

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, part 1;*
- 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 1831/2003), 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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