Note

This Interim Report is presented by the National Organic Standards Board Livestock Subcommittee on behalf of the Vaccines Made with Excluded Methods Working Group. This document has **not** been approved by the Livestock Subcommittee, but comments on this document are welcome, and will be supplied to the working group as they develop a final report.

Vaccines Working Group

Interim Report: Identifying Vaccines Made with Excluded Methods

Submitted to the National Organic Standards Board Livestock Subcommittee

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SUMMARY OF FINDINGS:

1. Organic livestock producers, certifiers and material evaluation programs can identify certain vaccines as being produced with excluded methods by the presence of the words “chimera,” “vector,” or “subunit” on the label of the vaccine.

2. The Center for Veterinary Biologics assigns a product code of D to DNA vaccines and R to recombinant vaccines. However, rules on confidential business information and differences in the definitions between the NOP’s “excluded methods” and CVB’s “recombinant” do not allow for the working group to identify these vaccines in the market or to verify that they are made with excluded methods.

3. The definition of excluded methods seems to be a less than ideal fit with vaccine production methods. The vaccines working group has developed two proposals for certifiers and material evaluation programs to sort between various vaccine products which have publicly described development methodologies. Under the first proposal, all technologies that could be used to create a targeted change or mutation in a genome would be considered excluded methods. The second proposal would take every technology on a case by case basis so that if a given technology can induce genetic mutations randomly or targeted, that technology would be allowed if mutations were random for the material in question.

FURTHER INVESTIGATION NEEDED

The working group suggests seeking comment on a number of issues:

1. The definition of "excluded methods" under the USDA organic regulations excludes "the use of traditional breeding" (emphasis added). However, the regulations do not give more detail on what is included in "traditional breeding". Therefore, it is difficult to determine what techniques used in vaccine development would be considered "traditional breeding", and, thus allowed under the regulations, versus those that would be considered "excluded". How should traditional breeding techniques be divided from modern breeding techniques as it pertains to vaccine production? Should the definition of excluded methods be changed or clarified specifically towards vaccine production?

2. The definition of excluded methods includes the use of methods that are not possible under natural conditions such as the use of recombinant DNA technology. However, recombination can be a naturally occurring event in many biological processes, such as occurs every time a plant or animal sexually reproduces. How should a pragmatic line be drawn between techniques which use recombination (allowed) and techniques which rise to the level of recombinant technology (excluded)?

3. The third criterion proposed to identify vaccines made with excluded methods is for certifiers and MEPs to analyze the methods used to create the vaccine. The working groups proposed two ways for this analysis of methods to be done. Should a given technique be declared excluded or allowed or should the effects of each method be analyzed so that all random genetic modifications be allowed and all targeted genetic modifications be prohibited?

4. If the use of biological mutagens to randomly modify the genome of the targeted pathogen is considered allowed (i.e. not an excluded method), do certifiers and MEPs need to consider whether the biological mutagen was produced through use of an excluded method? For example, if the use of transposons to create random genetic mutations is allowed, can the transposon have been produced using excluded methods? Essentially this question is asking how far back into the development or manufacturing should the excluded method prohibition apply? As a factual matter, how far back in the development process can an accurate assessment be made of the possible use of excluded methods?

INTERIM REPORT

I. Introduction

The NOP (National Organic Program) established the Vaccines Made with Excluded Methods (MWEM) working group in response to a request of the National Organic Standards Board (NOSB) for more information about the use and identification of vaccines MWEM. The working group includes two members of the NOSB, NOP staff, and staff from the Center for Veterinary Biologics (CVB), the division in the Animal Plant and Health Inspection Service (APHIS) that approves and regulates vaccines for use in livestock and pets. The working group prepared this discussion document to summarize its efforts to date for the NOSB Livestock Subcommittee. This document outlines an approach for how certifiers and material evaluation programs (MEPs) can identify some vaccines MWEM (i.e. genetically engineered vaccines and what some people incorrectly refer to as GMO vaccines) and summarizes questions that could be posed to the organic community about whether certain methods used to produce vaccines should be considered excluded or allowed under the USDA organic regulations.

