National Organic Standards Board
Materials/GMO Subcommittee
Discussion Document on Excluded Methods Terminology
August 22, 2014

The Materials/GMO subcommittee is posting this discussion document a second time to allow for more public input. Those who commented during the last posting do not need to re-submit comments. The Materials Subcommittee thinks that because this is a complicated subject it would be beneficial for other stakeholders to participate in the conversation so as to collect as much input as possible before they proceed.

Introduction and Scope

A year ago the project was started to grapple with the definition of "excluded methods" in the USDA organic regulations. This is the definition that appears in the rule (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)

The definition was based on the best efforts of the NOSB in 1995 and has provided adequate guidance to prohibit the use of the most obvious genetically engineered crops such as herbicide-resistant corn and soybeans and Bt cotton, as well as prohibit processing inputs such as genetically engineered yeasts and enzymes. However, this definition contains terms that are unclear, outdated and incomplete in light of new methods of recombinant DNA technology that have emerged since the definition was first adopted in 1995.

In 2011 and 2012 a number of confusing issues came before the NOSB and to the NOP which made it necessary to revisit the definition. These include genetically engineered vaccines for livestock, the use of cell fusion within plant families to create male sterility in brassica hybrids, whether or not GMOs could be used in biodegradable bioplastic mulches, and the question of whether mutated algae might therefore be genetically engineered. The current definition is inadequate to clarify these issues.

In 2013, NOSB first Discussion Document on excluded methods,[1] each of the terms in the above definition was discussed further, terms involved in traditional breeding, such as mutagenesis and conjugation, were defined and discussed, and new terms that may be considered to be genetic engineering were brought up. No conclusions were suggested except that there is a need to do more work on the subject. The discussion questions posed asked commenters to suggest principles on which to base GE distinctions, to offer opinions on what terms were and were not excluded methods, and to bring forward new terms that may need consideration. A list of the terms brought up is in Appendix 1.

[1] NOSB 2013. Excluded Methods Terminology Discussion Document. April 2013. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5102656

The NOSB received about 16 substantive public comments on the first discussion document, and also many general comments about keeping GMOs out of organic agriculture. The intention of this Second Discussion Document is to summarize the substantive public comments received on the previous one and to propose some further questions to move forward the issue of strengthening the Excluded Methods Terminology. The goal, as this effort continues, is to have concrete determinations for the National Organic Program, Accredited Certifiers, and organic producers to use in keeping GMOs out of organic food and farms.

This Discussion Document builds onto where the other one left off. The sections below titled "Relevance to Rulemaking", "Comments on Definition(s), Principles, and Criteria", "Process or Product" and "European Approaches" are all summaries of information that was submitted through public comments. The subsequent "Discussion" section includes the NOSB subcommittee analysis of the issues brought up. Finally, the questions at the end aim at collecting more input from the public on how to proceed.

Note: The Subcommittee recognizes that the usual public comment time period is not long enough to fully circulate, digest, discuss and respond to these issues. We strongly urge the NOP to create the ability for longer comment periods as was adopted by the NOSB in its Public Communications Recommendation on April 10, 2013.

Relevance to Rulemaking

In our first Discussion Document we did not state whether the subcommittee was proposing a change in the regulation or to address this subject through guidance.

Several commenters pointed out the language from the Senate report that accompanied OFPA, which was quoted in the first proposed rule. (62 Fed. Reg. 65850, 65875)

> While the OFPA mandates that the Secretary develop organic standards, it is silent on the issue of genetically engineered organisms (GEOs) and their products. However, the accompanying Senate report language states that ‘‘as time goes on, various scientific breakthroughs, including biotechnology techniques, will require scrutiny for their application to organic production. The committee is concerned that production materials keep pace with our evolving knowledge of production systems.[2]’’

This reference from the Senate report was quoted in the first proposed rule somewhat out of context. It appears to have been used by the congress as justification for a registration program for organic materials that was subsequently removed from the conference report. However, it implies the same need for flexibility as quoted below from the rule's preamble.

