National Organic Standards Board
GMO ad hoc Subcommittee
Discussion Document
Excluded Methods Terminology

February 6, 2013

Introduction

There is a fundamental contradiction at the heart of the regulatory approaches to organic agriculture and biotechnology. Organic certification is a **process-based** guarantee while biotechnology is subject to a **product-based** assessment (Caruso 2006).

One of the strengths of the process-based regulation is that it takes into consideration the entire production system. If biotechnology were assessed on the basis of its impacts on the system rather than the narrow focus of whether or not a particular transgenic crop poses a “plant pest” risk, regulators would have to take into account the environmental impacts of increased herbicide applications, increased use of more volatile and toxic herbicides, impacts on pollinator populations, reduction of vegetative diversity, introduction of novel proteins into soil and water ecosystems, likelihood of selecting for resistance in weed and pest populations, negative socioeconomic impacts, and reduction of transgene-free germplasm availability.

There are contradictions concerning what are defined as “excluded methods,” the phrase used in the USDA organic regulations to describe certain products of biotechnology. Furthermore, the concepts of "excluded methods" have widened to includequestions about what vaccines for livestock are being produced through use of “excluded methods”, issues around the use of micro-organisms in processed foods and the discussion leading up to a October 2012 NOSB recommendation to allow the use of biodegradable mulch film with a prohibition on the use of organisms or feedstock derived from “excluded methods”. Now that the USDA organic regulations have been fully implemented for 10 years, new issues and technologies in plant and animal breeding have been identified and it may be necessary to clarify the language in these regulations.

The purpose of this Discussion Document is to provide definitions of the terms included in the definition of “excluded methods” and to ask for stakeholder input on what changes may need to be made to the definition of “excluded methods” or what clarifications and interpretations may need to be made through guidance.

Background and Relevant areas in the Rule

Here is the definition of “excluded methods” in the National Organic Standards (7 CFR 205.2; Terms Defined):

> Excluded methods. A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a

> foreign gene, and changing the positions of genes when achieved by recombinant DNA technology). Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization, or tissue culture. (Federal Register / Vol. 65, No. 246 / Thursday, December 21, 2000 / Rules and Regulations p. 80639)

Since the rule went into effect in 2002, no changes have been made to the definition of "excluded methods" in the regulations. Due to the lack of specificity in the definition, ACAs (Accredited Certifying Agents) have made their own determinations of how far back in the ingredient and input chain (or breeding line) to verify compliance with the prohibition on excluded methods in organic production and handling and interpret what these terms within the definition mean. This has caused confusion among stakeholders at times, where other techniques not mentioned in the definition are also considered by some as excluded, while some terms within the definition are open to multiple interpretations.

Three separate topic areas that came up in the last two years have caused this confusion to become more important to try to address. The NOSB Livestock Subcommittee has been grappling with the subject of excluded methods used in vaccines and, to guide their further deliberations, a Vaccines Working Group[1] developed a parallel discussion of the terms relevant to vaccine production to solicit comment on what vaccine production methods may or may not be considered excluded. This discussion is posted as an Interim Report and the Working Group is seeking public input.[2] In December 2011, the NOSB's review of Docosahexaenoic acid (DHA) algal oil, an ingredient petitioned for use in organic handling, prompted discussion over whether the form of mutagenesis that may be used to develop this ingredient should be considered an "excluded method".[3] Lastly, the NOP recently issued Policy Memo 13-1 on February 1, 2013 concerning Cell Fusion Techniques used in Seed Production (discussed in the cell fusion section below) to clarify which cell fusion techniques should be considered use of an "excluded method".[4]

Some ACAs and groups such as Organic Materials Review Institute (OMRI) have developed their own decision making protocols for interpreting the definition of "excluded methods". With advances in biotechnology since the time the "excluded methods" definition was first adopted by the NOSB in 1995 and innovations in product development from biotechnology, the decision making is getting more and more complicated.

Discussion

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[1] In May 2012, the NOSB Livestock Subcommittee issued a resolution requesting that USDA provide information regarding which vaccine products were produced through the use of biotechnology. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5098924. In response, the NOP initiated a Vaccines Working Group comprised of NOP staff, NOSB members, and staff from APHIS' Center for Veterinary Biologics. The Working Group began meeting in July 2012 and provided an interim report to the Livestock Subcommittee in February 2012 on this issue.