II. Background

The USDA organic regulations at 7 CFR part 205 contain several references that are relevant to the discussion on the use of vaccines in organic livestock production. The first reference, under the "Livestock healthcare practice standard", requires that "the producer must establish and maintain preventative healthcare practices, including…administration of vaccines and other biologics" (205.238(a)(6)). The second reference on the National List of Allowed and Prohibited Substances allows the use of livestock vaccines, which are synthetics as follows: 205.603(a)(4) as follows: "Biologics – vaccines" (205.603(a)(4)) (without annotation). The third reference at 205.672 deals with emergency pest or disease treatment which is defined in 205.2 to include disease eradications programs. In the past, vaccines MWEM have been required as part of disease eradication programs. The working group is unclear as to the effects of these eradication programs on organic livestock producers.

The fourth reference is nested within the section of the USDA organic regulations that details the allowed and prohibited substances, methods, and ingredients in organic production and handling. Under this section (205.105(e)), the use of excluded methods is prohibited in organic production. Excluded methods are defined under the USDA organic regulations (205.2). The methods that are excluded and, thus, prohibited, are those 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. However, there is a specific reference to vaccines in the section on excluded methods. Section 205.105(e) of the organic regulations provides an allowance for vaccines produced through the use of excluded methods if the vaccines are reviewed and recommended for the addition to the National List by the NOSB. The review needs to be conducted in accordance with section 205.600 of the organic regulations. Section 205.600 specifies the evaluation criteria that the NOSB follows in their evaluation of allowed and prohibited substances, methods, and

ingredients. To date the NOSB has not recommended any vaccines made with excluded methods be added to the National List.

The preamble to the final rule (65 FR 80554) in 2000 discussed the NOP's response to comments about use of vaccines MWEM in organic livestock production. Some commenters wanted all vaccines MWEM to be completely prohibited from organic livestock production while others wanted all vaccines to be temporarily allowed until more information could be assembled in the future to determine if any of the vaccines MWEM were necessary for production. At the time, NOP chose to structure the provision so that vaccines MWEM could only be used by organic production if they are affirmatively included on the National List after review by the NOSB. But, with no information or guidance about how to identify vaccines MWEM, many organic livestock producers, with approval from their certifiers, have chosen vaccines based upon disease prevention and not based on whether they are made with excluded methods.

To rectify this divergence between regulatory language and industry practice, the NOSB, in 2009, recommended a change to section 205.105(e) to allow the use of vaccines made with excluded methods if vaccines made without excluded methods were not commercially available[1]. That recommendation stated that such a change would not require individual review of vaccines made with excluded methods. The NOP has not implemented this change into the USDA organic regulations. Therefore, the current exception at section 205.105(e) to allow vaccines made with excluded methods only applies to those that are reviewed according to 205.600. In September 2010, the NOP requested that the NOSB review vaccines made with excluded methods (i.e. GMO vaccines or genetically engineered vaccines) in accordance with section 205.600[2].

In response to the NOP's request, the NOSB began to review vaccines MWEM. The Livestock Subcommittee requested a Technical Review of GMO Vaccines[3], drafted a proposal and submitted the proposal to the full NOSB. The NOSB discussed the proposal pertaining to the use of vaccines MWEM at its May 2012 public meeting[4]. The NOSB received considerable public comment on this issue leading up to and at this public meeting. Comment was split with members of the general public advocating for a prohibition on vaccines MWEM and certifiers and producers asking for more detailed information about current vaccine use and clarification about which vaccines were MWEM. Due to the need for additional technical information before voting, the NOSB decided to table the proposal until a future meeting, but passed a resolution that included a request for more information from USDA[5]. The NOSB requested 1) NOP identify all vaccines registered with USDA as GMO or non GMO 2) Vaccine manufacturers voluntarily and truthfully label vaccines about their absence of GMO content, 3) NOP or other USDA

[1] http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5081499&acct=nosb
[2] http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5090932
[3] The technical review may be viewed at
http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5097326
[4]Information on the May 2012 NOSB meeting may be found at
http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateJ&page=NOSBMeetings
[5]May 25, 2012 NOSB Formal Recommendation on GMO Vaccine Information Request
http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5098924

agency publish a real time tracking system to identify GMO and non GMO vaccines. In response to the NOSB's May 2012 resolution, the NOP convened the Vaccines Made with Excluded Methods Working Group.