From the preamble to the current rule (65 Fed. Reg. 13512, 13521):

> We recognize that the phrases, ‘‘natural conditions or processes’’ and ‘‘not considered compatible with organic production,’’ may be subject to interpretation.
> ....

[2] U.S. Senate. 1990. Food, Agriculture, Conservation and Trade Act of 1990 - Report to Accompany S2830. Rpt 101-357, 101st Congress, 2nd Session. Government Printing Office, Washington, DC.

We recognize that industry and consumer expectations regarding the products of these techniques in organic production systems may evolve. We believe that, taken together, these phrases allow for a degree of flexibility to ensure that our regulations continue to accurately reflect industry practices and consumer preferences. In cases where questions may arise regarding a specific technique, we anticipate that such questions would be resolved by the Administrator based on recommendations from the NOSB.

The Materials/GMO subcommittee has discussed this issue and believes that NOP Guidance is the most appropriate form for any clarifications and interpretations to be made regarding excluded methods, for the very reasons mentioned by the Senate and the NOP.

Comments on Definition(s), Principles, and Criteria

This section is in two parts. Part 1 summarizes the public comment regarding principles and criteria to consider in clarifying or revising the excluded methods definition further. Part 2 consists of the additional terms brought up by commenters with some of their definitions provided. Appendix 1 contains the terms that were defined and discussed in the first Discussion Document.

1. Other definitions related to Excluded Methods to draw from –

A. The Cartagena Protocol definitions (CFS public comment):

"Living modified organism"

"[a] living modified organism is defined as any living organism that has a combination of genetic material obtained through the use of modern biotechnology.

"Modern Biotechnology" (also adopted by Codex Alimentarius):

(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 conventional breeding and selection."[3]

"While this language is more specific, the underlying theme of the definition is the same However, the distinctions presented by the Cartagena protocol definition could also be used to inform a newly created guidance document.[4]"

B. Proposed new definition of Excluded Methods (Dag Falck public comment):

Methods that change the genetic material of an organism through recombining DNA[5] through laboratory methods and in ways that are not dependent on the use of conjugating, sexual or asexual reproduction methods, including transgenic (intraspecific or intergeneric), or cisgenic (intrageneric) transfers of genes. Methods not included in the definition are: other natural, classical, or modern breeding techniques that depend on movement of genes only through a conjugative, sexual or asexual reproduction method with parent gene material from within the same taxonomic family[6].

C. Ethical Criteria (FiBL public comment):

1. The genome is respected as an indivisible entity and technical/physical invasion into the plant genome is refrained from (e.g. through transmission of isolated DNA, RNA, or proteins).

[3] Convention on Biological Diversity. 2013. The Cartagena Protocol on Biosafety. Available at: http://bch.cbd.int/protocol.

[4] Center for Food Safety 2013. Public Comment to NOSB. Docket AMS-NOP-12-0070

[5] http://en.wikipedia.org/wiki/Recombinant_DNA.

[6] Dag Falck, Nature's Path 2013. Public Comment to NOSB. Docket AMS-NOP-12-0070

2. The cell is respected as an indivisible functional entity and technical/physical invasion into an isolated cell on growth media is refrained from (e.g. digestion of the cell wall, destruction of the cell nucleus through cytoplast fusions).
3. The ability of a variety to reproduce in species-specific manner has to be maintained and technologies that restrict the germination capacity of seed-propagated crops are refrained from (e.g. Terminator technology).[7]

D. Operational criteria (Rich Theuer public comment):

It is very helpful that you set forth these operational criteria for implementing the phrase "without the use of excluded methods:"
1. Keeping genetically modified organisms out of organic livestock feed, crops, and food; and
2. Preventing the introduction of novel proteins into soil and water ecosystems.
This is the kind of guidance that certifiers, producers, and handlers can execute.[8]

2. Terms not in the prior Discussion[9]

The descriptions provided here are our best attempts to summarize very technical issues. More information can be found in the cited sources. While some of these techniques may seem to obviously be consistent with the existing excluded methods definition, others are not, and some may or may not be depending on specifics. These are presented only as examples to give readers the context and descriptions of some terms that will be evaluated in our future work.

