation in the case of maize. The two right-hand columns list all of the transgenic corn events or varieties that have been approved for cultivation in the U.S.A. Of these, the top 15 have actually been produced commercially on a large scale. Of these, four are no longer in commercial use (indicated by ‘terminated’). The remaining 10 events have been authorized, but never commercialized. To the right is the approval status of these products for human use in the EU and Japan, as examples of international markets. All of the currently commercialized events have been approved in Japan, but three of those events have not been approved in the EU.

Differences in approval status create a problem for grain exporters attempting to move maize or maize products into various markets. Not only is it necessary to determine whether GM material is present in order to comply with labeling regulations in these countries, but it is also necessary to insure that a given lot of product does not contain varieties or events that have not been approved for food use in the specific country of import.

The current status of labeling regulations in the European Union (EC Council Regulation 1830/2003, (European Commission 2003) exemplifies the situation encountered in many parts of the globe. Products of unknown composition or products that contain greater than 0.9% GM material must be labeled with a phrase such as ‘Produced through gene modification’. Products containing less than 0.9% GM material do not require labeling, as long as the producer can provide strong traceability documentation demonstrating that positive efforts were taken to avoid GMO admixture. For events not yet approved, but engaged in the approval process and having received a ‘favorable’ safety assessment, there is a transitional threshold (applicable for three

years after the date of application of the Regulation) of 0.5%, whereas, for other unapproved transgenic events, there is zero-tolerance. Thus, importers must demonstrate the absence of GM events that have not been approved for food use in Europe. Event specific methods are designed to provide this key information.

Each event-specific primer set defines an amplicon that spans a sequence junction unique to the transgenic event of interest. These are sites where two sequence elements have been joined in a manner unique to that event. Thus, a positive signal with such a primer set is definitive evidence for the presence of the respective event in the sample of interest.

Figure 33.10 is an example of a recombinant gene that has been created by splicing together five different pieces of DNA, from five different sources. The green bars, which flank sequences 1 and 5, represent maize genomic DNA sequences. The recombinant gene was inserted into a unique site within the maize genome, thus the sequences found at the junctions of the recombinant gene and these flanking sequences are unique to this transgenic event.

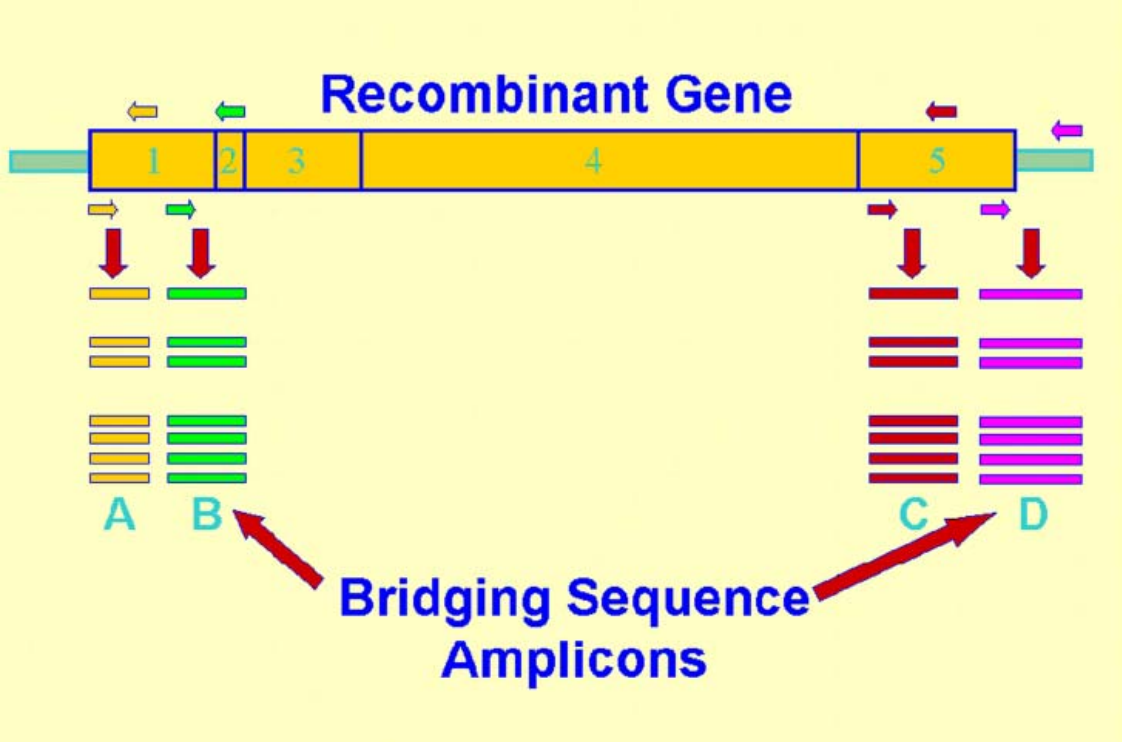

Figure 33.10. Bridging primers allow definitive detection of specific transgenic events
Primer sets B and D bridge junctions between two sequence elements. By selecting primer sets that bridge junctions that are unique to a given transgenic event or variety an assay can be developed that is highly specific for the detection of a single transgenic event. Primer set B bridges the junction between two internal elements in the transgenic construct, allowing construct-specific detection, while primer set D bridges the junction between the transgenic construct and the host genome, allowing event-specific detection.

We show four different primer sets that could be used to detect this gene. Primer sets A and C detect sequences that are wholly within a single sequence element of the transgene. As a consequence, they are unable to distinguish between different transgenic events that contain these genes.

Primer set B, however, consists of one primer that recognizes sequence 1 and another that recognizes sequence 2. These two sequence elements do not exist adjacent to each other in nature, so the only case in which a PCR product will be made from this primer set will be when sequence elements 1 and 2 are juxtaposed, as is the case in the recombinant gene shown in Figure 33.10. Thus, this primer set is specific for DNA isolated from any recombinant organism transformed with this particular recombinant DNA construct. Such a primer set B is termed 'construct-specific.'

In contrast to primer set B, primer set D includes one primer specific for sequence 5 and one specific for the maize genomic sequences flanking the recombinant gene. Because currently available methods insert transgenes randomly into the host genome, the host genomic sequences flanking a transgene will be different in every transformation event. Thus, primer set D will be capable of amplification only when presented with genomic DNA from the transformation event depicted in Figure 33.10. Other transformation events will result in other sites of insertion and their detection will require that the primer specific for sequence 5 be paired with a different primer, which would be homologous to the maize genomic sequences flanking the recombinant gene in that particular event. Thus, primer set D is truly 'event-specific'.

In developing event-specific methods, the specificity of each event-specific primer set must be verified by running PCR reactions containing that primer set and containing DNA isolated from all commercialized transgenic events for that species, as well as reactions containing DNA from all common food plants and gene modification events of other species. It is also important to sequence the event-specific amplicons to verify that the correct, expected transgenic sequences are, in fact, being amplified.

The use of event-specific primer sets is the only approach to definitive quantification of GMO content. The typical approach to quantification that is carried out in GMO analytical laboratories around the world is to quantify based on analysis using one or more broad spectrum primer sets that recognize common transgenic elements, such as the CaMV 35S promoter, the nos terminator, the Cry 1Ab gene, etc. Because these elements are present in different copy numbers in different transgenic events, as illustrated in Table 33.4, they cannot be used for accurate quantification of percent GMO in samples in which more than one event is, or may, be present. Since complex mixtures that contain multiple GMOs in unknown proportions are not the exception but the rule for real-world samples, such broad-spectrum primer sets seldom provide accurate quantification. Only event-specific PCR analysis is capable of providing definitive GMO quantification: event-specific primers can be used to achieve accurate quantification based on definitive quantification of each individual transgenic event present in the sample.