[2] Vaccines Working Group Interim Report: Identifying Vaccines Made with Excluded Methods. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5102576

[3] December 2011 NOSB Recommendation on DHA algal oil. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5097102

[4] National Organic Program, Policy Memo 13-1: Cell Fusion Techniques Used in Seed Production. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5102380

The GMO ad hoc Subcommittee believes that strengthening and clarifying the definition of "excluded methods" in the USDA organic regulations will help all stakeholders with implementation of the regulations and strengthen processes behind keeping GMOs out of organic food. To do this, the GMO ad hoc subcommittee has prepared this discussion document to 1) examine the language currently in the "excluded methods" definition (Part A), and 2) outline other terms that could be added to the definition (Part B), providing context for regulatory definitions from organic standards of other countries (Appendix I). The Subcommittee is seeking public input on which of these terms belong in a revised definition for "excluded methods" under the USDA organic regulations, as well as the guiding principle(s) to use in crafting a new definition.

Part A

Examination of the exact language of the excluded methods definition at 7 CFR 205.2 will bring out the key issues.

1. A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production.

The phrase "not possible under natural conditions or processes" has become problematic in the context of "traditional" breeding methods that involve disruption of normal plant cell growth. For example, mutagenesis can be a process in which chemical or radiation stress is applied on a cell to force mutation to happen, but it also commonly occurs in nature and at least some of the mutagenesis chemicals are derived from nature. (More on mutagenesis under *5. traditional breeding*). The concept of "natural" is not defined in any regulations and is very blurred after centuries of humans manipulating the environment and plants, animals and microbes.

This brings up the question, what exactly is it about a genetic modification process that is objectionable in the organic context? This larger question is what the GMO Subcommittee is re-visiting here in order for organic stakeholders to clarify the basis of objection to the technology because **even acceptable breeding methods could very well not be possible under natural conditions**. It may be that the species line is where people object to genetic exchange occurring. If this is the case, the terms *interspecific* (between species) and *intraspecific* (within species) or *intergeneric* (between genera) and *intrageneric* (within a genus) may come in handy. So many different techniques are used now that wording must be very carefully chosen or some crops already accepted in organic cultivation might be ruled out. Examples include triticale (created from breeding two different genera), bananas and seedless watermelon from somatic doubling, and more.

Dutch organic plant breeder Edith Lammerts van Bueren has proposed that organisms have "intrinsic value" and "integrity" represented by an intact genome (Lammerts van Bueren et al. 2003). While this argument may seem to have more of a philosophical than scientific basis to it, it may be a useful organizing statement in describing what the organic community finds acceptable means of plant and animal breeding. It also may be relevant to consider that while the prohibition on genetically modified organisms allows for certain traditional methods used to produce nonorganic seeds, (e.g. mutagenesis), the standards for organic plant breeding can be considered more restrictive, since the chemicals or irradiation techniques used are not permitted methods under organic standards.

2. Such methods include cell fusion

Cell fusion: The process in which two different cells fuse into one single cell. The resulting cell has all of the contents from the original cells and has one nucleus containing the genetic material from both of the original cells. This occurs naturally during sexual reproduction, when gametes (eggs and sperm) fuse to produce the zygote, and can also occur under laboratory conditions between somatic cells (any cell other than a gamete). (Websters Online Dictionary[5])

Protoplast fusion
A technique in which protoplasts (plant cells from which the cell wall has been removed by mechanical or enzymatic means) are fused into a single cell. (National Academies 2004 glossary)
or
The fusing of two protoplasts (a bacterial or plant cell deprived of its cell wall but having an intact plasma membrane).- (CAN/CGSB-32.315-2004 **Voluntary** Labelling And Advertising of Foods That Are and Are Not Products of Genetic Engineering)

Also relevant here:
Somatic hybridization : "The technique of hybrid production of plants through the fusion of isolated somatic (body) protoplasts under in vitro conditions and subsequent development of their product (heterokaryon) to a hybrid plant is known as somatic hybridization." (Chawla, H.S. 2001. Introduction to Plant Biotechnology, 2nd ed. Science Publishers, Inc.)

The NOP was asked to clarify its position on cell fusion because it has been used as an "acceptable" means of developing varieties of Brassica and citrus crops among others. Cell fusion has been used in traditional breeding and hybridization programs as well as in general propagation using tissue culture. It can be used within a genus or species or between very different species.

So why might cell fusion be considered an excluded method? A form of cell fusion called *somatic cell hybridization, somatic hybridization,* or *protoplast fusion* involves destruction of cell walls using chemical or electrical stimuli, which then allows the genetic material to be fused. This approach has been used to develop both *intraspecific* and *interspecific* crosses (see selected citations A).

In 2012, the NOP received questions about whether seed varieties produced through cell fusion techniques are allowed in organic production. The issue for certifiers is complicated by the fact that it is not disclosed publically which varieties may have been bred using cell fusion and so enforcement of any prohibition is extremely problematic. Additionally the cell fusion event may have happened up to 30 years ago and the resulting trait simply passed to subsequent generations by traditional breeding methods.

