BEYOND PESTICIDES
701 E Street, SE ■ Washington DC 20003
202-543-5450 phone ■ 202-543-4791 fax
info@beyondpesticides.org ■ www.beyondpesticides.org

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Ms. Michelle Arsenault
National Organic Standards Board
USDA-AMS-NOP
1400 Independence Ave. SW.,
Room 2648-S, Mail Stop 0268
Washington, DC 20250-0268

Docket # AMS-NOP-22-0071

Re. MS: Excluded Methods

These comments to the National Organic Standards Board (NOSB) on its Spring 2023 agenda are submitted on behalf of Beyond Pesticides. Founded in 1981 as a national, grassroots, membership organization that represents community-based organizations and a range of people seeking to bridge the interests of consumers, farmers, and farmworkers, Beyond Pesticides advances improved protections from pesticides and alternative pest management strategies that eliminate a reliance on pesticides. Our membership and network span the 50 states and the world.

The organic community is unanimously opposed to genetically engineered (GE) products in organic food—hence classifying them as excluded methods. Unfortunately, some proponents of GE have sought to confuse the issue by refusing to label some GE methods as GE. It is, therefore, especially important for the NOSB project of defining and classifying novel methods of breeding to continue—and to the extent possible, keep up with new technologies. The Materials Subcommittee (MS) asks about several definitions, which are discussed below.

In some cases, a given "method" (or umbrella term) may encompass two or more approaches that differ in their acceptability under review and labeling requirements. It is important to distinguish these different approaches so that decisions are not made that lump different techniques together and mistakenly allow practices that run contrary to the organic standards and law, while disallowing others that may be compliant.

TILLING (Targeted Induced Local Lesions IN Genomes):

TILLING (Targeted Induced Local Lesions IN Genomes) is a reverse genetic technique of creating and then identifying large numbers of mutations, specifically point mutations (aka single nucleotide polymorphisms, or SNPs). As noted by its name, the TILLING combines mutagenesis with a sensitive mutation detection system to speed up the detection of useful mutations.[1] The first step in TILLING is to create many mutants by exposing plant material, usually seeds, to a mutagen—a chemical mutagen, radiation, or environmental stressors such as heat or salinity. The mutant plants (M1) are self-fertilized to create the M2 generation, and seeds from these plants are tested for point mutations (SNPs).

The second step is to use polymerase chain reaction (PCR) techniques to detect the SNPs. TILLING allows one to detect all the genetic variability that exists at a specific locus, thereby allowing one to more easily connect various mutations with potential desirable phenotypic outcomes, such as disease resistance or changed organoleptic properties like taste, color, odor, or feel.

An example of the need to avoid lumping different techniques together is the difference between TILLING and Eco-TILLING. Ecotype TILLING (Eco-TILLING) identifies natural genetic variation in genes of interest related to useful agronomic traits in diverse crop germplasm.[2] Thus, Eco-TILLING identifies natural genetic variation in a population while TILLING identifies primarily induced mutations. Since there is no mutagen involved with Eco-TILLING, it can be argued that Eco-TILLING does not meet the definition of an excluded method. The potential issue with TILLING is that such techniques violate the first criteria for determining whether a method should be an excluded method, since the mutagens do not respect the genome as indivisible. Environmental stresses, such as heat, cold, and increased salinity, when applied to the whole organism, are naturally occurring and so could be allowed. However, it can also be argued that even Eco-TILLING, by focusing on specific genes, rather than the whole organism, is also contrary to organic principles. Nevertheless, TILLING and Eco-TILLING should be considered as two separate methods for the purpose of identifying excluded methods.

Double Haploid (DH)

As noted by the MS, a double haploid is a genotype of two identical chromosomes, constituting a pure homozygous or inbred line. As noted by the MS, doubling may result from spontaneous or artificial means.

DH plants may be produced by *in vivo* or *in vitro* methods. The *in vivo* techniques involve the living plant. The *in vivo* method consists of cross pollination techniques using either

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[1] McCallum CM, Comai L, Greene EA and S Henikoff. 2000. Targeting Induced Local Lesions IN Genomes (TILLING) for Plant Functional Genomics. *Plant Physiology* 123(2): 439-442. At: https://academic.oup.com/plphys/article/123/2/439/6087556.