Table 33.4. Copy Number of Common Transgenic Crop Sequences

Variety	35S-P	35S-T	nos-T
Maize			
Mon 810	1	0	1
Bt 11	2	0	2
Mon GA21	0	0	2
Aventis T14	3	3	0
Aventis T25	1	1	0
Aventis CBH-351	4	1	4
DeKalb DBT418	3	0	0
DeKalb DLL25	1 inc.	0	0
Event 176	2	0	2

Soy			
Mon 40-3-2 (RR)	1	0	1
Aventis A5547-127	1	1	0
Aventis GU262	2	2	0
Aventis A2704-12	2	2	0
Aventis A5547-127	1	1	0
Dupont A2396	1	0	1

Maintaining Uniformly High Standards of Performance in the GMO Testing Laboratory

The following section is based on Genetic ID's experience in maintaining the uniformity of quality of GMO analytical services within the laboratories of the Global Laboratory Alliance, which includes three laboratories that Genetic ID owns and operates, in the USA, Germany, and Japan, and 18 additional private and government laboratories around the world that have licensed Genetic ID's GMO testing technology.

Standard operating procedures—To standardize GMO testing and ensure consistency and quality, a thorough and comprehensive system of standard operating procedures (SOPs) is essential. This system must be well embedded within a document control system so that all amendments to methods, and the incorporation of new methods, occur in a uniform, orderly way. It is essential that all individuals that use the methods receive all changes at the same time, so that uniformity can be maintained. SOPs should be established not only for laboratory procedures, but also for analyzing data and reporting results. It is critical that the uniformity of the entire analytical process be maintained.

Laboratory performance assessment—Performance assessment programs or proficiency testing schemes are required to ensure that all procedures are performed consistently, correctly and accurately. Typically, both internal and external performance assessments are conducted. The internal program introduces into the analytical stream of the laboratory blind 'check samples' at a frequency proportional to the total number of commercial samples of that kind analyzed by the laboratory. A range of sample types and analysis types are included in the check sample program, thereby assessing the entire scope of methods used in the laboratory. Technicians should not be aware of which are authentic samples and which are check samples. Results of the check sample program must be reviewed and audited on a regular basis and results shared with both technicians and with management as part of the ongoing quality improvement program of the laboratory. The laboratory should participate in external performance assessment schemes on a frequent basis. Several organizations offer such programs at this time, including the US Department of Agriculture, the UK Food Analysis Performance Assessment Scheme, the American Oil Chemists Society, and the American Association of Cereal Chemists.

Laboratory accreditation—The most widely accepted standard for analytical laboratories is ISO/IEC 17025 (International Organization for Standardization 1999). Regular on-site evaluation of the analytical laboratory by an independent third party accreditation body to verify that the laboratory is operating in compliance with this or an equivalent standard is very important. Accreditation to ISO 17025 includes: (a) inspection of laboratories on a yearly or appropriate basis; (b) evaluation of the laboratory's quality system and technical operations; (c) evaluation of all quality documentation; and (d) evaluation of extensive validation data for each analytical method that is to be included within the scope of accreditation. Accreditation of each method is laboratory-specific and thus a method must be validated independently in each laboratory in which it is used.

The credibility of accreditation is highly dependent on the level of acceptance and recognition of the accreditation body. For instance, reciprocity has not been established between EU and US accrediting bodies. Thus, if a laboratory wishes to provide testing services in Europe, it is prudent to undergo accreditation by an EU-recognized accreditation body.

International standardization of GMO testing methods—Laws and international accords requiring the monitoring of GMOs released into the environment and the labeling of foods consisting of, or containing ingredients derived from, GMOs have created the need for standardization of methods for analysis of GMOs.

The standardization of methods for GMO testing has not progressed at the same pace as the introduction of GMOs into the food system and the enactment of labeling laws. Japan (Japanese Ministry of Health Labour and Welfare 2002), New Zealand (New Zealand Ministry of Agriculture and Forestry 2002), Germany (German-Federal-Foddstuffs-Act 1998), and Switzerland (Scheweizerisches Lebensmittelbuch 1998) have all established official testing methods for some GMOs. Unfortunately, no country has established a comprehensive set of testing methods nor a system for updating methods to assure that they cover all GMOs currently in the marketplace. A global unification and standardization of GMO testing methods needs to be achieved to properly service the food and agricultural industries, which are global in nature. GMO testing services must be available globally that are consistent, reproducible, and reliable in sensitivity, specificity, and accuracy. Only then can exporters be confident that the products released on the basis of test results from one port, will be found acceptable when tested by the buyer in a distant port on the other side of the globe.

Several initiatives are in motion to develop, standardize, and validate methods. Most prominent are the CEN and ISO efforts. In Europe, a network of official government reference laboratories for GMO testing, known as the European Network of GMO Laboratories (ENGL), has been established by the Joint Research Centre of the European Commission. This is a group of more than 50 official control laboratories, each appointed by the national authority of the corresponding EU member state. One of the objectives of this network is to develop and standardize testing methods that will respond to testing needs evolving out of EU legislation on GMO labeling. There are also initiatives in progress within various industries, including the seed, tobacco, and cereals industries.

It is important to note that the aforementioned initiatives will all require years to achieve completion. In the meantime, the food and agricultural industries must find interim strategies to assure consistent compliance with labeling laws and to provide products that are responsive to consumer's expectations. One initiative that is designed to address this need is the Global Laboratory Alliance. This network of more than 18 laboratories from around the world has methods for all commercialized GMOs, and is already operating to uniform standards, with a quality assurance system in place to maintain compliance and assure consistency in testing globally.

Future Technologies

PCR and immunological methods are both too limited to deal with future genetic analytical needs in the food and agricultural industries. We will need technologies that can analyze hundreds of genetic targets simultaneously, quantify accurately, and are highly sensitive. Ideally, these methods should be rapid, inexpensive, effective with diverse food matrices, and field operable. These requirements cannot be fulfilled by either PCR or immunological methods, nor do presently available alternatives meet this need. For example, near infra-red spectroscopy (NIR) is

fast and inexpensive, and can detect GM grain or soybeans, if relatively high levels are present (Hurburgh et al. 2000). However, this approach is not sufficiently sensitive, and its discriminative capability is not sufficient. Moreover, it is not universal in its applicability. Another approach that has been explored, DNA microarrays (Grohmann 2002), is useful for gene discovery, but is not well adapted to GMO analysis. Microarrays can handle many targets simultaneously, but this technology is neither quantitative nor sufficiently sensitive for GMO analysis. Because of their lack of sensitivity, microarrays must be coupled with PCR amplification if they are to be used even for qualitative GMO analysis. Thus, at best, microarrays can only replace the electrophoresis step of current qualitative GMO analysis.

Biosensors are a third technology that may have potential for GMO testing. Biosensors have not found routine use in GMO testing to date. However, three different kinds of biosensors have been evaluated for their suitability. These include surface plasmon resonance (SPR) (Minunni et al. 2001, Feriotto et al. 2002, Mariotti et al. 2002), piezoelectric (Minunni et al. 2001), and electrochemical biosensors (Minunni et al. 2001). When used in conjunction with PCR amplification all three of these approaches were found to provide technically adequate levels of detection (Minunni et al. 2001, Feriotto et al. 2002, Mariotti et al. 2002).

Other features of these biosensors offer advantages for GMO detection. First, they can be multiplexed to screen for many targets simultaneously. Second, the detection process used in these biosensors is nucleic acid hybridization, which is highly selective. Third, other work has shown that these biosensors can function quantitatively. Fourth, because they operate on simple physical principles, detection is rapid and economical. Fifth, commercial instrumentation based on these biosensors should be easy to use and automatable. Finally, in some cases, with further work, portability and field-operability should be achievable.