[5] http://www.websters-online-dictionary.org/definitions/Cell+Fusion

In NOP Policy Memo 13-1, the type of cell fusion used to transfer traits such as male sterility within a plant family was determined to be possible with natural breeding techniques and compatible with organic production.

> "... the NOP further concludes that cell fusion (including protoplast fusion) is not considered an excluded method when the donor cells/protoplasts fall within the same taxonomic plant family, and when donor or recipient organisms are not derived using techniques of recombinant DNA technology."

This Policy Memo also explains that cell fusion techniques are considered an “excluded method” when the donor cells/protoplasts do not fall within the same taxonomic family. Cell fusion is also an “excluded method” when the donor or recipient organism is derived using techniques of recombinant DNA technology and techniques involving the direct introduction into the organism of hereditary materials prepared outside of the organism.

Here again it seems as if the technology itself is not the issue so much as the crossing of the species or taxonomic family line.

3. *microencapsulation and macroencapsulation*

Micro-encapsulation: a process in which tiny particles or droplets are surrounded by a coating to give small capsules many useful properties. (Wikipedia)
Macroencapsulation: (cell and molecular biology) The envelopment of a large mass of xenotransplanted cells or tissue in planar membranes, hollow fibers, or diffusion chambers to isolate the cells from the body, thereby avoiding the immune responses that the foreign cells could initiate, and also to allow the desired metabolites (such as insulin and glucose for pancreatic islet cells) to diffuse in and out of the membrane.
(McGraw Hill Science and Reading Dictionary)
http://www.answers.com/topic/macroencapsulation#ixzz2E2MQK67W

It may be time to remove these processes from the definition of “excluded methods” because they don’t involve recombining genes. They may be more appropriately classified as “nonagricultural” or “synthetic” materials or as a form of *nanotechnology*. Micro- and macroencapsulation appear to be cellular packaging mechanisms for engineered genes, food additives, or pesticides rather than a form of genetic engineering (see selected citations B). Specifying them as excluded methods is questionable and has been ridiculed by at least one academic commenter (Eisen 2012).

4. and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology).

DNA, Recombinant
Biologically active DNA which has been formed by the in vitro joining of segments of DNA from different sources. It includes the recombination joint or edge of a heteroduplex region where two recombining DNA molecules are connected.
Year introduced: 1977 (MeSH)

Gene Deletion

A genetic rearrangement through loss of segments of DNA or RNA, bringing sequences which are normally separated into close proximity. This deletion may be detected using cytogenetic techniques and can also be inferred from the phenotype, indicating a deletion at one specific locus. Year introduced: 1993. (MeSH)

Genetic engineering
Changes in the genetic constitution of cells resulting from the introduction or elimination of specific genes via molecular biology (i.e., recombinant DNA) techniques. (National Academies 2004 glossary)

Recombinant DNA techniques
Procedures used to join together DNA segments. Under appropriate conditions, a recombinant DNA molecule can enter a cell and replicate there. (National Academies 2004 glossary)

Recombination naturally occurs between chromosomes during the process of meiosis to form gametes for sexual propagation, in plants, animals and other organisms. Recombination naturally occurs during high frequency recombinant (Hfr) conjugation in which part of the chromosome from one bacterium is transferred to another bacterium, resulting in homologous recombination which genetically modifies the target bacteria. These are just two examples of genetic modifications through recombination events which may be allowed by the current definition of excluded methods.

This language seems valid and on point in addressing the main concerns most organic stakeholders have with transgenic technologies; however, the specifics could probably be updated to include other recombinant technologies and to remove phrasing that is not in common usage. For example, “gene doubling” is not often found in the literature.

Such methods do not include the use of

5. traditional breeding,

This term is assumed to include breeding methods that have been used prior to the emergence of transgenic technologies. It is not clear at which point traditional breeding techniques are divided from modern or non-traditional breeding techniques. Is there a time point at which all techniques before that time are considered traditional and all new techniques developed after that time are not considered traditional? The use of transposons (see below Part B) since the 1930's or chemical, physical, and biological mutagens since the 1940's are blurring the distinction between traditional breeding and biotechnology.

One form of traditional breeding that has not been formally defined and has been called into question is *mutagenesis.*

Mutagenesis (or mutation breeding)
A process whereby the genetic information of an organism is changed in a stable, heritable manner, either in nature or induced experimentally via the use of chemicals or radiation. In

agriculture, these genetic changes are used to improve agronomically useful traits. (National Academies 2004 glossary)

The “problem” with mutagenesis as an acceptable practice in organic breeding is that it sometimes relies on processes that would not occur naturally, but sometimes does involve naturally derived substances and processes. Additionally, some mutagenesis is now accomplished by inserting DNA or other genetic material into a cell (*insertional mutagenesis).* Because of its widespread usage in plant breeding since the 1940s, however, there may be a need to clarify which types of mutagenesis are acceptable under the organic standards. See selected citations C.