The working group first collected information regarding the use of vaccines, government programs that may require the use of vaccines, technical information about how vaccines are made and how vaccines are regulated. In response to requests from the NOP, CVB and Veterinary Services (VS) from APHIS elaborated on regulations that could require livestock producers to use vaccines. The working group's understanding is that the Secretary of Agriculture has the authority to declare emergencies at various levels depending upon the severity of the outbreak. Emergency declarations allow both state and the federal government to require livestock producers to use specific vaccines, including vaccines MWEM. The only regional emergency in the past decade was an Exotic Newcastle outbreak in unvaccinated backyard poultry and game fowl. No vaccination program was used in this emergency because USDA determined that most commercial poultry operations in the area, whether conventional or organic, had already vaccinated their birds for this disease. It is difficult to ascertain whether vaccines MWEM would be needed in future emergencies but VS stated it is likely that most new vaccines would be made with such methods and these could be selected as the most effective option in future disease outbreaks. However, no such forced vaccination program in response to an emergency has occurred recently.

The working group also learned that disease eradication programs authorized by the federal government may include mandated use of vaccines. The two recent eradication programs, Brucellosis in cattle and Pseudorabies in swine both required vaccines. These two eradication programs used vaccines that allow blood tests to differentiate between those animals that have an immune response due to the vaccine and those animals that have an immune response due to the disease. In order to differentiate between vaccinated animals and animals which had the disease, producers must use a modified live vaccine that results in a strong immune response, has mutations that alter at least one epitope and is not virulent. The Brucellosis vaccine was developed using cell culture passages, a presumably allowed technology in organic production. The Pseudorabies vaccines, several vaccines were approved for this eradication program, were developed using excluded methods. Based on our discussions with APHIS, the working group believes that vaccines made with excluded methods may be USDA's preferred vaccine choice in future eradication programs.

APHIS' CVB regulates vaccines and vaccine manufacturers under the Virus-Serum-Toxin Act, CVB's primary role is to review and license vaccines based upon purity, safety, potency, and efficacy. CVB requires certain label terms depending upon specific configurations of the vaccine seed (form of the agent used to create the vaccine). CVB also tracks vaccines that are made through the use of biotechnology. However, CVB's evaluation of whether a vaccine is produced through "biotechnology" does not align with how "excluded methods" is defined under the USDA organic regulations. Because of this lack of alignment, it is difficult to know the extent to which vaccines on CVB's list of biotechnology derived vaccines overlaps with what could be considered produced through an "excluded method". CVB does review the use of biotechnology in manufacturing of the vaccines, e.g. if a vaccine is produced using cells made with excluded methods. However, if only the cell line used to culture the vaccine seed has a genetic insertion, deletion or other mutation, the vaccine itself is not considered to be a

recombinant. [6] Finally, the working group could not identify a comprehensive path of "partial" alignment such that if a vaccine were identified as biotechnology derived by CVB then it is was definitely considered made with "excluded methods" as defined by the NOP.

III. Working Group Deliberations

After considering background research, information from other USDA agencies and public comments, the Working Group came to the conclusion that developing criteria for certifiers and MEPs to use to identify vaccines MWEM would be the only approach to allow the organic industry to determine which, if any, vaccines made with excluded methods are being used and if there are reasonable alternatives to these vaccines. The working group has identified three criteria that could be used by certifiers and MEP's to determine the excluded or not excluded status of vaccines. The working group developed how one of the criteria would be used but requests input from the NOSB and the organic community on clarifying the two other items.

The working group considered creating a list of all vaccines produced with (or without) use of excluded methods. This would be the easiest resource for organic livestock producers and certifiers to use. However, creation of a negative and/or positive list is difficult for a variety reasons, including the lack of precise criteria to decide whether something should be considered produced through excluded methods. Furthermore, for such lists to be useful, the lists would need to specify the branded vaccine products that livestock producers purchase and use, not just generic names of the disease or pathogen that is being used to create the vaccine. Another reason the working group chose not to create a list is that the CVB does not differentiate vaccines based upon excluded methods. USDA is concerned that creating such a list would imply a deficiency of vaccines MWEM, which would not be scientifically accurate within USDA's responsibility to regulate the purity, safety, potency, and efficacy of vaccines. The working group was also concerned 1) with liabilities due to the possibility of inaccurately placing a specific vaccine on a list, and 2) the possibility of not being able to obtain necessary vaccine manufacturing information, which is often submitted as confidential business information to APHIS CVB.