With currently available biosensors, as with microarrays, sensitivity of detection is the primary limitation, and it may not be possible to upgrade the sensitivity of the biosensors tested to date to achieve the sensitivity required for stand-alone use, independent of PCR. As discussed, elimination of the need for PCR is essential, if a detection method is to constitute a genuinely fundamental advance in GMO detection technology. Although this may not be achievable with currently available biosensors, as research in biosensors continues over the next few years, innovative designs and detection principles may lead to development of novel biosensors having sufficient sensitivity to adequately fulfill future GMO analytical requirements.

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Chapter 34

Public Participation in Biosafety Issues

LIM LI CHING
THIRD WORLD NETWORK

1. Introduction

'Public participation' is a buzzword that has been gaining increasing popularity in discussions about genetic engineering, in part due to obligations laid down in the Cartagena Protocol on Biosafety, and in part due to the debate that has surrounded the issue. A discussion on public participation in the context of biosafety necessitates an examination of the following questions: *What* is the purpose of participation? *Why* engage in it? *In what* should the public participate? *How* should this be done? *When* should the public participate?

This chapter will explore some of these questions, and will argue that public participation, at all stages in decision making involving genetic engineering and genetically modified organisms (GMOs), is critical for the effectiveness of regulatory frameworks and to inform policy and decisions. However, to truly have public participation, an appreciation and understanding of values as well as socio-cultural, economic and political contexts are necessary. As a consequence, concerns about socio-economic impacts or ethical issues will also have to be taken into account alongside scientific criteria and technical measurements. Ensuring public participation, however, is not an easy task. There are no templates or toolkits for participation, neither is there a universal prescription or standard formula.

This chapter starts with a short overview of the legal frameworks that support the need for participation. It then explores other reasons why public participation is encouraged and practiced in relation to biosafety, and gives some examples of what the public has participated in. Finally, it describes some experiences and lessons that have been learnt.

2. International agreements that support public participation

There is an increasing trend for multilateral environmental agreements to contain provisions on public participation, which place the responsibility on governments to engage in awareness raising and participation activities. The rationale is that public involvement is critical to the effectiveness of any regulatory framework. Some relevant examples from the multilateral arena, which enshrine public participation, are given in the following.

2.1 Rio Declaration on Environment and Development

One of the outcomes of the United Nations Conference on Environment and Development (the 'Earth Summit') in 1992 was the Rio Declaration on Environment and Development. The Rio Declaration comprises a series of principles that define the rights and responsibilities of States in the area of environment and development.

Principle 10 of the Rio Declaration (UNCED 1992) states:

> *Environmental issues are best handled with participation of all concerned citizens, at the relevant level. At the national level, each individual shall have appropriate access to information concerning the environment that is held by public authorities, including information on hazardous materials and activities in their communities, and the opportunity to participate in decision-making processes. States shall facilitate and encourage public awareness and participation by making information widely available. Effective access to judicial and administrative proceedings, including redress and remedy, shall be provided.*

Principle 10 thus clearly links environmental issues with public participation. The key elements are appropriate access to information, facilitating awareness and participation in decision-making processes, and access to judicial and administrative proceedings.

2.2 Aarhus Convention

The UN Economic Commission for Europe Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters, also known as the Aarhus Convention, entered into force in October 2001. The Convention covers Parties from the Pan-European region, including Europe, Caucasus and Central Asia region (EECCA). It has been ratified by 39 countries, including the European Community.

The Aarhus Convention grants the public rights, and imposes obligations on Parties and public authorities regarding access to information and public participation. There are three pillars to the Convention: access to information, public participation and access to justice (UNECE 1998). Public participation relies upon the other two pillars – the information pillar to ensure that the public can participate in an informed fashion, and the access to justice pillar to ensure that participation happens in reality.

Activities involving GMOs were not initially subjected to the Convention's participation requirements, but were referred to national legislation. However, in May 2005, agreement was reached on an Amendment that provides a legal obligation for Parties to provide the public with early and effective information, and public participation prior to making decisions on whether or not to authorize a GMO release for experimental and for commercial purposes. When decisions are made, due account has to be taken of the public participation outcomes.

2.3 Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety (Secretariat of the Convention on Biological Diversity 2000) places a clear obligation in Article 23 (see Box 34.1) on Parties to promote and facilitate public awareness, education and participation, including access to information, and also requires mandatory public consultation and disclosure of results of decisions to the public in the decision-making process (Chee & Lim 2005).

Box 34.1 Article 23. Public Awareness and Participation

1. The Parties shall:

(a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies;

(b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.

2. The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of

such decisions available to the public, while respecting confidential information in accordance with Article 21.
3. Each Party shall endeavour to inform its public about the means of public access to the Biosafety Clearing-House.

There are common elements in the aforementioned three multilateral instruments. Important among these is that they refer to the *active* provision of information, that is, the right of the public to receive information and the obligation of authorities to proactively collect and disseminate information of public interest, without the need for a specific request (Goven 2004). They also refer to public participation across different stages (in policy making, specific decisions, etc.). Obligations are placed on governments to ensure transparency and accountability of response. Nonetheless, there are other elements crucial to participation that are not addressed, which are discussed further below.

3. Ensuring informed policy and decisions through public participation

Ensuring informed policy and decisions is a key reason for public participation. Participation is integral to a good policy and biosafety regulatory framework. This is because, in many cases, regulators will not be dealing with GMOs that have been developed in their particular countries. Instead, different countries will have different local environments and agroecosystems, in which there may be no previous field release and hence experiences with the GMO in question. Participation by local people with knowledge of local conditions thus becomes important (Goven 2004).
To ensure informed policy and decisions, we also need information on diverse experiences and perspectives that are relevant to assess and manage the risks and impacts of GMOs (Goven 2004). Past experiences with introduced technologies, and the power relations associated with them are relevant. Technologies themselves are not neutral instruments as they are embedded in particular cultural worldviews and contexts.

It is not only the physical environment that is relevant, as there is also the social environment, and the economic, political and cultural characteristics of the society concerned (Altieri 2004; Goven 2004). For instance, the release of genetically modified (GM) crops involves interactions with social systems such as agricultural practices and farming. This ultimately raises questions as to whether farmers' rights to use, save, exchange, and sell seeds – practices vital to the farming communities in most developing countries – would be affected by proprietary GM crops. What are the cultural implications of crossing cultural boundaries, or the significance of manipulating organisms for which peoples have relationships with, and custodianship over? What are the implications of an instrumental treatment of maize, for example, which is integral to the cultural and spiritual life of many indigenous peoples?

Public participation can help to provide answers to these questions by mapping local knowledge, i.e., information on diverse local conditions, practices and cultures that are relevant for the assessment of risks and impacts of GMOs (Altieri 2004). The authorities have to know which ecological and social systems the technology is going to interact with, and have to engage with the people who live in those conditions, as they will have a key role in evaluating the risks and impacts of GMOs.

This suggests that assessments of GMOs must include an assessment of risks to cultural integrity (Altieri 2004). However, neither 'risks' nor 'benefits' can have any meaning without reference to social values. Risk is embedded in values, in that it is a situation or event in which something of

value to people is at stake and the outcome is uncertain. So, assessing risk requires knowledge of what people value, why they value what they do, and who decides upon the value. It is thus not possible to evaluate the impacts of new technologies without reference to social concerns. At the very least, it means that assessments where this cultural significance is absent are not applicable (Goven 2004).