6. conjugation,

Conjugation, Genetic: A parasexual process in BACTERIA; ALGAE; FUNGI; and ciliate EUKARYOTA for achieving exchange of chromosome material during fusion of two cells. In bacteria, this is a uni-directional transfer of genetic material; in protozoa it is a bi-directional exchange. In algae and fungi, it is a form of sexual reproduction, with the union of male and female gametes. Year introduced: 1968 (MeSH)

Conjugation can be used to transfer genetic information (via plasmids) between different genera of bacteria. Might this violate the guiding principle of not crossing taxonomic lines? (See Jones and Woods 1986 for background.)

7. fermentation,

Fermentation: Anaerobic degradation of GLUCOSE or other organic nutrients to gain energy in the form of ATP. End products vary depending on organisms, substrates, and enzymatic pathways. Common fermentation products include ETHANOL and LACTIC ACID. (MeSH)

Should inclusion of fermentation on this list be reconsidered? While the process of fermentation can be used to multiply transgenic organisms and some fermentation processes are done with transgenic organisms, it is not a breeding technology. (See Jones and Woods 1986 for background on the use of microbes to manufacture solvents via fermentation.)

8. hybridization,

Hybridization, Genetic: The genetic process of crossbreeding between genetically dissimilar parents to produce a hybrid. (MeSH)

Hybrid
Progeny of genetically different parents, usually of the same species, that has enhanced productivity over either parent. Generally, the more genetically diverse the parent lines, the more hybrid vigor, or heterosis, is observed in the hybrid progeny. (National Academies 2004 glossary)

Here are some other types of hybridization defined on the MeSH site for reference.

Nucleic Acid Hybridization: Widely used technique which exploits the ability of complementary sequences in single-stranded DNAs or RNAs to pair with each other to form a double helix. Hybridization can take place between two complimentary DNA sequences, between a single-stranded DNA and a complementary RNA, or between two RNA sequences. The technique is used to detect and isolate specific sequences, measure homology, or define other characteristics of one or both strands. (Kendrew, Encyclopedia of Molecular Biology, 1994, p503). Year introduced: 1972(1971) (MeSH)

In Situ Hybridization
A technique that localizes specific nucleic acid sequences within intact chromosomes, eukaryotic cells, or bacterial cells through the use of specific nucleic acid-labeled probes. Year introduced: 1993. (MeSH)

9. in vitro fertilization,

Fertilization in Vitro
An assisted reproductive technique that includes the direct handling and manipulation of oocytes and sperm to achieve fertilization in vitro. Year introduced: 1979. (MeSH)

10. tissue culture

Tissue Culture Techniques
A technique for maintaining or growing TISSUE in vitro, usually by DIFFUSION, perifusion, or PERFUSION. The tissue is cultured directly after removal from the host without being dispersed for cell culture. Year introduced: 2005. (MeSH)
Another one that was defined long before the "year introduced"

Tissue culture does not "disperse the tissue for cell culture." But cell culture is used in breeding and the process of culturing cells can stimulate genetic variability that can provide further breeding material. Cell culture can be a means of generating "natural" genetic variability under "unnatural" conditions along the lines of mutagenesis. See selected citations D. Here are related definitions.

Cell Culture Techniques
Methods for maintaining or growing CELLS in vitro.Year introduced: 2005 (1996) (MeSH)

Primary Cell Culture
The initial culturing of cells derived directly from fresh TISSUES. Year introduced: 2012 (MeSH)

Batch Cell Culture Techniques
Methods for cultivation of cells, usually on a large-scale, in a closed system for the purpose of producing cells or cellular products to harvest. Year introduced: 2012. (MeSH)

Part B
Contemporary breeding methods that may be candidates as "excluded methods", but may not be.

This section was compiled by looking through critiques of the current organic standards regarding transgenic technologies and from there following the discussions on the internet. A couple of other terms are relevant to the work of the Vaccines Working Group. One science blogger in particular (von Mogel 2010) dissects the rationale Jim Riddle presented in 2010 for why genetic engineering is incompatible with organic farming. Von Mogel includes a figure that purports to show relative risks of unintended consequences by breeding method (National Academies 2004). The figure is shown in Appendix II.