IV. Working Group Proposal

The working group has identified criteria that would allow certifiers and MEPs to identify vaccines MWEM. The three criteria to be used in in conjunction are:

- Label Guidelines
- Product Codes
- Methods of Production Analysis

A. Label Guidelines

[6] European organic standards allow the use of all vaccines if they are needed to prevent a disease in the area. Canadian organic standards forbid genetically engineered vaccines outright. In addition, Canadian organic livestock producers may only use a nongenetically engineered vaccine that was grown in a cell culture system that included genetic modifications if no other vaccine is available.

CVB regulations require that certain vaccine seed configurations have specific terms on the labels of branded vaccine products. These terms are required for a subset of biotechnology derived vaccines. While these terms are not added to the labels because an excluded method was used, CVB states that all such vaccines were created using methods that the NOP would exclude. The terms on labels that identify vaccines were made with excluded method are "Subunit," "Vector," and "Chimera." Because these vaccines are labeled with the identified terms, CVB can disclose a trade names list for all of these vaccines.

Vaccines must be labeled with the term "Subunit" when the vaccine is an extracted or purified protein that was expressed in a recombinant system. These vaccines do not contain any genetic information (DNA). These vaccines only contain the protein antigen that induces an immune response. To create "Subunit" vaccines, the gene for the antigenic protein is inserted into an expression vector or expression system. The gene from the pathogenic organism may be expressed in prokaryotic or eukaryotic cell culture systems. The expressed protein is then extracted or purified and used in the vaccine. Currently there are no active licenses for subunit vaccines.

Certain modified live vaccines must be labeled with the term "Vector" or "Chimera" to denote that the vaccine contains DNA from two pathogens. These vaccines are created by identifying a viral structure that induces a strong immune response. This viral structure is termed the expression vector. In many cases, the expression vector is a virus that in its unaltered form can cause a disease in the target species. The vector will then have at least one gene from another disease causing agent inserted into the viral genome. Vaccines labeled with "Vector" may be efficacious against two diseases, the disease caused by the unaltered vector and the disease caused by the source of the gene that was inserted into the vector or only be efficacious against the disease caused by the source of the gene that was inserted into the vector. Vaccines labeled with "Chimera" are similar to "Vector" labeled vaccines, except that certain genes required for replication competency are supplied by the added genes and not contained in the expression vector.

B. Product Code

The CVB requires that every biologic, including vaccines, produced must have a product code. The CVB guide on true names and product codes[7] notes that the 5th digit of the product code may contain "D" or "R." The letter "D" in the fifth digit signifies that the vaccine is a nucleic acid vaccine. Such vaccines, also called DNA vaccines, are made with excluded methods and depend upon foreign genes being expressed in some of the cells of the vaccinated animals. The letter "R" in the fifth digit signifies the vaccine has a recombinant component or is a subunit protein derived from a recombinant organism. The recombinant designation only applies to components in the vaccine and not to methods used to make the vaccine such as genetically engineered cells that are used for cell culturing the vaccine seed.

In public comments, some certifiers stated that they were aware of the R code in the fifth digit of the product code as designating that a component in the vaccine was recombinant or recombinant-derived. However, these certifiers were not able to translate the product code information to actual vaccines on

[7] http://www.aphis.usda.gov/animal_health/vet_biologics/publications/pel_1_3.pdf

the market. CVB is unable to provide a list of the trade names of the vaccines with a "D" or "R" in the product code because confidential business considerations will not permit discussion of production methods, unless the biologics firm specifically agrees to disclose the information. The working group was unable to develop a method to identify the trade names of vaccines and other biologic products that have a D or R in the product code other than the trade names that are already identified as MWEM, e.g. are labeled as containing a "Vector" or "Chimera." Vaccines that have a "D" or "R" in the product code may or may not be made with excluded methods since the production methods may not be identified for evaluation. The working groups is requesting input from the NOSB and organic community to identify methods of linking product codes to trade names in a manner that clearly identifies whether or not an excluded method was used.

C. Method of Production Analysis

Some firms have waived confidentiality by describing how the vaccines were made in public comment to the NOSB. However, some vaccines were and in the future may be made with methods that are not clearly excluded or allowed in organic production. The working group is requesting input from the NOSB and the organic community to provide comments on this issue.

Modified live vaccines generally have been found to produce greater immune responses in vaccinated animals and have become more common in new vaccines than killed vaccines. Live vaccines require that the genome of the disease causing organism be modified to create a living, but not virulent, pathogen which can be packaged in the vaccine. The excluded methods definition (205.2) includes methods which genetically modify organisms or influence their growth and development by means not possible under natural conditions or processes which are not considered compatible with organic production. The definition identifies some of the methods that are excluded including recombinant DNA technology (gene deletion, gene doubling, introducing a foreign gene and changing the positions of genes when achieved by recombinant DNA technology). The definition states that some methods to genetically modify organisms are allowed, including traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization or tissue culture.