- Doubled Haploid Technology – A breeding technique used to create homozygous inbred lines in one generation instead of the many required by traditional methods. Used widely in wheat, canola and corn, it involves the following steps: emasculation, pollination, 2,4-D treatment, embryo culture, and colchicine treatment. It often involves crosses between wheat and corn.
- Targeted genetic modification (TagMo) – a collective term for the zinc finger nuclease techniques that create DNA double-stranded breaks at specific genomic locations that can then be used to alter the target gene. The genetic modification would not necessarily involve transfer of nucleic acids from another species, nor would it be easy to detect in a final product. It is unclear how these would be regulated in the U.S.
- "FasTrack" – a breeding scheme that has so far been used in plums where an early-flowering gene from poplar is inserted into a plum tree. When the plum flowers in less than a year, it is crossed with non-transgenic varieties carrying desirable traits. Markers are used to identify the right traits and, at the end of the breeding program, only those are selected that do not have the transgene.
- Synthetic Biology – practitioners generate new DNA sequences the way computer programmers write code, creating new life-forms. Called by one of its founders "genetic engineering on steroids[10]". So far it has been used to generate a yeast that produces a malaria drug and to make synthetic vanilla.

[7] FiBL Research Institute of Organic Agriculture 2013. Public Comment to NOSB. Docket AMS-NOP-12-0070

[8] Richard Theuer 2013. Public Comment to NOSB. Docket AMS-NOP-12-0070

[9] Among many sources used for definitions are the following: Kuzma J, Kokotovich A (2011) Renegotiating GM crop regulation. EMBO reports 12: 883–888; Podevin N, Devos Y, Davies HV, Nielsen (2012) Transgenic or not? No simple answer! EMBO reports 13: 1057 – 1061; Waltz E (2012) Tiptoeing around transgenics. Nature biotechnology 30: 215–217; Wikipedia for each term.

[10] Phillpot, Tom 2014. Now your Food Has Fake DNA in It. Mother Jones http://www.motherjones.com/environment/2014/08/food-fake-dna-synbio-vanilla-ice-cream

- Cisgenics – A genetic modification of a recipient organism with a gene (cisgene) from a crossable (sexually compatible) organism. This is not always interpreted as a prohibited technique because such crossing may be able to occur in nature.
- Intragenesis – genetic modification of a recipient organism that involves the insertion of a reorganized, full or partial coding region of a gene, often with a promoter and/or terminator from another gene of the same or crossable species.
- Plastid transformation – Plastids are semi-autonomous organelles within higher plants with a small, highly polyploid genome. Technology has been developed for genetic modification of this genome independent of nuclear DNA. Currently used commercially in tobacco, and widely researched.[11]
- Gene silencing via RNAi and DNA methylation – Interfering with the regulation of gene expression through inserting methyl groups onto RNA and DNA that then suppress the expression of the gene. Can occur in nature, but is used as a recombinant technique in cancer research and plant breeding.
- RTDS (Rapid Trait Development System) – the next generation precision gene editing technology developed by Cibus company. Similar to the oligonucleotide targeted DNA modification (below) it does not leave behind transgenic material, only uses it to create a change in a precise area of a gene.
- Site directed mutagenesis via oligonucleotides, zinc finger nuclease (ZFN) – an introduction of recombinant DNA through transient molecules that are identified by zinc-finger nucleases, with or without a repair template. The techniques resemble transgenesis but the end products are similar to, and indistinguishable from, conventionally bred plants.
- Agro-infiltration – Similar to the zinc finger nuclease technique above, but using an *Agrobacterium* to inject several foreign DNA molecules into the plant cell.
- Reverse breeding – A process that uses several other techniques such as RNAi to suppress meiotic recombination, tissue culture, and then double haploidization to create parental lines that are homozygous to use in breeding F1 hybrids.
- Embryo transfer of animals – a technique used in animal breeding. It involves inducing superovulation of donor with gonadotropins, artificial insemination, recovery of embryos, isolation and storage of embryos, transfer of embryos back into animals, and then pregnancy.
- Marker Assisted Selection (MAS) – a process whereby a marker is used for indirect selection of a genetic trait. Markers are usually DNA but they can be morphological (such as seed color) or biochemical (specific enzymes). Very commonly in use is the antibiotic resistance marker so that any population can be exposed to antibiotics and the organisms that survive have the marker. This technique may not necessarily be considered genetic engineering in itself, but can be used in conjunction with other transgenic techniques or involve inserting recombinant markers.