This is a list of other emerging breeding strategies that may need to be included in the recitation of "excluded methods." These are:

- Gene silencing—occurs naturally and may also be engineered

Silencing
Shutdown of transcription of a gene, usually by methylation of C residues. (National Academies 2004 glossary)

- Embryo rescue

Embryo rescue
A sequence of tissue culture techniques used to enable a fertilized immature embryo resulting from an interspecific cross to continue growth and development, until it can be regenerated into an adult plant. (National Academies 2004 glossary)

This fairly common technique is used to clean plant tissue from viruses (such as potatoes) and is quite beneficial to organic agriculture.

- Microinjection—clearly an excluded method. Need to be specified?

Microinjection
Introduction of DNA into a cell by injection through a very fine needle. (National Academies 2004 glossary)

- Biolistic transfer—already covered in the definition of excluded methods. Need to be specific? Also known as *microprojectile bombardment.*

Biolistic device
A device that bombards target cells with microscopic DNA-coated particles. Familiarly known as the Gene Gun, it was first developed in the early 1980s. (National Academies 2004 glossary)

- Somaclonal variation (analogous to mutagenesis in that it is a form of natural genetic variation forced by unnatural conditions [cell culture])

Somaclonal variation
Epigenetic or genetic changes, sometimes expressed as a new trait, resulting from in vitro culture of higher plants. (National Academies 2004 glossary)

- Transposons — naturally occurring, double stranded DNA sequences with a defined structure. They are present in plant, animal and bacterial species.

Transposons

Each end of the transposon includes inverted repeats. In prokaryotes, the internal structure includes at least one gene for transposase and may contain many more depending upon the type of transposon. When the transposase gene is expressed, the protein binds to the inverted repeats of the transposon, cleaves the genomic DNA and excises the transposon. Transposase can then cleave the genomic DNA at another spot and recombine the transposon into a new position in the genome. By moving from one location to another in the genome, transposons can cause gene deletions or change expression patterns through gene deletion, resulting in changed phenotypes. (Ivics, Z. and Z. Ivsvak. 2010; MeSH)

Transposons were initially identified as jumping genes by Barbara McClintock in research on variegation of corn kernels in the 1930's (Pray & Zhaurova, 2008). More recently researchers have used transposons as a vector for inserting specific foreign genes into the genomes of various species. The transposon system called "Sleeping Beauty" was used to genetically modify swine cells with genes from rice (Carlson et. al., 2011). The Vaccine Working Group has more detail on transposons as used to produce vaccines.

- Transduction— while this theoretically could occur in nature, the specific purpose of its intentional use is in biotechnology applications.

Transduction
The process through which the genomes of bacteria can be modified with the use of bacterial virus, called a phage. Some types of phage attach to the bacterial cell wall and insert the viral genome into the cell. The viral genome may then be inserted into the bacterial genome through a recombination event which is part of the lysogenic cycle. After receiving a trigger, the viral genome will be excised and the lytic cycle will be triggered (MeSH).

This method can stably introduce genetic mutations into the new bacteria. This technique is widely used to create gene deleted vaccine products.

Discussion Questions
The GMO ad hoc Subcommittee is seeking response from the organic community on the issues presented in this discussion. A few of the questions to be addressed are:

1. Does the definition of "excluded methods" in the Organic Rule need to be revised? Please provide reasoning for either a "yes" or a "no" answer.

2. On what general principle(s) should practical and consistent distinctions be made between “excluded” and permitted methods of breeding that could apply to plants, animals and micro-organisms? Under such general principles should we further define or replace terms such as "natural conditions" and "traditional breeding"?

3. Are there other terms beyond those discussed here that should be addressed in the context of excluded methods?

4. Of the terms and practices discussed here, which ones should be in the definition of excluded methods and which not excluded? Why?

5. How far back into the development or manufacture of a substance, or in the development of vaccines, or in the lineage of a breeding line, should the excluded methods prohibition apply? How far back is practical and verifiable?

Subcommittee Vote
Motion to adopt the proposed Discussion Document on Excluded Methods

Motion by: Jean Richardson Second: Colehour Bondera
Yes:7 No:0 Absent:0 Abstain:0 Recuse:0

Appendix I. Other GE and GMO definitions

USDA Advisory Committee on Biotechnology and 21st Century Agriculture (AC21)
http://www.usda.gov/documents/ac21_report-enhancing-coexistence.pdf

—**Genetically Engineered** is meant to include biotechnology-derived organisms produced through the application of 1) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles or 2) fusion cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection.