Many of the older non-biotechnology derived modified live vaccines were made by using bacterial culture, cell culture or tissue culture with multiple passages to induce genetic modifications to the disease causing pathogen. The various cultures were then screened to identify a modified version that induced an immune response but that was no longer virulent. This is a process of random genetic modification followed by screening for the desired phenotype. The Brucellosis vaccine that is part of the Brucellosis eradication program was produced by growing the parent strain in various concentrations of an antibiotic cocktail over several passages to induce random mutations in the genome of the bacteria. These random mutations resulted in a non-virulent bacterial strain that did not produce the O-chain component of the lipopolysaccharide that was one of the epitopes for immune response.[8] This change in at least one epitope was required for eradication programs so that vaccinated animals could be differentiated from animals infected by the actual pathogen.

[8] Schurig, G., R.M. Roop, T. Baghi, S. Boyle, D. Buhrman and N. Sriranganathan. 1991. Biological properties of RB51; a stable rough strain of Brucella abortus. Veterinary Microbiology 28: 171.

The working groups assumed other genetic modification methods that would be allowed are exposure to chemical or physical mutagens. Physical mutagens include ionizing radiation, UV radiation and radioactive decay. These mutagens create genetic modifications in a random manner through a variety of ways. Some chemical mutagens break the double stranded DNA, allowing a recombination event to occur which can cause gene deletion and changing the position of genes. Other mutagens cause DNA bases to switch to other bases, errors in DNA repair or errors in replication. These mutagens all genetically modify organisms in a random manner that is not targeted. Generally, the vaccines working group considered chemical and physical mutagens to be traditional breeding techniques.

Biological mutagens are excluded if they are considered to be a recombinant technology. Recombination is the process by which double stranded DNA is broken, rearranged and then rejoined. 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 are allowed by the current definition of excluded methods.

Some biological mutagens are clearly excluded by the current definition. Restriction enzymes are naturally occurring proteins in many bacteria that will cleave DNA at specific sequences. These enzymes are defense against phage (viruses that target bacteria) which insert their genetic material, usually but not always DNA. Restriction enzymes have been used to cleave a gene of interest and then through a targeted recombination event create a specific gene deletion, clone the gene in a vector or cause a changing of positions of genes in a controlled, nonrandom manner.

Other biological mutagens are neither explicitly allowed or excluded and may be allowed when used one way but not when used in a different way. Specifically, the working group discussed the methods used to create a vaccine which the manufacturer has stated, in public comments to the NOSB, was not made with excluded methods. This particular gene-deleted product was created using transposons and phage transduction. Transposons and phage transduction both result in genetic modifications mediated through recombination events. However, the working group was divided as to whether or not these methods were excluded. Are these methods considered traditional breeding techniques? Are these methods considered a technology as techniques that involve recombination are allowed in organic production but recombinant technologies are not allowed? The working group recognized that the definition of excluded methods did not appear to clearly fit with the methods and technologies used to produce vaccines. The working group is requesting input from the organic community in regards to how these biological mutagens should be classified in regards to the definition for excluded methods as well as how to evaluate biological mutagens in general.

Transposons[9], 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 genes within the transposon 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.

Eukaryotic transposons are more complicated as they are first copied to RNA. The RNA is then converted to cDNA by a reverse transcriptase, which is coded for by a gene on the transposon. Another gene on the transposon is an integrase, which then inserts the transposon cDNA back into the genome at a new position.[10] 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.

Transposons have played a large role in the formation of the genomes of many species. Inactivated transposons and transposon repeats are estimated to make up 44% of the human genome, though only a small fraction are still active.[11] Transposons are present in plant, animal and bacterial species. Transposons mediated recombination events from transposon activity will occur in most if not all species used in organic production, including agricultural, handling and lab based species.

In order to evaluate the use of transposons in vaccine production, the working group considered if transposons would fit into the allowance for traditional breeding techniques. The working group was 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 definition of excluded methods allows all traditional breeding techniques, so the distinction is important for organic producers.