Process or Product?

Public commenters offered several papers from Europe that discussed the difference between a processed-based standard for GMOs and a product-based standard. This is relevant to the current discussion because the Federal Rule for organic is based on a process-based approach to all of organic production, yet there are some areas where the process is intertwined with the product or a quantitative tool can be used to assess the validity of a process approach. See discussion section for more.

[11] Maliga, P. 2004. Plastid transformation in higher plants. Annu Rev Plant Biol. 2004;55:289-313. http://www.ncbi.nlm.nih.gov/pubmed/15377222

"The US oversight system was built mostly around the idea that GM plants should be regulated on the basis of characteristics of the end-product and not on the process that is used to create them."[12]

"The first challenge is to make sure that regulatory frameworks remain fit for purpose. However, frameworks that use process-based definitions as a trigger for regulatory oversight might not be functional over time (Sidebar B). Several authors have argued that new biotechnology-based plant breeding techniques might not fit into, or might rapidly outgrow, the established definitions for GMPs *[COGEM 2006 (9), Morris SH 2008 (10) as cited in original]* or other narrowly defined product definitions [*Kuzma, J.2011 (8), Ledford, H. 2011 (11), Waltz, E. 2012 (12) as cited in original]*. NPPs *(new plant products*) blur the sharp distinction between GMP and non-GMP, and introduce a new continuum between genetic engineering and conventional breeding. Process-based legislation will require not only updates to the lists of new biotechnological plant breeding techniques but also debate on their classification as GMP or non-GMP. However, such flexibility is rarely evident in regulatory frameworks.[13]

> ***"Sidebar B*** | Process-based compared with product-based regulatory frameworks[14]
> Process-based regulatory frameworks
>
> Argentina, Brazil, the EU and many other countries have put new process-based regulatory systems in place to regulate the use of genetically modified organisms (GMOs), as the techniques used for their production were thought to raise specific safety concerns. In these jurisdictions, a GMO is mainly characterized by the transformation techniques used in its production. The definitions of GMOs used by these countries are often partly or fully based on those put forward by international organizations such as the United Nations Food and Agricultural Organization (FAO) and international treaties such as the Cartagena protocol.
>
> Product-based regulatory frameworks
>
> Canada and the USA opted to regulate all plants or products with new traits developed either through genetic engineering or any other plant breeding techniques under the same, yet existing, regulatory system [26,27]. The transformation techniques were not considered inherently risky. Therefore, the focus of product-based regulatory systems is on the risks of products and new traits or attributes introduced into a plant, rather than the method of production."

European Approaches to Classifying Genetic Manipulation Methods

The EU has made the distinction between "traditional" breeding methods and conventional (transgenic) breeding.[15]

FiBL submitted a comment that included a chart that describes methods with a yes/no column for compatibility with organic standards for both plants and animals. The NOSB could work on something similar and the methods that receive consensus can be incorporated into guidance. A subset of this chart is presented here as an example:[16]

[12] Kuzma J, Kokotovich A (2011) Renegotiating GM crop regulation. EMBO reports 12: 883–888

[13] Podevin N, et. al. (2012) Transgenic or not? No simple answer! EMBO reports 13: 1057 – 1061

[14] *ibid.*

[15] (Directive 2001/18/EC. and an EU background paper 'Current plant breeding techniques', DOC.XI/464/92.
- Clemens van de Wiel, Jan Schaart, Rients Niks & Richard Visser, "Traditional plant breeding methods", 2010 - http://edepot.wur.nl/141713)

[16] FiBL Research Institute of Organic Agriculture 2013. Public Comment to NOSB. Docket AMS-NOP-12-0070