Codex Alimentarius Commission, International Food Standards
http://www.codexalimentarius.org/standards/list-of-standards/en/

CAC/GL 44-2003
PRINCIPLES FOR THE RISK ANALYSIS OF FOODS DERIVED FROM MODERN BIOTECHNOLOGY
Adopted in 2003. Amendments 2008, 2011

SECTION 2 – SCOPE AND DEFINITIONS

7. The purpose of these Principles is to provide a framework for undertaking risk analysis on the safety and nutritional aspects of foods derived from modern biotechnology. This document does not address environmental, ethical, moral and socio-economic aspects of the research, development, production and marketing of these foods3.

8. The definitions below apply to these Principles:

"Modern Biotechnology" means the application of:

i) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or

ii) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection[4].

[3] This document does not address animal feed and animals fed such feed except insofar as these animals have been developed by using modern biotechnology.

[4] This definition is taken from the Cartagena Biosafety Protocol under the Convention on Biological Diversity.

European Union
Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 Deliberate Releases into the Environment of Genetically Modified Organisms
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001L0018:20080321:EN:PDF

Article 2

Definitions - (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;

ANNEX I A
TECHNIQUES REFERRED TO IN ARTICLE 2(2)
Techniques of genetic modification referred to in Article 2(2)(a) are inter alia:

(1) recombinant nucleic acid techniques involving the formation of new combi- nations 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 fertilization,
(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.

Canadian Standards:

CAN/CGSB-32.315-2004, Voluntary Labelling And Advertising of Foods That Are and Are Not Products of Genetic Engineering
http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/032-0315/index-eng.html

Genetic engineering (Génie génétique): Refers to techniques by which the genetic material of an organism is changed in a way that does not occur naturally by multiplication and/or natural recombination. Examples of the techniques used in genetic engineering include but are not limited to the following:

1. recombinant DNA (rDNA) techniques that use vector systems
2. techniques involving the direct introduction into the organism of hereditary materials prepared outside the organism
3. cell fusion (including protoplast fusion) or hybridization techniques that overcome natural physiological, reproductive, or recombination barriers, where the donor cells/protoplasts do not fall within the same taxonomic family

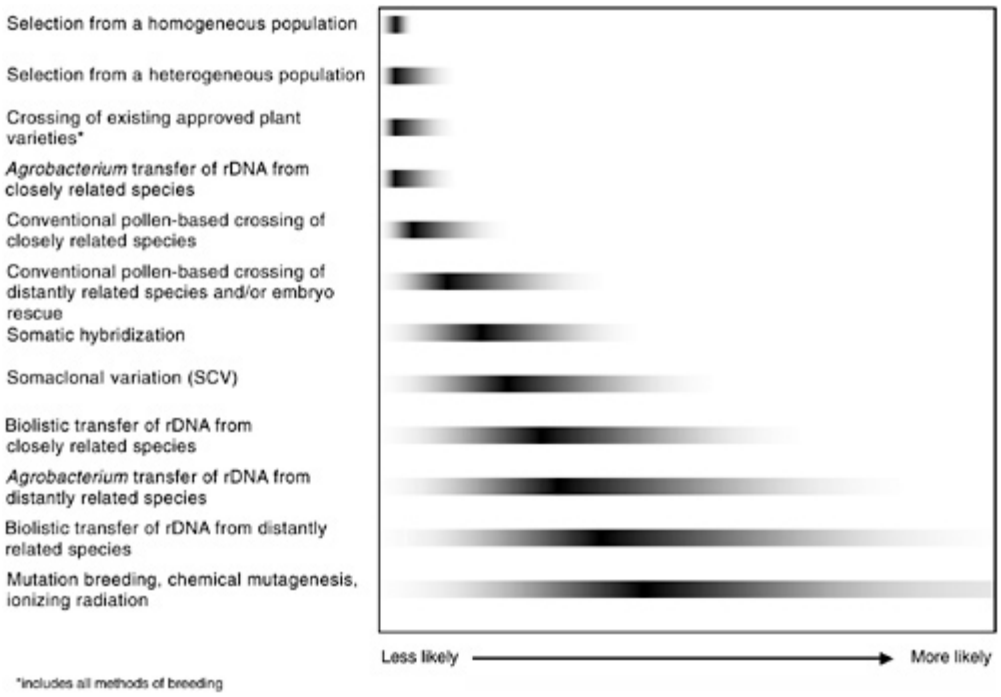
Unless the donor/recipient organism is derived from any of the above techniques, examples of excluded techniques include but are not limited to the following:

1. in vitro fertilization
2. conjugation, transduction, transformation, or any other natural process
3. polyploidy induction
4. mutagenesis
5. cell fusion (including protoplast fusion) or hybridization techniques where the donor cells/protoplasts fall within the same taxonomic family.