Transposons were initially identified as jumping genes by Barbara McClintock in research on variegation in corn kernels which began in the 1930's. [12] As the transposons moved and genetically modified the genome, various genes would be turned on or off, altering the phenotype of the expected breeding. The activity of the transposons was part of the plant breeding resulting in the phenotype. Some of the working group considered the use of transposons to fall under the category of traditional breeding.

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[9] MeSH (Medical Subject Headings), the NLM [National Library of Medicine] controlled vocabulary thesaurus used for indexing articles for PubMed.
http://www.ncbi.nlm.nih.gov/mesh

[10] http://chemistry.umeche.maine.edu/CHY431/Genome4.html

[11] Mills RE, EA Bennet, RC Iskow, and SE Devine. 2007. Which transposable elements are active in the human genome? Trends Genet 23(4): 183

[12] Pray, L. & Zhaurova, K. 2008 Barbara McClintock and the discovery of jumping genes (transposons). Nature Education 1(1)

Others on the working group felt that traditional breeding techniques did not provide a clear demarcation between allowed and excluded methods.

More recently, researchers have used transposons as a vector for inserting specific foreign genes into the genome of various species. One of the more widely cited methods is with a transposon system called "Sleeping Beauty."[13] Transposons have been used to genetically modify a variety of agricultural species from plants such as rice to swine cells. This use of transposons would be excluded in organic production. Figure 1. provides an illustrated explanation of how transposons are now used to insert genes of interest into genomes.

[13] Carlson, DF, JR Garbe, W Tan, MJ Martin, JR Dobrinsky, PB Hackett, KJ Clark and SC Fahrnekrug. 2011. Strategis for selection marker-free swine transgenesis using the Sleeping Beauty transposon system. Transgenic Res 20(5): 1125

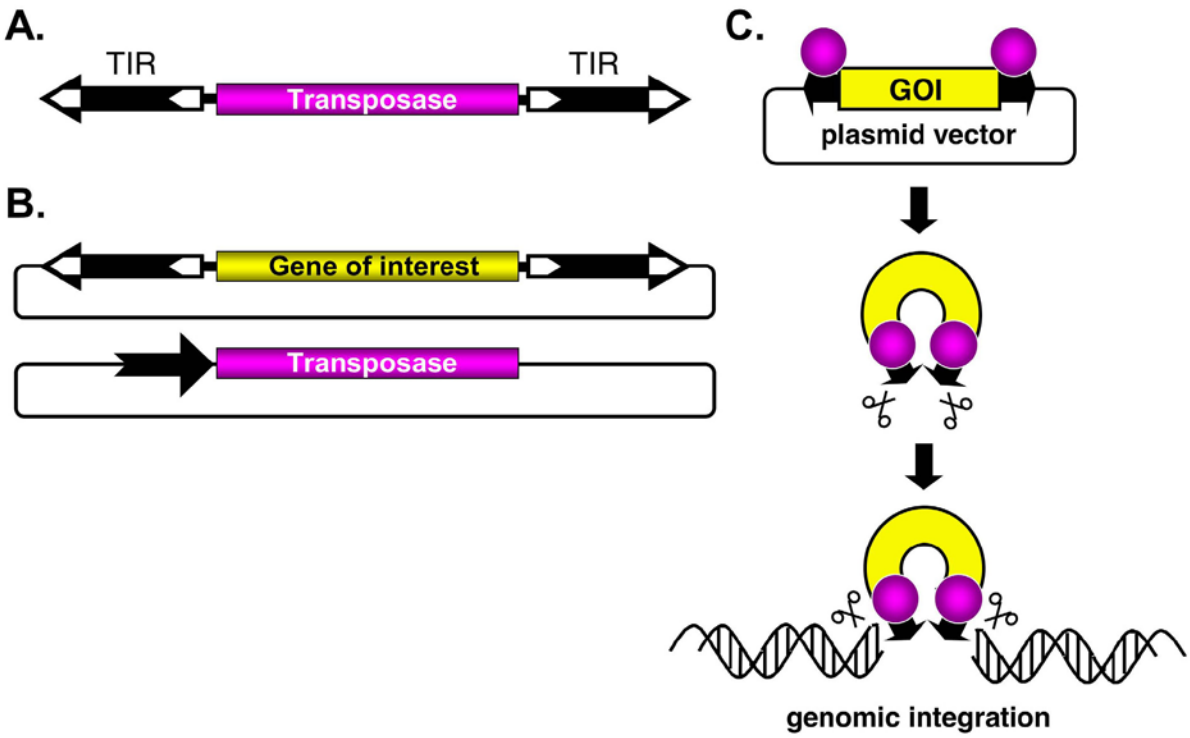

Figure 1.