Method	Excluded (by FiBL)	Why
Embryo rescue	No / YES in animals	Plants: Embryo is maintained on artificial media, but no genetic changes occur. Animals: Embryo transfer on organic farms is rejected, therefore also embryo rescue
Microinjection	YES	Invasive technique that violates integrity of a cell
Biolistic device	YES	Invasive technique that violates integrity of a cell
Somaclonal variation	YES, if artificially introduced	Somaclonal variation results from mutation and is identified during in vitro culture, but might not necessarily be introduced by the tissue culture...
Transposons	Yes if artificially introduced	Transposons are a regulatory element influencing gene silencing and mutation rate. Transposons can be artificially introduced by genetic engineering, see genetic engineering
Transduction	No	Is a natural phenomenon

This type of evaluation in Europe has led to an independent effort to define and certify "Organic Varieties" and even Organic Animal Breeding.[17] In this idea (which has not yet been written into any regulations), only approved non-GMO plant breeding methods would be used to create what could be certified as an Organic Variety or Organically Bred Animal. In this country, a parallel idea has been floated that organically grown seeds be held to different criteria regarding GMOs than conventional seeds, even those not called GMO.[18] If such ideas were adopted, then a set of organic plant breeding standards could be developed, or at least organically produced varieties may be distinguished from other varieties, such as not being able to have used cell fusion for Cytoplasmic Male Sterility (CMS) or double haploid technologies.

Discussion

Definition(s), Principles, and Criteria

The subcommittee likes the definitions regarding biotechnology from the Cartagena Protocol for several reasons. First, it is more specific than the current definition regarding recombinant DNA and direct injections or fusion between families. Second, it is well accepted internationally and therefore provides the NOP with good justification for adopting into guidance. Third, it provides a better framework than the existing definition to further elaborate the various technologies that would be allowed as well as those which would be prohibited. This will be discussed further below.

The definition proposed by Mr. Falck in B above also attempts to make it clearer, but is not as widely accepted or known.

The criteria and principles in comments C and D above are valid points that the subcommittee appreciates the input on. The points raised in D as operational are accepted as part of our goals for how to interpret the principles and definition adopted, but they would be the subsequent step

[17] Neff, A.S. & Augsten, F. 2009. Assessing Reproductive and Breeding Techniques in Organic Agriculture using Cattle Breeding as an Example. FiBL Discussion Paper. Submitted with FiBL public comment to docket AMS-NOP-12-0070

[18] Still, Andrew, 2013. Adaptive Seed Catalog and Seed Ambassadors Blog. http://www.seedambassadors.org/

after the broader issues of definition and principles. Perhaps other operational criteria would be helpful as well. Operational criteria for determining acceptability of crop inputs derived from GMO feedstocks and/or fermenting organisms (potentially including corn gluten meal, corn steep liquor, and biodegradable mulch, for example), disinfectants like alcohols, and processing aids would be appropriate in guidance to certifiers and materials review organizations and/or in the materials listings. The subcommittee will be looking at this point in developing future work plans.

We are posing further discussion questions on some of the criteria raised in the FiBL comment (point C above) to see if these criteria are useful and realistic.

Process or Product?
Since the whole underpinning of the U.S. organic regulations is a process-based system, it would make sense that this concept carry over to defining excluded methods. This is indeed the basis of the current definition. However, this is not currently how U.S. government agencies regulate GMOs, as noted above, or handle other issues such as pesticide residues or water quality standards.

While some commenters seemed to feel that there might be advantages to a product-based definition, such a structural revision is beyond the scope of this current effort. Therefore, the rest of this discussion will assume a continuation of a process-based approach.

European Classification Concepts applied in the U.S.
It would seem to make sense to try to distinguish between traditional and transgenic breeding techniques for both plants and animals. The FiBL suggestion of doing this through a chart has some strong benefits, including:

- The chart could be developed over time, with the terms everyone agrees to adopted first and then the more controversial ones hammered out over time.
- such a chart can follow logically from the Cartagena Protocol definition to indicate recombinant DNA, direct injection, cell fusion outside of families and other guidance provided by that definition that is somewhat lacking in the current definition.
- A chart such as this would be easier for the NOP to maintain as instruction or guidance and would not be as lengthy as a list of crop varieties and inputs. Additionally, it does not have to be updated as frequently.
- It maintains a transparency to all stakeholders that is now somewhat lacking in how GMOs are regulated.
- It gives ACAs clear instruction on how to evaluate seeds, vaccines, microorganisms and other potential GMOs.