Although the debate may sometimes seem polarized due to the controversies surrounding GMOs, experience suggests that open engagement with different opinions and values helps reveal a more complex and diverse picture of public attitudes, interests, needs, and priorities, allowing policy makers to see ways forward (Glover et al. 2003).

3.1 Public awareness

Public awareness plays a crucial role in ensuring informed policy and decisions. However, 'public awareness' may be misused as a code for public education about the benefits and safety of GMOs. In this respect, proponents of the technology see participation as a key to ensuring that a skeptical and worried public accepts the technology. This is not what we are talking about here. One purpose of public participation is to increase the awareness of the public and the regulators. Such increased awareness increases the ability to identify relevant social and ecological changes. An awareness and sense of the known issues, questions and concerns related to biosafety in a country helps participants to identify the knowledge they have that is relevant, and thus to inform policy. It is an iterative process.

Public participation and public awareness are thus intrinsically linked. Participation is impossible without information being shared and accessed effectively. On the other hand, sharing information and raising awareness invites participation because it enables the public to consider issues and form opinions on them.

4. Participation in what?

Public participation is relevant throughout all stages of assessment and regulation of genetic engineering and GMOs. This includes participation in national policy discussions, in the development, implementation and review of national biosafety frameworks (NBFs), policies and laws, in evaluation of risk assessments and specific applications, and in monitoring processes.

The identification of problems, needs, priorities, and options in relation to the sustainable use of biodiversity is critical at an early stage of any discussion about GMOs and biosafety. Public participation is important at this stage, as the use of biodiversity needs to be *socially* as well as environmentally sustainable, for example, in relation to food security, cultural integrity and poverty reduction. Hence, a wider public debate on the role of genetic engineering and on what alternatives and options are available for a country is also needed. While, for example, the Cartagena Protocol on Biosafety's framework on public participation may be limited (to the transfer, handling and use of GMOs in relation to conservation and sustainable use of biodiversity, but with reference to human health risks and socio-economic conditions) once participatory exercises on biosafety are initiated, wider socio-economic, ethical and moral issues are invariably raised. Processes that are unresponsive to such public demands for a more broadly defined approach to regulation are likely to lack credibility and legitimacy (Glover 2003; Glover et al. 2003). Such a broadly defined approach is needs-driven (Goven 2004), rather than technology-driven. Questions such as 'Who determines the needs?' and 'What determines the arrival of a new technology?' should be addressed. The public, not the technology developers, should make that determination.

Public participation is also crucial in the development, implementation and review of national biosafety frameworks (NBFs,) policies and laws. However, it is not just a matter of inviting the public to participate once an NBF has been developed. What is needed is also public input into the determination of the proper scope of an NBF. Questions such as what should be addressed within the NBF and who gets to frame the framework all need to link with the relation to society's needs, problems, priorities, and options.

In the implementation of the NBF, policies and laws, regulators will have to take decisions on specific applications and conduct risk assessments. Here, public participation is also important, as local conditions, local knowledge and social practices matter.

Furthermore, the NBF and decisions related to GMOs need to be reviewed as conditions change, scientific understanding evolves, problems emerge, and new challenges arise. Even though a GMO may not be approved for release in a particular country, there could still be contamination or inadvertent release, and associated effects, which need to be monitored. Public participation is relevant in monitoring, particularly in providing local knowledge and experiences (especially of those who are affected by particular decisions) that can inform decision making, helping governments adjust decisions and policies accordingly. For instance, public participation in monitoring can help local people to work with policy makers to decide how changes should be monitored (for example, in helping to design and adapt methodology, in collecting and analyzing data), what criteria should be used, and how results should be acted upon (Guijt & Gaventa 1998). It can reveal valuable lessons and approve accountability (see also Chapter 32 and the case study presented in the following).

4.1 Participation in monitoring: Bt cotton in Andhra Pradesh, India as a case study

Three varieties of Bt cotton were commercially planted for the first time in 2002 in central and southern India. From the first year of planting, there were conflicting reports as to what benefits or otherwise were associated with Bt cotton. In such situations, it is clear that local level information and knowledge are needed, based on farmers' actual experiences. Participation in monitoring can help shed light on what may be a confusing situation, as it provides an opportunity for those directly affected to make their experiences known.

The Deccan Development Society (DDS) and the Andhra Pradesh Coalition in Defence of Diversity (APCIDD), a coalition of over 140 civil society groups in Andhra Pradesh, began monitoring Bt cotton, focusing particularly on the cotton district of Warangal. The aim was to assess the performance of Bt cotton vis-à-vis the claims made of increased yield, reduced pesticides use and higher profits, and to make the experiences available for public debate.

The study involved all the stakeholders in the district – farmers who cultivated Bt and non-Bt cotton, cotton scientists, officials of the State agriculture department and the agricultural market committee, and the manager of a ginning factory. Data collectors were village-based grass-roots researchers from eleven local NGOs, who stayed continuously with farmers and farming communities to record changing perceptions on Bt cotton throughout the growing season. The focus was on small farmers who farm under rain-fed conditions.

The three-year study was carried out over each growing season, with interviews conducted with farmers every two weeks (Qayum & Sakkhari 2005). Focus group discussions were also carried out. There was fortnightly recording of data on field operations, use of fertilizers and pesticides, and status of crop and pest damage, while scientists regularly visited the fields to verify data collection. Participatory video was used by the DDS Community Media Trust, a rural women's media collective. The women, themselves poor and marginal farmers, filmed and interviewed

farmers, documenting changes and analyzing the reasons for these changes with the farmers (DDS Community Media Trust 2004). Hundreds of farmers testified in the study and on film.

The methodology used over the three years was broadly the same, but with some modifications made on the basis of experience, and to focus the study more specifically on the experiences of small farmers. The needs and priorities of poor and marginal small farmers were very much related in economic terms, so the main indicators used were economic in nature and related to yield (and hence profit), costs (of seed, pesticides, irrigation, etc.), pesticide reduction (because pesticides are expensive inputs), and net income.

The study obtained results showing that non-Bt cotton yielded more than Bt cotton, but incurred less expense (Qayum & Sakkhari 2005). There was, however, a slight, but insignificant reduction in pesticide use for Bt cotton farmers, compared to non-Bt cotton farmers. As a result of the higher yields with non-Bt cotton, non-Bt cotton farmers earned 60% more on average than Bt cotton farmers.

4.1.1 Monitoring leading to policy shifts

In May 2005, the Indian regulatory authority, the Genetic Engineering Approval Committee (GEAC), decided not to extend the approval for commercial cultivation of the three varieties of Bt cotton in question (Mech-162, Mech-12 and Mech-184) in Andhra Pradesh. In addition, the GEAC decided not to renew the approval for commercial cultivation of Mech-12 Bt cotton in South India. The three varieties can still be cultivated in other parts of India, and other varieties of Bt cotton are approved by the GEAC, including for Andhra Pradesh.

A GEAC official said: 'This decision was taken on receiving adverse reports from about twenty farmers' organizations. The Andhra Pradesh government had given adverse reports on the performance of Bt cotton while other states like Karnataka, Tamil Nadu, Maharashtra, and Madhya Pradesh have sent mixed reports.' Moreover, Andhra Pradesh farmers who had suffered poor results after planting Bt cotton had also protested on the streets, and burnt seed outlets that stocked the Bt cotton.

4.1.2 Some lessons

The study initiated by DDS and APCIDD is a good example of participation in monitoring. It had captured farmers' experiences over the three years and was a channel by which farmers' experiences with Bt cotton could be fed back to the government. However, this does not mean that the burden of monitoring should be off-loaded to civil society or farmers or the people who are affected by decisions (Goven 2004). Moreover, in this case, farmers still took to the streets in protest. Hence, one lesson is the need for mechanisms by which a regulatory authority can take into account new information, developments in science, etc. that come to light because of a monitoring process.