[2] Comai L, Young K, Till BJ, Reynolds SH et al. 2004. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant Journal* 37: 778-786. At: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.0960-7412.2003.01999.x.

irradiated pollen, pollen of different species, or using haploid inductor or inducer lines of the same species to produce seeds with a haploid embryo.[3]

For most crops except for maize, there will need to be an in vitro step, where the haploid cells are raised in plant tissue cultures, often with phytohormones to stimulate growth. Even if the plant can produce a haploid embryo, these embryos usually need to be raised in plant tissue culture, since the embryos often will die if they remain in the seed and are not put in a cell culture system. A synthetic chemical, such as colchicine, is often used to create chromosomal doubling, thereby turning a haploid plant into a double haploid, although some of the embryos will spontaneously duplicate their chromosomes.

Some *in vivo* methods, particularly the use of haploid inductor or inducing lines, might not be considered to be using an excluded method, as long as none of the ingredients used in the *in vivo* methods are produced using modern biotechnology. For the *in vivo* method that uses irradiated pollen, the use of irradiation violates the first criterion for determining whether a method should be an excluded method, since the use of chemicals and irradiation does not respect the genome as indivisible.

The *in vitro* methods using synthetic chemicals such as plant hormones to spur growth, as well as use of colchicine to result in chromosomal doubling to create the double haploid, should fall into the category of excluded methods.

Therefore, we suggest that the NOSB definitions must distinguish four categories of DH—those produced *in vivo* and those produced *in vitro*, each produced with or without irradiation or prohibited synthetic chemicals.

Induced Mutagenesis

The definitions of mutation, mutagen, and induced mutagenesis are satisfactory, but the definition of induced mutagenesis must distinguish cases in which mutations are induced by synthetic chemicals or irradiation from those induced by environmental stressors. The NOSB has already said that mutations developed using *in vitro* nucleic acid techniques are excluded methods. Environmental stressors, such as heat, cold, increased salinity, UV light, etc. are naturally occurring and could therefore be considered an allowed method.

Definitions, including induced mutagenesis, should distinguish cases that may be viewed as excluded methods from those that may not.

Transposons or Transposable Elements (TE)

[3] Murovec J and B Bohanec. 2011. Haploids and Doubled Haploids in Plant Breeding. Chp. 5, pp. 87-106 in Plant Breeding, Ed. IY Abdurakhmonov. At: https://www.researchgate.net/profile/Ibrokhim-Abdurakhmonov/publication/263162927_Plant_Breeding/links/00b4953ad3045a295b000000/Plant-Breeding.pdf#page=99.

The definitions of transposon/TE are satisfactory, but, as with other definitions, should distinguish cases in which transposons are developed by synthetic chemicals or irradiation from those induced by environmental stressors. The NOSB has already determined that a transposon developed by use of in vitro nucleic acid techniques is an excluded method. TEs, once thought to be "junk DNA," are now known to play a major role in driving genome evolution.[4] Since they can readily move within the genome, they can create new mutations and can play a key role in regulating gene expression. In addition, the TEs can comprise a significant fraction of the genome. In addition to causing mutations, TEs can induce epigenetic alterations that modify gene expression, which can result in phenotypic variation and adaptation to stress.[5] Environmental stressors such as heat, cold, increased salinity, and UV light can activate TEs to move within the genome. Since such environmental stressors are natural, they could be considered an approved method. If a synthetic chemical or irradiation is used to stimulate TE movement within the genome, thereby causing mutations, the process falls in the category of an excluded method.

Definitions, including induced mutagenesis, should distinguish cases that may be viewed as excluded methods from those that may not.

Beyond Pesticides thanks Michael Hansen at Consumer Reports and Jaydee Hanson at Center for Food Safety for assistance in understanding these technologies. With their analysis, the NOSB can honor the intent and letter of the Organic Foods Production Act and preserve the key standard of excluded methods.

Thank you for your consideration of these comments.

Sincerely,

Terry Shistar, Ph.D.
Board of Directors

[4] Durbin MJ, Scheid OM and C Becker. 2018. Transposons: a blessing curse. *Current Opinion in Plant Biology* 42: 23-29. At: https://www.sciencedirect.com/science/article/pii/S1369526617301577

[5] Song X and X Cao. 2017. Transposon-mediated epigenetic regulation contributes to phenotypic diversity and environmental adaptation in rice. *Current Opinion in Plant Biology* 36: 111-118. At: https://www.sciencedirect.com/science/article/abs/pii/S1369526616301820