At this juncture, before we even start to create a table of excluded methods terms, we invite input from the public on whether or not this is a worthwhile effort and any ideas for how to implement such an idea.

Unresolved Issues
Exploring this issue has brought to the attention of the subcommittee that engineered genetic manipulation of plant breeding materials has already occurred in many of the crop varieties that are currently being used in organic farming. A partial list:

- Disease resistant tomatoes (embryo rescue to introduce resistance genes)
- wheat and barley (double haploid technology using wheat and corn crosses along with embryo rescue and colchicine gene doubling)
- hybrid corn parent lines (double haploid to get homozygosity in 1 generation)
- Seedless tangerines and mandarins (mutations through irradiation)

- Brassica hybrids (cell fusion from radish traits)[19]

Many of these techniques that were used in initial crosses that have now passed down through many generations may not be traceable any longer. There are also many new varieties in development that will strongly challenge any definitions or regulatory scheme. Without a revised definition and some guiding principles to use for past and future determinations about excluded methods, there may not be effective ways to regulate either past or future techniques and their products.

Discussion Questions
The Materials/GMO Subcommittee is seeking response from the organic community on the issues presented in this discussion. A few of the particular questions to address are:

1. Are the definitions presented from the Cartagena Protocol an appropriate basis for guidance to further enable NOP and the NOSB to sort out terminology? (on page 3)

2. Among the criteria suggested, we would like feedback on the ones mentioned below and ask whether there are any other important criteria to use in genetic engineering determinations.
   - o Technical/physical invasion into the plant genome is refrained from (e.g. through transmission of isolated DNA, RNA, or proteins).
   - o The cell is respected as an indivisible functional entity and technical/physical invasion into an isolated cell on growth media is refrained from (e.g. digestion of the cell wall, destruction of the cell nucleus through cytoplast fusion).
   - o The ability of a variety to reproduce in species-specific manner has to be maintained and technologies that restrict the germination capacity of seed-propagated crops are refrained from (e.g. Terminator technology).

3. Would it be a good approach to continue a process-based evaluation of the terms and techniques, determine whether they are a result of genetic engineering, and then list both the GE and non-GE terms in a chart maintained by the NOP in the public record? If so, please offer suggestions on how this could be implemented. If not, please suggest any alternatives.

4. Are there terms or methods not included in appendix 1 that should be added to the discussion? Briefly explain.

Subcommittee Vote (Recorded August 26, 2014)
Motion to adopt the proposed Second Discussion Document on Excluded Methods Terminology

Motion by: Zea Sonnabend
Seconded by: C. Reuben Walker
Yes: 6 No: 0 Absent: 1 Abstain: 0 Recuse: 0

[19] for detail on this issue, please see: Myers, Jim 2014. in Proceedings from the 7th Organic Seed Growers conference. https://seedalliance.org/index.php?mact=DocumentStore,cntnt01,download_form,0&cntnt01pid=30&cntnt01returnid=139

Appendix 1
Terms defined and discussed in the first Discussion Document. They are presented in the same order they were in the document.

<u>In current definition of Excluded Methods:</u>
Cell Fusion
    Protoplast Fusion
    Somatic hybridization
Micro-encapsulation
Macro-encapsulation
Recombinant DNA
Gene Deletion
Genetic Engineering
Mutagenesis (mutation breeding)
Conjugation, genetic
Fermentation
Hybridization
    Hybrid
    Nucleic Acid Hybridization
In Vitro Fertilization
Tissue Culture
    Cell Culture
    Primary and Batch Cell Culture

<u>Not in Definition of Excluded Methods</u>
Silencing
Embryo Rescue
Microinjection
Biolistic device
Somaclonal variation
Transposons
Transduction

Approved by C. Reuben Walker, Subcommittee Chair, to transmit to NOSB February 25, 2015