Feedback mechanisms need to be created, in order to ensure that monitoring results are communicated back to the decision maker, as well as procedures for acting on the feedback. People affected by particular decisions need an avenue by which to inform the authorities of their experiences and the implications of the decisions. Are there, for example, advisory review committees that can review new information and recommend changes? How would the public be involved in these processes? Equally critical is assigning responsibility for determining what changes may be necessary in the regulatory system. Will this be a strictly governmental decision or will there be input from a broader range of interests? Where the public has been involved in making recommendations and offering opinions, will there be any explanation of which options have been rejected and which have been taken forward, and why?

5. The context of 'participation'

5.1 Why does context matter?

In biosafety science, we miss the point if we focus only on the transgenic construct apart from its context. This applies to participation as well. Technology development is imbued with power relations and science is not value-free. Even if this was potentially true, science never occurs outside society. Science is embedded in societal power relations and vice versa. Questions such as 'What determines what research gets done?', 'What drives the research agenda?', 'Who does the research?', 'What is done with the results?', and 'What is the fate of inconvenient findings?' need to be asked.

5.2 Understanding the context and its implications

'Participation' as a notion has become increasingly popular – it is specified in international agreements, advocated by international organizations (e.g. World Bank), and is increasingly embraced in GMO policy by Northern governments. It is, however, rarely acknowledged that meaningful participation requires the possibility to bring about change in policy.

There has also been emphasis on the procedural aspects of participation, with increasing focus on techniques, e.g. toolkits, best practice standards and harmonization. However, there is actually no universal template for participation; instead participation needs to be framed by local needs and concerns (Goven 2004). Imposing a formal template or formulaic techniques will not ensure true participation.

The focus on procedures can also obscure important contextual issues – both the local context and the power context. It can facilitate participation becoming a tool of legitimation rather than investigation, and it obscures the inherently political nature of participation itself. Framed and promoted this way, there is a danger that 'participation' will become another disempowering technology – governments' 'performance' of the technique will be judged externally (by donors and development agencies), while it de-legitimates bottom-up self-organized civil society participation.

Institutionalized participation is not a substitute for self-organizing civil society organizations. It is not meant to displace them, but should exist alongside. If institutionalized participation processes are used to marginalize or de-legitimize civil society, this is not compatible with participation as a means to effective and informed policy and decisions (Goven 2004).

An obvious reason for public participation is that people who are likely to bear the consequences of a decision should have a say in the decision. Participation is not just a means, but a democratic right that everyone has. Yet, one of the most important rights to participation is the right *not* to participate. Some people or civil society organizations may choose not to participate in processes they do not consider to be legitimate. This decision has to be respected by the authorities.

Dilemmas do exist for civil society; if it rejects participation, then decision making would likely be dominated by experts and elites. Alternatively, active civil society can be a crucial asset in the sustainability of participation, and in the processes of gathering relevant information and recognizing risks. Civil society can bring important issues to public attention and are often repositories of enormous amounts of relevant knowledge. It can also create spaces for participation, on its own, or facilitated by government.

6. Making participation happen

Participation does not just happen; it needs to be made to happen. External actors can either strengthen or limit participation. Hence, a key question is: Who creates the space for participation? This has implications for what can happen within the space and the impact it can have. Governments would be more likely to take up and use the outcomes from participatory deliberations in a space it created itself, than in a space created by civil society, for example. Yet, it is important to recognize that the two kinds of spaces are not necessarily antagonistic (Glover et al., 2003). Furthermore, governments can play two roles – initiating participatory and awareness-raising activities, and creating an enabling environment for others to take the initiative.

What is needed for participation to happen? Legal avenues and obligations (e.g. in EU Directive 2001/18 the public is given opportunity to comment on each GMO application), constitutional provisions, the attitudes of those in charge, and guarantees of access to information all affect whether participation is helped or hindered. For example, access to good, complete information is crucial for effective participation.

Furthermore, enabling processes need to be in place for eliciting local and distributed knowledge, including enabling processes for minority or marginalized groups (Goven 2004). Special efforts may be needed to reach these groups directly; otherwise inclusion may be restricted to a narrow circle of participants, potentially reproducing social inequalities. The following issues also need to be considered: translation into relevant languages, means of communication (non-print for non-literate), resources (including time), and terminology to be avoided (e.g. obscure words, jargon).
Experience shows that the public is capable of discussing scientific issues. However, ways must be found to make scientific knowledge accessible and useful to 'non-scientists'. The challenge is to provide people with the opportunity to engage on their own terms, to ask their own questions on the technology and on what forms of regulation may be appropriate for managing associated risks.

Transparency is needed, not just in making the timing or location of a decision process apparent, but also in the decision trail and reasoning, so that a decision can be justified *in relation to public input*. If participation is to be sustainable, the process must be considered legitimate, and for that the public needs to know what happens to their inputs, and the reasons why their inputs are adopted or not. The credibility of public participation initiatives is dependent on the degree of accountability and responsiveness of the convening institutions.

7. Some mechanisms used in relation to GMOs

7.1 Advisory boards and committees

Advisory boards and committees are a common tool used to advise governments on biosafety decisions (see for instance Chapter 24, which discusses the Norwegian Gene Technology Act). The authority and credibility of such bodies depend heavily on their independence from government and industry, the extent to which they include the perspectives of non-scientists, and their ability to represent a broad range of interests. The composition of these advisory boards and committees is important. Some advisory boards and committees may include civil society representatives and lay people, but this alone is not evidence of public participation. Moreover, while it is critical to have technical and scientific advice, there are also socio-economic, cultural, ethical, and other concerns related to GMOs, which have also to be taken into account.

Advisory boards and committees can be required to hold public hearings and consultations. How they go about doing so will determine the extent of public participation.

7.2 Public debates

A public debate can raise awareness of biosafety issues, as well as is a means to feed back public opinion to the authorities. An example is the United Kingdom's 'GM Nation?' debate (GM Nation 2003), which originated from a recommendation of the Agricultural and Environment Biotechnology Commission (AEBC), an independent body which advises the government on biotechnology issues and their impact on agriculture and the environment. The debate was not treated as a simple exercise to say 'Yes' or 'No' to GM crops. It tried to establish the nature and full spectrum of the public's views on genetic engineering and the possible commercialization of GM crops, and any conditions it might want to impose.

Nine 'foundation discussion workshops' were held prior to the debate, to enable the public to help frame the debate, and to identify the questions that should be asked. The 'GM Nation?' debate was a large exercise, involving 675 public meetings all over the United Kingdom. At least 8,324 people attended a public meeting (based on feedback forms received), but this was thought to be an underestimate, and the total attendance may have been nearer 20,000. Over 1,200 letters and e-mails expressing views on GM issues were received, and 36,557 feedback forms were returned. 'Narrow but deep' group discussions were also organized, where a comparatively small number of randomly selected people were given an opportunity to study the issue in-depth and respond accordingly.

7.3 Citizens' juries and scenario workshops

Citizens' juries and scenario workshops are collectively known as 'deliberative and inclusive (or inclusionary) policy processes' (DIPs) (Glover et al. 2003). DIPs have the potential to widen the circle of participation, and may enable a deeper form of participation in which choices are deliberated and cross-examination of expert opinion is encouraged, which would not normally feature in conventional consultative processes. The aim is to facilitate meaningful collective deliberation among participants, rather than merely to collect information or solicit opinions out of context. They are distinguishable from events such as hearings or public meetings where a panel of officials or experts seeks information or views and answers questions, without enabling opportunity for collective discussion or open dialogue among various participants.

