National Organic Standards Board
Materials/GMO Subcommittee Proposal
Excluded Method Determinations October 2018
August 14, 2018

Introduction and background

At the November 18, 2016 in-person NOSB meeting, the NOSB recommended that the National Organic Program (NOP) develop a formal guidance document for the determination and listing of excluded methods. This 2016 recommendation, entitled "Excluded Methods Terminology," clarified the excluded methods definitions and criteria in response to increasing diversity in the types of genetic manipulations performed on seed, livestock and other biologically-based resources used in agriculture. Genetic engineering is a rapidly expanding field in science. The NOSB recognizes the need to continually add methods to the list for review and to determine if the methods are or are not acceptable in organic agriculture. In addition to the 2016 recommendation, a discussion document provided a list of technologies needing further review to determine if they should be classified as excluded methods or not. At the Fall 2017 NOSB in-person meeting, the NOSB passed a recommendation to add three technologies as excluded methods to the NOP guidance document.

Goals of this proposal/document

This proposal for the October 2018 NOSB meeting addresses three additional methods listed as "To Be Determined" in the November 2016 discussion document. Using the NOSB's proposed improved definitions of excluded methods, the NOSB Materials Subcommittee identified one technology as an excluded method in organic agriculture and another technology as a method that should not be excluded in organic agriculture.

Public comment at numerous NOSB meetings over the years continues to stress the view that technologies used to manipulate the genetic code in a manner that is outside traditional plant and animal breeding should remain prohibited in organic production. Among all of the organic stakeholders, there is a strong belief that genetic engineering is a threat to the integrity of the organic label. Both organic producers and consumers reject the inclusion of genetic engineering in organic production. This document represents the continuing work of the NOSB to clarify which methods, in the expanding field of genetic engineering, can be used under the USDA organic seal.

Definitions

The NOSB previously recommended the use of the following definitions to determine whether or not a method should be/is excluded.

Genetic engineering (GE) – A set of techniques from modern biotechnology (such as altered and/or recombinant DNA and RNA) by which the genetic material of plants, animals, organisms, cells and other biological units are altered and recombined.

Genetically Modified Organism (GMO) – A plant, animal, or organism that is from genetic engineering as defined here. This term will also apply to products and derivatives from genetically engineered sources. (Modified slightly from IFOAM Position)

Modern Biotechnology – (i) in vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or (ii) fusion of cells beyond the taxonomic family, that overcomes natural, physiological reproductive or recombination barriers, and that are not techniques used in traditional breeding and selection. (From Codex Alimentarius)

Synthetic Biology – A further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems. (Operational Definition developed by the Ad Hoc Technical Expert Group on Synthetic Biology of the UN Convention on Biological Diversity)

Non-GMO – The term used to describe or label a product that was produced without any of the excluded methods defined in the organic regulations and corresponding NOP policy. The term "non-GMO" is consistent with process-based standards of the NOP where preventive practices and procedures are in place to prevent GMO contamination while recognizing the possibility of inadvertent presence.

Classical/Traditional plant breeding – Classical (also known as traditional) plant breeding relies on phenotypic selection, field-based testing and statistical methods for developing varieties or identifying superior individuals from a population, rather than on techniques of modern biotechnology. The steps to conduct breeding include: generation of genetic variability in plant populations for traits of interest through controlled crossing (or starting with genetically diverse populations), phenotypic selection among genetically distinct individuals for traits of interest, and stabilization of selected individuals to form a unique and recognizable cultivar. Classical plant breeding does not exclude the use of genetic or genomic information to more accurately assess phenotypes, however the emphasis must be on whole plant selection.

Criteria

Below are the criteria listed in the previous NOSB recommendations to determine if methods should be excluded:

1. The genome is respected as an indivisible entity and technical/physical insertion, deletions, or rearrangements in the genome is refrained from (e.g. through transmission of isolated DNA, RNA, or proteins). *In vitro* nucleic acid techniques are considered to be invasion into the plant genome.
2. The ability of a variety to reproduce in species-specific manner has to be maintained and genetic use restriction technologies are refrained from (e.g. Terminator technology).
3. Novel proteins and other molecules produced from modern biotechnology must be prevented from being introduced into the agro-ecosystem and into the organic food supply.
4. The exchange of genetic resources is encouraged. In order to ensure farmers have a legal avenue to save seed and plant breeders have access to germplasm for research and developing new varieties, the application of restrictive intellectual property protection (e.g., utility patents and licensing agreements that restrict such uses to living organisms, their metabolites, gene sequences or breeding processes are refrained from).

The NOSB has voted and determined these to be excluded methods.