Note: (Descriptions of most of these techniques are found in Appendix A.)
Appendix A
Cell fusion (Fusion cellulaire): The fusing of two cells to form a single cell.
Macroinjection (Macro-injection): The introduction of larger molecules into single cells.
Microencapsulation (Micro-encapsulation): The enclosure of small DNA molecules into a capsule, which could be any fatty, fibrous, or membranous structure.
Microinjection (Micro-injection): The introduction of DNA or other compounds into single cells with a microscopic needle.
Mutagenesis (Mutagenése): The induction of genetic mutation through chemical, physical, or radiation treatment, causing nucleotide(s) of the exposed organism's DNA to be altered. This occurs naturally at a very low rate of occurrence, or can be accelerated with in vitro methods.
Plasmid (Plasmide): A circular DNA molecule found in bacteria. Plasmids can transfer genes between bacteria and are important transformation tools.
Polyploidy (PolyploÏdie): The condition where more than two copies of chromosomes are present within a cell - this is caused either by the prevention of cell division or by reproduction of extra copies of chromosomes.
Protoplast fusion (Fusion de protoplastes): The fusing of two protoplasts (a bacterial or plant cell deprived of its cell wall but having an intact plasma membrane).
Recombinant DNA (rDNA) techniques (Techniques de l'ADN recombinant): The transfer, in vitro, of spliced genes between different organisms of the same or different species, or the transfer of synthetic genes, which in turn changes the heritable traits of the organism. Such transfer of genes can be accomplished using vector systems or by direct introduction using a number of techniques including but not limited to chemoporation, electroporation, liposome fusion, macroinjection, microencapsulation, microinjection, and transduction.
Taxonomic family (Famille taxonomique): An orderly classification of living organisms according to their presumed natural relationships, in which a group of related living organisms form a category ranking above a genus and below an order, and usually comprising several to many genera.

Transduction (Transduction): The transfer of DNA from one micro-organism to another via a virus that infects bacteria.
Transformation (Transformation): A process whereby a cell incorporates foreign DNA into its genome.
Vector (Vecteur): An organism, plasmid, or virus that is used to deliver selected foreign DNA into a host cell.

Appendix II. Graph from National Academies study assessing the safety of genetically engineered foods, p. 64. (National Academies 2004).



References

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Selected citations and abstracts
A. cell fusion, somatic cell hybridization, somatic hybridization, protoplast fusion

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Somatic hybrids produced by protoplast fusion between S. tuberosum and S. brevidens: phenotypic variation under field conditions. S. Austin, M. K. Ehlenfeldt, M. A. Baer and J. P. Heigeson. Theor Appl Genet (1986) 71:682-690.
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Somatic hybrids between Solarium brevidens and Solarium tuberosum: Expression of a late blight resistance gene and potato leaf roll resistance. (USA 1986)
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Somatic hybridization in citrus: An effective tool to facilitate variety improvement.
J. W. Grosser, P. Ollitrault, O. Olivares-Fuster. In Vitro Cellular & Developmental Biology - Plant November–December 2000, Volume 36, Issue 6, pp 434-449
Download PDF (354 KB)
Summary: **Citrus somatic hybridization and cybridization via protoplast fusion has become an integral part of citrus variety improvement programs worldwide.** Citrus somatic hybrid plants have been regenerated from more than 200 parental combinations, and several cybrid combinations have also been produced. Applications of somatic hybridization to citrus scion improvement include the production of quality tetraploid

breeding parents that can be used in interploid crosses to generate seedless triploids, and the direct production of triploids by haploid + diploid fusion. ....Several allotetraploid somatic hybrid rootstocks are performing well in commercial field trials, and show great promise for tree size control. Seed trees of most of these somatic hybrid rootstocks are producing adequate nucellar seed for standard propagation. Somatic hybridization is expected to have a positive impact on citrus cultivar improvement efforts.

B. microencapsulation, macroencapsulation

Microencapsulation: Methods and Industrial Applications. By Simon Benita.
http://books.google.com/books?hl=en&lr=&id=sz-669oFo6AC&oi=fnd&pg=PP1&dq=pesticide+microencapsulation&ots=_x0RTSoIAI&sig=mJQZ6Ox-CfpRkBpASH8YzS6CJ90#v=onepage&q=pesticide%20microencapsulation&f=false
Chapter 2. Advances in the Technology for Controlled-Release Pesticide Formulations

Microencapsulation: Is listed under Drug Compounding (MeSH)
The preparation, mixing, and assembling of a drug. (From Remington, The Science and Practice of Pharmacy, 19th ed, p1814)