Participatory deliberation aims to go beyond formal and perfunctory approaches to consultation that generally succeed in involving only conventional stakeholder groups, such as academics, unions, industry, and pressure groups (Glover et al. 2003). DIPs aim to provide for meaningful participation by individuals and groups from a broad and diverse range of perspectives. The question of how participants are selected is therefore crucial. It is also important to appreciate the risk of reproducing power inequalities.

For example, a combination of a citizens' jury/scenario workshop on food and farming futures for Andhra Pradesh, India was held in 2001 (Pimbert & Wakeford 2002). GM crops were a major topic of deliberation. Participants were marginal-livelihood citizens. Selection was facilitated by independent researchers and based on selection criteria – small or marginal farmers living near or below the poverty line; open-minded, with no close connection to non-governmental organizations or political parties; and likely to be articulate. The jurors were presented with three different scenarios or visions for the future. Invited specialist witnesses defended a particular vision, and were open to cross-examination. The jurors considered all three visions, assessing pros and cons on the basis of their own knowledge, priorities and aspirations, taking into account the specialist witnesses' contributions. They did not choose one of the three visions per se, but assessed critically the viability and relevance of all elements of each scenario, and constructed their own unique vision.

DIPs are often complex and resource-intensive processes, but they point to the possibility of developing an approach defined first by needs, followed by a consideration of how the technology fits in with those needs. They can thus provide valuable insights that help both to define questions and to evaluate solutions. DIPs can therefore be used both to support effective, informed decisions and to enhance the transparency, democracy and legitimacy of decision-making processes (Glover et al. 2003).

8. Some key issues for public participation

* Early involvement of the public – The credibility, legitimacy and effectiveness of any public participation process depends strongly on the extent to which it enables the public to help frame the issues to be considered.

* Independence and transparency – These are critical factors that foster legitimacy, trust, credibility, and confidence. The public needs to know what happens to their inputs, and the reasons why their inputs are not adopted.

* Access to good, complete information – This is crucial for effective participation. Participation is impossible without information being shared and accessed effectively. Access to information allows the public to make decisions that are informed.

* Adequate allocation of resources – Participatory processes can be resource-intensive, especially in the short term. Sufficient time and resources are needed to enable meaningful consultation and deliberation. However, if better-informed and more widely supported decisions are made through meaningful participation, this may reduce the political, social and economic costs in the long term. Such costs could include breakdown in pubic trust, loss of confidence in regulatory bodies, and loss of legitimacy that may result either from a lack of participation or ill-conceived participatory processes.

* Recognition that participation is a process – Participation should not stop with the creation of an NBF or legislation, or just because a particular exercise has been successfully carried out. It is an ongoing process that feeds into the implementation of NBFs, policies and laws, and their monitoring and review. Furthermore, experience shows that broad, open-ended, dynamic and responsive processes are better able to accommodate the range of concerns on GMOs (Glover et al. 2003). Processes that succeed in accommodating this diversity are more likely to command public credibility and respect.

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Chapter 35

Biosafety Forecast Service: The Precautionary Approach in practical Biosafety

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'In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.'

(Principle 15, Rio Declaration on Environment and Development)

Biosafety Forecast Service (BFS)

The Precautionary Approach and the BFS

The Cartagena Protocol on Biosafety is an international treaty regulating primarily the transboundary movement of living modified organisms (LMOs). The Protocol, adopted as a supplement to the Convention of Biological Diversity, seeks to protect biological diversity from the potential risks posed by LMOs resulting from modern biotechnology. The Biosafety Protocol emphasizes the precautionary approach, allowing a country to reject or place restrictions on the importation or release of an LMO when the science on the potential benefits and hazards to human health and the environment is uncertain.

The Biosafety Forecast Service (BFS), a research-based risk identification and analysis project, was conceived with the principles of the Biosafety Protocol and the precautionary approach in mind. The Service is designed to support scientific risk assessment and holistic decision making by countries meeting their obligations under the Protocol, identifying areas of scientific uncertainty (Box 35.1) in applications for the release of LMOs (and more generally, genetically modified organisms, GMOs) as food, feed and medicine into the environment. It is also intended to assist regulatory authorities, non-governmental organizations (NGOs), civil society leaders, and citizens operating within their National Biosafety Frameworks.

Decisions taken on LMOs by countries party to the Biosafety Protocol should be preceded by a scientific risk assessment. They may also take into account socio-economic considerations, especially with regard to indigenous and local communities. The BFS is planned to support decision-making and the evaluation of LMO applications through both guidance for scientific risk assessment and the analysis of potential socio-economic and legal impacts.

Box 35.1 Examples of areas of scientific uncertainty

The identification of areas of scientific uncertainty permits the recognition of fields of biosafety where more research is needed. A few examples of these areas include:

Effect of novel RNAs.

- Gene silencing caused by RNA interference (RNAi)

Post-translational modification.
- In vitro studies using the in-planta produced protein in comparison to the bacterial version.
- Detection of minor variants.

Effects on non-target species.
- For bio-pesticides.
- For pharma crops.

Scientific risk assessment
A scientific risk assessment is based on risk identification, which may be customized on a case-by-case basis depending on the modified organism, its modification or its intended application. The BFS produces briefings on generic scientific risk issues as well as case studies that include custom assessments.

Risk identification can include aspects of an LMO from its production to its release into the environment and its use as food or feed. It includes aspects such as the molecular biology of the modification, genetic stability and effects of out-crossing and potential environmental and food hazards (Box 35.2).

Box 35.2 Examples of risk identification issues

Molecular issues include identifying and characterizing changes to:
- the genome, e.g. insertions, mobile elements, DNA processing sites, regulatory DNA sequences and introns;
- the transcriptome, e.g. novel mRNA molecules, silencing effects and regulatory RNA molecules;
- the proteome, e.g. novel polypeptides, modifications, and structures, unanticipated loss of a protein.

Genetic issues include:
- stability of the modification, gene and gene product across tissues and over generations;
- stability of the modification, gene and gene product in hybrids;
- impact of horizontal gene transfer.

Food hazard issues include:
- equivalence of modified and conventional counterparts;
- analysis of novel products and metabolites/catabolites;
- potential allergens, toxins, anti-nutrients, carcinogens, and co-carcinogens;
- uptake of DNA and other products specific to the GMO through food;
- factors undermining the sustainability of alleged benefits.

Environmental hazards include:
- horizontal gene transfer in the environment;
- effects of co-technologies (e.g. herbicides) used with the GMO;

- impacts on biodiversity;
- factors undermining the sustainability of alleged benefits.

Analysis of potential socio-economic impacts
An evaluation of socio-economic impacts covers a wide range of issues, often specific to an area, organism, or an organism's intended application. The BFS generates briefings on general socio-economic issues (Box 35.3) as well as case studies that include customized assessments.

Box 35.3 Examples of socio-economic issues
Socio-economic issues are diverse; they may include:

- the economic costs and benefits associated with the production and use of the LMO/GMO (including an assessment of the impacts on market access);
- the resource demands of adequate monitoring and containment of the LMO/GMO;
- the compatibility of the management procedures required by LMOs/GMOs with valued socio-cultural practices and resources;
- the socio-economic impacts of GMO-related farming regimes (e.g. changes related to intellectual property, co-practices, capital inputs, size of functional landholding), especially on indigenous and local communities;
- the socio-economic implications arising in the event of a loss of biodiversity, (e.g. through introgression of transgenes into traditionally important species or landraces);
- the potential impacts of the LMO/GMO on the rights of indigenous communities.

Analysis of legal implications
Any decision taken on LMOs should consider both domestic and international legal obligations, including those concerned with intellectual property protection and biodiversity.
An analysis of legal implications covers the conditions and constraints that may arise in conjunction with the purchase and use of LMOs. It also assesses state-level rights and obligations under international agreements, such as the Convention on Biological Diversity and the WTO Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS).