General organization and use of class II transposable elements as gene vectors.

(A) Autonomous transposable elements consist of terminal inverted repeats (TIR; black arrows) that flank the transposase gene.

(B) Bi-component transposon vector system for delivering transgenes that are maintained in plasmids. One component contains a DNA of interest between the transposon TIRs carried by a plasmid vector, whereas the other component is a transposase expression plasmid, in which the black arrow represents the promoter driving expression of the transposase.

(C) The transposon carrying a DNA of interest is excised from the donor plasmid and is integrated at a chromosomal site by the transposase.[14]

[14] Ivics, Z. and Z. Ivsvak. 2010. The expanding universe of transposon technologies for gene and cell engineering. Mobile DNA. 1:25

The other method used by the vaccine manufacturer under discussion was transduction[15], which is 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. The excision of the viral genome is not perfect and in some cases, parts of the bacterial genome will be excised and packaged into the new phage. These phage can then be used to infect additional bacteria. The bacterial genetic material in the phage will be inserted into the newly infected cell. A homologous recombination event may occur so that some of the genes from the originally infected cell's genome will replace the genes in the newly infect cells. This method can stably introduce genetic mutations into the new bacteria.

These two, briefly described methods of transposons and transduction were used to create a gene deleted vaccine product that the manufacturer has stated is not made with excluded methods. Specifically, the manufacture stated that the transposon Tn10, which codes for tetracycline resistance as well as transposase was used to introduce genetic modifications. The tetracycline resistance gene allows for selection for stable recombination events by adding tetracycline to the media to kill all those bacteria which were not mutated by transposons. Bacteria, which had transposon linked mutations to the genes that needed to be inactivated in order to knock out virulence, then underwent transduction by Phage P22Htint to create the mutated strain used for the vaccine. These methods resulted in bacteria that had stable genetic modifications that rendered the bacteria avirulent, but able to induce a strong immune response in vaccinated animals.[16]

The working group did not come to a decision about the status of vaccines developed using these methods. Certifiers and MEPs who examine vaccines for compatibility with the organic regulations will need guidance on future determinations of other vaccines as well. The working group considered two proposals for methods that could be used for this final determination of methodologies that are not clearly covered in the current definition of excluded methods. While outside the scope of the working group's mandate, a third option briefly discussed is that the definition of excluded methods could be revised to more clearly demarcate technologies used in vaccine production as being allowed or excluded. The vaccines made with excluded methods working group would encourage the GMO Subcommittee to consider changing the definition of excluded methods in 205.2 based on some of the issues addressed in this document.

The first proposal would be technique based. The working group or the NOSB would assess the methods used to genetically modify genomes for vaccine production and then state which methods are excluded. For example, every vaccine that used a transposon or polymerase chain reaction to create the

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[15] MeSH (Medical Subject Headings), the NLM [National Library of Medicine] controlled vocabulary thesaurus used for indexing articles for PubMed.
http://www.ncbi.nlm.nih.gov/mesh

[16] Curtis, R. and S Kelly. 1987. Salmonella typhimurium Deletion Mutants Lacking Adenylate Cyclase and Cyclic AMP Receptor Protein Are Avirulent and Immunogenic. Infection and Immunity. 55(12): 3035

vaccine seed would be excluded. This proposal has the limitation of not allowing all uses of a given method, even if only certain uses are excluded. For example, because transposons can be used to create transgenic plants and animals, all use of transposons would be excluded. This proposal would provide greater clarity, but less flexibility.

The second proposal would be based upon how the genetic mutations were introduced to the genome. Most of the allowable mutagens such as chemicals or radiation introduce genetic modifications randomly. Under this proposal, any biological mutagen that created genetic modifications randomly would be allowed and biological mutagens which are targeted (i.e. genetically engineered) to specific places in the genome or specific genes would not be allowed. This proposal would require more work and effort by certifiers to identify not just which method was used, but how that method altered the genome of the pathogen. Restriction enzymes typically cleave DNA at a specific sequence. However that specific sequence may be repeated and randomly distributed across the genome. How should certifiers make a determination when a technique used to mutate or modify a genome should be considered random versus targeted?