Sher et al. 1999. Microencapsulation of pesticides by interfacial polymerization utilizing isocyanate or aminoplast chemistry.http://onlinelibrary.wiley.com/doi/10.1002/%28SICI%291096-9063%28199812%2954:4%3C394::AID-PS829%3E3.0.CO;2-S/abstract - fn1
Summary: Interfacial polymerization microcapsulation processes based on isocyanate or aminoplast chemistry, where all wall-forming reactants are placed in the dispersed oil phase are described.
Pesticide microcapsule formulations can be used to reduce mammalian toxicity and extend activity, to control evaporation, to reduce phytotoxicity, to protect pesticide from rapid environmental degradation, to reduce leaching and to reduce pesticide levels in the environment.
http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1096-9063(199812)54:4%3C394::AID-PS829%3E3.0.CO;2-S/abstract

Microencapsulation is a cell-based method of gene therapy, using genetically-modified cells to provide a novel protein for the treatment of various inherited or somatic diseases. In contrast to conventional gene therapy using viruses to transfer therapeutic genes into the patients' own cells, this method uses universal cell lines genetically engineered to secrete high levels of the therapeutic gene product. These cells are implanted within immuno-protective microcapsules into patients to act as a continuous source of the desired gene product, thus providing a potentially safer, reversible and more economic treatment than viral gene therapy. http://fhs.mcmaster.ca/gene/

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Macroencapsulation—MeSH search gives no results

Patrick Aebischer (http://len.epfl.ch/) is cited as the developer of macroencapsulation, which appears to be a packaging technology for genetic therapies. From Neuronal Degeneration and Regeneration: From Basic Mechanisms to Prospects. By F W Van Leeuwen.
http://books.google.com/books?id=516KNxhxd8UC&pg=PA518&lpg=PA518&dq=Macroencapsulation+genetic&source=bl&ots=eoaX_4ZZv7&sig=ngahF3GMkDNcpOXNk6tedviHcXE&hl=en&sa=X&ei=qki8UKPCJ6mu0AGgrYDwDg&ved=0CE4Q6AEwBDgU#v=onepage&q=Macroencapsulation%20genetic&f=false

C. Mutagenesis

Wieczorek, A. M. & Wright, M. G. (2012) History of Agricultural Biotechnology: How Crop Development has Evolved. Nature Education Knowledge 3(10):9. Cites FAO as stating that "More than 2,500 plant varieties (including rice, wheat, grapefruit, lettuce and many fruits) have been developed using radiation mutagenesis."
http://www.nature.com/scitable/knowledge/library/history-of-agricultural-biotechnology-how-crop-development-25885295

Falk, R. 2010. Mutagenesis as a Genetic Research Strategy. Genetics. August; 185(4): 1135–1139. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2927745/

Alonso et al. 2003. Genome-Wide Insertional Mutagenesis of Arabidopsis thaliana. Science 301 (5633): 653-657. Abstract online at
http://stke.sciencemag.org/cgi/content/abstract/sci;301/5633/653

D. cell culture, somaclonal variation

Somaclonal variation — a novel source of variability from cell cultures for plant improvement. P. J. Larkin, W. R. Scowcroft. Theoretical and Applied Genetics 16. X. 1981, Volume 60, Issue 4, pp 197-214.
Abstract: It is concluded from a review of the literature that **plant cell culture itself generates genetic variability (somaclonal variation).** Extensive examples are discussed of such variation in culture subclones and in regenerated plants (somaclones). A number of possible mechanisms for the origin of this phenomenon are considered. The phenomenon may be employed to enhance the exchange required in sexual hybrids for the introgression of desirable alien genes into a crop species. It may also be used to generate variants of a commercial cultivar in high frequency without hybridizing to other genotypes.
http://link.springer.com/article/10.1007%2FBF02342540?LI=true

Somaclonal variation - Genetic basis and breeding applications. David A. Evans.
Somaclonal variation, the recovery of genetic changes in plants regenerated from tissue culture, offers an opportunity to uncover natural variability and to use this variability for the development of new varieties. This review focuses on the unique variation generated by this technique and the current use of somaclonal variation to develop new plant varieties.
Abstract—pay for full text.
http://www.sciencedirect.com/science/article/pii/0168952589900218

Somaclonal variation as a tool for crop improvement. Angela Karp. Euphytica

February 1995, Volume 85, Issue 1-3, pp 295-302. Abstract: Somaclonal variation is a tool that can be used by plant breeders. The review examines where this tool can be applied most effectively and the factors that limit or improve its chances of success.... Somaclonal variation is cheaper than other methods of genetic manipulation. At the present time, it is also more universally applicable and does not require ‘containment’ procedures. It has been most successful in crops with limited genetic systems and/or narrow genetic bases, where it can provide a rapid source of variability for crop improvement.
Full article online at http://link.springer.com/article/10.1007%2FBF00023959?LI=true