The Biosafety Assessment Tool (BAT)

Context
A comprehensive survey of the resources already freely available to support decision making and risk assessment processes for GMOs was conducted in November and December 2004 by the team of the Biosafety Forecast Service. This survey revealed that existing resources mainly comprised databases of highly technical literature for specialists; comprehensive, but not quality assured, databases associated with distribution services; and decision-tree formats providing little or no background support for the user.

The BFS is purposefully different to existing services in several important ways. First, it will be quality assured. Most of the content development for the BFS involves work at the leading edge

of the research literature. New knowledge is also produced by the team, to meet the highest international standards of peer review. For example, two technical summaries have already been published, one on monitoring GMOs and one evaluating designs of experiments purporting to assess the impacts of horizontal gene transfer.

Second, the BFS goes beyond simply providing summaries of risk issues and reviews of technical literature. To allow users to make their own interpretations of the information provided by applicants and regulatory agencies, the BFS is developing the Biosafety Assessment Tool, also called BAT.

BAT: The tool
The BAT will be a free-to-the-public electronic resource, designed as a practical tool for the risk assessment of GMO applications for food, feed, medicine, or environmental release. By using the BAT, policy and regulatory officials in government, non-governmental organizations (NGOs), citizens, and researchers will be able to customize biosafety information from the elite scientific and technical literature and apply it to their own risk assessments, or to evaluations of assessments done by others. It will help both the identification of relevant risk issues and assist with the evaluation of technical information provided in GMO import/release applications. Unlike a decision-tree approach that leads to a certain conclusion based on an analysis 'behind-the-scenes', the BAT is designed to make explicit the connection between the actual data supplied to regulatory authorities (e.g. by applicants) and considerations of risk so that the user can learn to recognize uncertainties in the evaluation of GMOs.

The aim is to make it possible for a user to construct a comprehensive and context-specific assessment of the technical information, as well as to identify what additional issues or uncertainties should be addressed by either regulatory authorities or the applicant.
The BAT will not only support the writing of scientific risk assessments but also assessments related to the socio-economic impacts. This emphasizes the holistic and independent approach of this tool. The tool will not tell the user whether to accept or reject a GMO; rather, it will assist the user to carry out GMO risk assessment and holistic decision making.

The organization of the BAT
The information within the BAT is organized as three different 'gates'. These gates have been customized to the needs of different users, or of the same user at different stages of risk evaluation.

Gate 1. Practical Assessment
Gate 1 will serve those prepared to assemble a final assessment of an application. The information in this section will be structured to reflect the organization of a typical application. It will explain the terms used in applications and the information that is, or should be, provided by the applicant.

Gate 2. Risk Assessment Guides
Gate 2 is based on a series of 'guides' designed to provide a comprehensive view of GMOs from production to release (Table 35.1). It could be used to complete an assessment or to gain a broad overview of GMOs and their implications. This Gate provides the rationale and references for the recommendations in Gate 1.

The guides that form part of this gate provide a more holistic view than the information displayed in Gate 1. These guides will provide information to assist decision makers and citizens with their

consideration of their own environmental, social, political, and economic context as well as scientific risk.

Table 35.1. Description of guides.

Guide	Description
GMO: The basics	The aim of this guide is to describe and explain the main scientific concepts used in applications and assessments. This guide will serve as a primer for all other guides.
GMO from DNA to insert	The aim of this guide is to suggest what could be considered in the assessment of the molecular characterization of a GMO. This will include the description of the risk spectrum of the transgene and the event.
GMO from protein to trait	The aim of this guide is to describe the risks and considerations from the RNA to the protein level of the molecular characterization of a GMO. This will include description of the transcriptome and proteome.
GMO and human safety	The aim of this guide is to make an assessment pertinent to human health. Main components of this guide will include: compositional analysis, allergenicity data and toxicological studies.
GMO and environment	The aim of this guide is to describe the risks and considerations for the release of a GMO into the environment. This can include gene flow, weediness, containment, coexistence, and effects on non-target organisms.
GMO management and monitoring	The aim of this guide is to assess strategies to monitor or contain a GMO once it has been approved for use in human food or for release into the environment.
GMO regulatory and legal issues	The aim of this guide is to illustrate models of existing regulatory frameworks, and to introduce new initiatives.

Gate 3. Risk Assessment Checklist

Gate 3 takes the form of a 'checklist'. The information presented in the BAT will be organized in this gate according to questions that may need to be considered by decision makers. It will explain the significance of the questions and point to information that may help the decision maker to address these questions in relation to their own country. This section is recommended for users that have finished their risk assessment.

The development of the BAT

In order to provide a model for the construction of the BAT, the BFS team has conducted extensive risk assessment analyses on glyphosate resistant wheat and LY038, a GM corn also called High Lysine Corn (Box 35.4). These analyses were used to plan the BAT, covering the steps taken to evaluate each scientific study and the costs and benefits of the proposed policy decisions.

Box 35.4 LY038: A case study

In 2004 an application (A549) was submitted to the food safety authority for New Zealand and Australia (Food Standards Australia New Zealand – FSANZ) to allow the introduction of LY038 high lysine corn into the human food supply. The BFS team assessed this application in the form of two submissions* to FSANZ. These submissions have been used as a training tool for the BFS

team and as a source of case studies for the development of content for the Biosafety Assessment Tool (BAT).

LY038 is a genetically modified corn that accumulates lysine and free lysine in the grain. Free lysine and lysine metabolites accumulate to levels with no historical precedent in comparison to conventional corn, making LY038 one of the first nutritionally enhanced GM organisms that food safety approval has been sought for.

More than 15 studies were included in A549, ranging from the molecular characterization to bioinformatics and feeding studies. These studies were assessed to answer two main questions:

- Do the scientific data made available by the applicant conform to the best international standards?
- Was the safety assessment conducted using the best available science?

The scientific risk assessment was accompanied by an analysis of the potential costs and benefits of changing the food code to permit LY038 in the human food supply.

The first submission was made in February 2005, with the second released in June 2006. In the latest submission, 95 recommendations were made to FSANZ highlighting concerns mainly related to food hazards and the cost-benefit analysis.

To allow the submissions to be used as the basis for the development of the BAT, the team has customized them for a wider audience, using more accessible scientific language and adding a discussion of the process used to identify risk issues. The documents will be reconfigured as practical resources, outlining the steps taken in evaluating each scientific study and allowing BAT users to apply the same process to other pending applications.

*The submission to the Initial Assessment Draft for A549 and the submission to the Draft Assessment Report for A549 are freely available at http://www.inbi.canterbury.ac.nz/ly038.shtml

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As part of the testing and evaluation of the BAT, several prototypes have been developed and more are still to come. Each prototype has a specific feature to be tested in evaluation sessions. This allows us to optimize the tool for our users.

Prototype version 1

The first prototype of the BAT was designed to get feedback about the usefulness of the tool itself – the quality and relevance of its information; the level or difficulty of the information; the prototype's organization and style – and to assess the prototype's sensitivity to different country needs.

For this first version, an easy-to-handle format was preferred, highlighting content rather than sophisticated functionalities. Microsoft PowerPoint was chosen as the platform and Prototype version 1 was launched in August 2005. This prototype demonstrated the approach of the BAT and the kind of information that will be provided in its interactive format, prompting valuable feedback to aid future technical development.

Two venues for testing and evaluation were used (Box 35.5): the Solomon Islands Regional Biosafety Course held in Honiara, Solomon Islands and the Holistic Foundations for Assessment

and Regulation of Genetic Engineering and Genetically Modified Organisms international biosafety course held in Tromsø, Norway.