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Targeted genetic modification (TagMo) syn. Synthetic gene technologies syn. Genome engineering syn. Gene editing syn. Gene targeting	• Sequence-specific nucleases (SSNs) • Meganucleases Zinc finger nuclease (ZFN) • Mutagenesis via Oligonucleotides • CRISPR-Cas system (Clustered regularly interspaced short palindromic repeats) and associated protein genes • TALENs (Transcription activator-like effector nucleases) • Oligonucleotide directed mutagenesis (ODM) Rapid Trait Development System	YES	1, 3, 4	Most of these new techniques are not regulated by USDA and are currently difficult to determine through testing.
Gene Silencing	RNA-dependent DNA methylation (RdDM) Silencing via RNAi pathway RNAi pesticides	YES	1, 2, 4	
Accelerated plant breeding techniques	Reverse Breeding Genome Elimination FasTrack Fast flowering	YES	1, 2, 4	These may pose an enforcement problem for organics because they are not detectable in tests.
Synthetic Biology	Creating new DNA sequences Synthetic chromosomes Engineered biological functions and systems	YES	1, 3, 4	
Cloned animals and offspring	Somatic nuclear transfer	YES	1, 3	
Plastid Transformation		YES	1, 3, 4	

Cisgenesis		YES	1, 3, 4	Even though the genetic manipulation may be within the same species; this method of gene insertion can create characteristics that are not possible within that individual with natural processes and can have unintended consequences.
Intragenesis		YES	1, 3, 4	Even though the genetic manipulation may be within the same species; this method of gene rearrangement can create characteristics that are not possible within that individual with natural processes and can have unintended consequences.
Agro-infiltration		YES	1, 3, 4	*In vitro* nucleic acids are introduced to plant leaves to be infiltrated into them. The resulting plants could not have been achieved through natural processes and are a manipulation of the genetic code within the nucleus of the organism.

The following genetic engineering methods were found by the NOSB NOT to be excluded methods.

Method and synonyms	**Types**	**Excluded Methods**	**Criteria Applied**	**Notes**
Marker Assisted Selection		NO		
Transduction		NO		

Discussion

The Materials Subcommittee recognizes the topic of genetic engineering and evaluation of excluded methods will remain on our work agenda to determine if new technologies do or do not meet our current definitions. We may also need to incorporate additional criteria into our current definitions to evaluate new and unique technologies.

We are aware that specific laboratory tests are not currently available to detect the use of several new excluded genetic modification technologies in organisms. However, we still believe that the technology should be listed as an excluded method, when appropriate, and anticipate the development of tests or other methods that can detect the presence of these technologies. The Materials Subcommittee may put forward another discussion document in the future to aid the NOP in determining how to enforce this prohibition when there is no means to detect an excluded method was used in production.

Public comment received has been positive regarding the listing of all proposed excluded and non-excluded methods listed above. At the November 2017 meeting, when three new items were added to the excluded methods list, there were numerous comments requesting a clear description of the methods used in these excluded technologies. The three items added were cisgenesis, intragenesis, and agro-infiltration. Basic descriptions of these technologies are described below.

- Cisgenesis—the intact DNA of a plant is directly modified through gene transfer, and the integrity of the nuclear genome is disturbed. The introduced gene is from the same taxonomic family. Cisgenesis is a form of genetic engineering where genes are artificially transferred from the same species, or between closely related organisms. Unpredictable outcomes can occur through gene transfer, random or otherwise, even within the same species, through the introduction of intended or unintended change to genetic sequences and unintended insertions of novel bacteria, viruses or DNA to the host.
- Intragenesis—the intact DNA of a plant is directly modified through gene transfer, and the integrity of the nuclear genome is disturbed. The introduced gene or multiple genes are from the same taxonomic family, and the gene sequence may be rearranged. The same inherent risks of cisgenesis caused by the unpredictable outcomes of gene modification technology, are also risks associated with intragenesis.
- Agro-infiltration—in vitro nucleic acids, usually via a bacterial transporter, are introduced to plant leaves by direct injection or by vacuum infiltration into the plant. This method manipulates the genetic code within the nucleus of the organism. The goal is to produce a desired protein not present in the plant. The bacteria can make a hole in the cell wall, move into the nucleus of the plant and integrate into the plants' chromosomes. Stable integration into the plant's cell structure is not guaranteed. Agro-infiltration can also be used to silence specific genetic traits, although exact knowledge of how this is working at a genetic level is not currently known.

Proposal

Two items were considered for this proposal. One item was determined to be an excluded method depending on the method used to cause the change to the organism. The other method was determined to not be an excluded method.

Transposons, when produced from chemicals, ultraviolet radiation or other synthetic methods, are to be added to the list of excluded methods.

- **Transposons** are jumping genes that occur in nature and are responsible for mutations. Transposon activity can be modified to increase mutation rates. This can be done by chemicals or by physical

stress like drought or heat. Changes or mismatches to the individual nucleotides occurs, altering the cell's genetic identity and genome size. When the transposon cleaves from its original location to another location, there is also a change to the genetic makeup at the site where it no longer resides.