Overall, the feedback sessions reinforced the merit of the general approach of the BAT in providing easy-to-follow, holistic information from the world of biosafety research in a format useful to those producing risk assessments. Participants commented that the prototype version 1 content and visual aids clarified complex scientific ideas that had previously confused them, suggesting that the BAT had the potential to fill a need within the risk assessment community for authoritative yet accessible biosafety resources. The holistic nature of the prototype engaged participants with disparate specialized backgrounds.

Box 35.5 Evaluation sessions for Prototype version 1

Figure 35.1 Prototype version 1 of the BAT

The first focus group that provided feedback on Prototype version 1 (Fig. 35.1) was assembled at the Solomon Islands Regional Biosafety Course held in Honiara, Solomon Islands, in August, 2005.* Participants representing the private sector and different areas of the Solomon Islands public sector, non-governmental organizations and educators were brought together during this course to use the prototype. This version of the BAT was used by participants in a workshop to assess a fictional application (for a GM fruit) of the type that could be received under the Biosafety Protocol (Fig. 35.2).

A second focus group was assembled at the Holistic Foundations for Assessment and Regulation of Genetic Engineering and Genetically Modified Organisms international biosafety course, held in Tromsø, Norway in September 2005.

Figure 35.2 Evaluation of the Prototype version 1 of the BAT held in Honiara, Solomon Islands as part of the Solomon Islands Regional Biosafety Course

A day of client feedback on Prototype version 1 was conducted, again assessing the fictional GM application. This feedback session aimed to evaluate the conceptual basis of the BAT, its usefulness, and its requirements in terms of function and design. The pool of participants at these biosafety courses was identified as an ideal group of potential BAT users. Positive feedback was received on the prototype from these sessions, with constructive comments for the simplification, organization and expansion of the information within.

* A full report of this course can be found at
http://www.inbi.canterbury.ac.nz/news_biosafety_solomons.shtml

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Prototype version 2

The main concerns that emerged during the evaluation of Prototype version 1 included the organization of the information within the limitations of the PowerPoint programme. Prototype version 2 addresses this by using a web-based interface. This allows the introduction of features such as menus, structured pathways and a search engine. Further components have been designed for this new version, such as an interactive window and a toolbar (Box 35.6). It is important that the effectiveness of these innovations is tested in future feedback sessions.

Box 35.6 Prototype version 2 of the BAT

Prototype version 2 of the BAT was designed in a web-based format. The layout of the tool is divided into three parts:

- The toolbar includes several features, including search and map functions. It will also include features that will allow users to write and save comments, quotes and references and export the gathered information into other programs for further use (Fig. 3A).
- The main screen, where information will be displayed, allows navigation using active links (Fig. 3B).
- The interactive window displays additional information related to the content of the main screen. It also displays definitions of highlighted words included in the glossary (Fig. 3C).

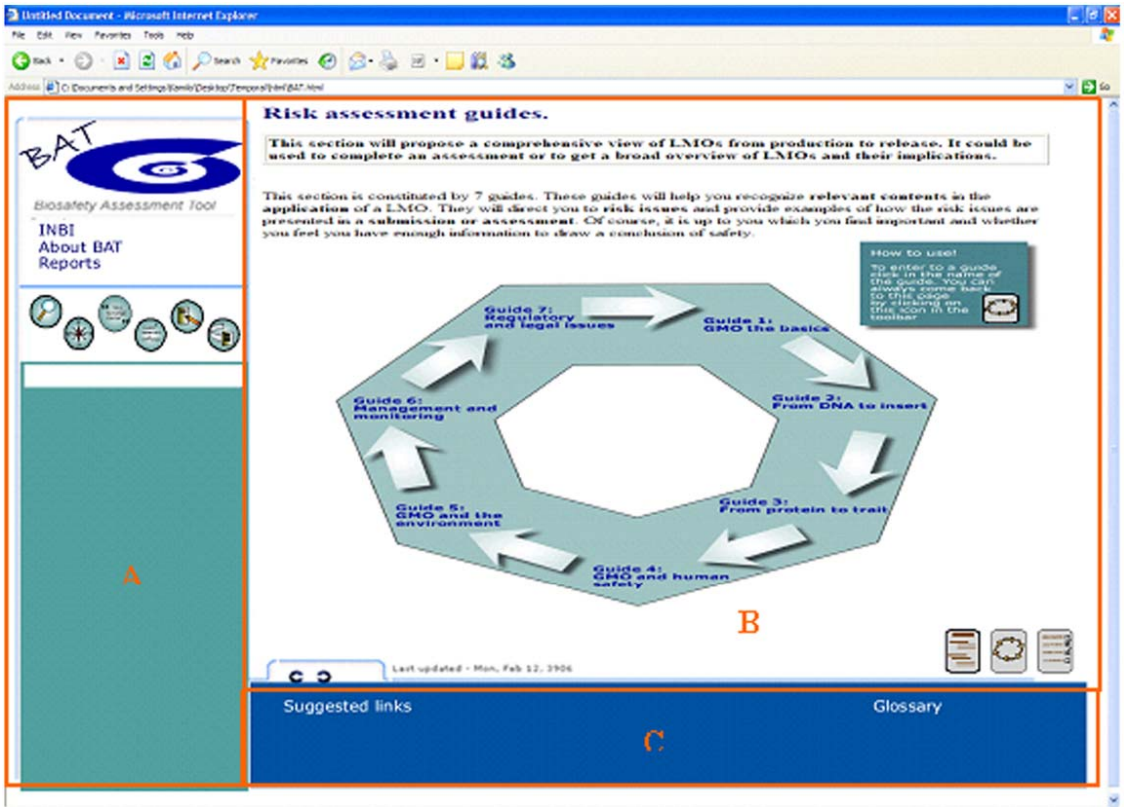

Fig 35.3 Layout of Prototype version 2. A)Toolbar B) Main screen C) Interactive window

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Prototype version 2 already has many features that will be released in the final version of the BAT. This version was first tested for its functionality and usability at the international Biosafety Course in Tromsø in August 2006 (Box 35.7). Other evaluation sessions are planned to take place in 2007.

Box 35.7 Evaluation session for Prototype version 2

Prototype version 2 was evaluated in three workshops on the molecular, health and environmental assessment of an application for the approval of LY038 corn (see Box 35.4). This evaluation session took place at the Holistic Foundations for Assessment and Regulation of Genetic Engineering and Genetically Modified Organisms international biosafety course, held in Tromsø, Norway in August 2006.

Feedback from this session was overwhelmingly positive.* Participants expressed their interest in the use of the BAT in a professional capacity, not only as a regulatory assessment tool but also as research database and teaching and training resource.

Figure 35.4 Participants in the evaluation session of the Prototype version 2 of the BAT

* A full evaluation report can be found at http://www.inbi.canterbury.ac.nz/news_bat2006.shtml

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Conclusion

Following the principles of the Cartagena Protocol on Biosafety, the BFS aims to support countries in LMO assessment by providing a holistic approach to decision making. The BAT was born from discussions with NGOs, policy makers, regulators and, ordinary citizens from all over the world interested in contributing to a robust biosafety framework in their countries. The technical nature of scientific risk assessment and the limited distribution of information mean that there can be significant barriers to participation in GM decision making. It is hoped that the BAT will help reduce the elitism of scientific risk assessment, promoting a more informed and critical analysis of GMOs.

The Biosafety Assessment Tool is practical. Users are assisted to form an assessment based on issues that they find relevant. Unlike decision-tree approaches, issues of risk will not be set and pre-ordered, but identified and evaluated by the user for their specific context.

The development funding for the BFS is scheduled to end in 2007. However, the Tool is a living resource. It will need constant attention and updating to maintain it at the leading edge of risk identification and social change.

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