- IFOAM's 2018 position paper on Techniques in Organic Systems considers transposons caused by physical stress to be compatible with organic systems.
- The NOSB livestock subcommittee discussed transposons in an August 2014 Memorandum to the NOP on Livestock Vaccines Made with Excluded Methods. Transposons were described as follows in that document.

  Transposons, also called transposable elements, are naturally occurring, double stranded DNA sequences with a defined structure. 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. Genes for antibiotic resistance, one example of the types of transposon genes, occur both naturally and sometimes as a marker in lab modified transposons. 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.

The method below has been determined to be an excluded method based upon the criteria listed above.

Method and synonyms	Types	Excluded Methods	Criteria Used	Notes
Transposons		YES - when produced by any means other than physical stress	1, 3, 4	Can be naturally occurring due to drought, heat or other means of physical stress, are not an excluded method. Transposons produced through chemical, artificial ultra violet radiation or other synthetic stress or interaction is considered to be an excluded method.

The following technology was found to not be an excluded method.

- **Embryo rescue in plants**—a technique that recovers plants from sexual crosses in which the majority of the embryos cannot survive *in-vivo* or may have gone dormant. The method helps to overcome embryo unviability, due to inherent weakness, immaturity or hybrids that degenerate. This method can combine desirable traits of complementary parents, such as in the development of seedless fruits. This method aids in shortening the breeding cycle. Embryos are placed in a controlled sucrose culture, for specific times and under controlled temperatures and light, to aid in the successful recovery of viable plants. The IFOAM position paper "Compatibility of Breeding Techniques in Organic Systems states, "In order to improve frequency of progeny of wide crosses, the embryo is transferred to artificial media. The embryo is derived from natural fusion of an egg and pollen cell. However, in wide crosses, the endosperm is often not well developed to feed the embryo. This method was used to produce triticale (Triticum aestivum x Secale cerale)." IFOAM also states this is compatible with organic systems.

Method and synonyms	Types	Excluded Methods	Criteria Used	Notes
Embryo rescue in plants		No		IFOAM's 2018 position paper on Techniques in Organic Systems considers this technique compatible with organic systems.

The following methods will continue to be researched.

Terminology				
Method and synonyms	Types	Excluded Methods	Criteria Used	Notes
Protoplast Fusion		*TBD*		There are many ways to achieve protoplast fusion, and until the criteria about cell wall integrity are discussed and developed, these technologies cannot yet
Cell Fusion within Plant Family		*TBD*		Subject of an NOP memo in2013. The crops subcommittee will continue to explore the issue of detection and testing.
TILLING	Eco-TILLING	*TBD*		Stands for "Targeted Induced Local Lesions In Genomes." It is a type of mutagenesis combined with a new screening procedure.
Doubled Haploid Technology (DHT)		*TBD*		There are several ways to make double haploids, and some do not involve genetic engineering while some do. It is difficult or impossible to detect DHT with tests.
Induced Mutagenesis		*TBD*		This is a very broad term and needs to be classified based on what induces the mutations, such as chemicals, radiation, or other stresses.
Embryo transfer in animals	Embryo rescue in animals	*TBD*		FiBL distinguishes embryo rescue in plants from animals. A technique used in animal breeding, FiBL involves inducing superovulation of the donor with gonadotropins (glycoprotein polypeptide hormones), artificial insemination, recovery of embryos, isolation and storage of embryos, and transfer of embryos into an animal, which results in a pregnancy and hopefully a birth of a live animal at maturity. More research is needed to clarify if use of hormones is essential to this technique.

Future Work on this Topic

The Materials Subcommittee will discuss embryo transfer in animals, study the various methods of induced mutagenesis, and will review the NOP February 2013 policy memorandum on cell fusion techniques used in seed production. We will continue to review new technologies as they are introduced to the marketplace, such as GAANTRY (Gene Assembly in Agrobacterium by Nucleic acid Transfer using Recombinase Technology*),* as additions to our list of methods for discussion and classification.

Subcommittee Proposal:

The NOSB recommends the NOP add the following to the table of excluded or not excluded methods in the NOP excluded methods guidance.

1. "Transposons, when produced from chemicals, artificial ultraviolet radiation or other synthetic methods," is to be added to the table listing excluded methods.
2. "Embryo rescue in plants" should be listed "not an excluded method".

Subcommittee Vote:

Motion to accept the proposal on excluded methods as stated above
Motion by: Harriet Behar
Second: Dan Seitz
Yes: 4 No: 0 Absent: 3 Abstain: 0 Recuse: 0

Approved by Harriet Behar, Materials Subcommittee Chair, to transmit to NOSB August 21, 2018