Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Submitted Electronically via Federal eRulemaking Portal (http://www.regulations.gov)

Re: Docket Number: FDA-2016-N-4389; Genome Editing in New Plant Varieties Used for Foods; Request for Comments[1]

Dear Dr. Kux:

The Biotechnology Innovation Organization (BIO) appreciates this opportunity to provide comments to the Food and Drug Administration (FDA) on its request for comments on genome editing in plants. BIO is the world's largest biotechnology trade association, representing small and large companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO members are involved in the research and development of healthcare, agricultural, industrial and environmental biotechnology products. BIO represents its members in a number of matters related to agricultural biotechnology, and in particular, has a strong interest in the sound regulation of plant biotechnology.

BIO fully supports the comments to this docket submitted by the American Seed Trade Association (ASTA), and we reference those comments a number of times in this document. In addition to voicing our full support for the comments submitted by ASTA, BIO adds the following.

INTRODUCTION

This is a critical time in the development of the newest biology-based technologies: an array of genome editing techniques that target sites in an organism's genetic material with remarkable precision. They represent the next step in a continuum of genetic modification methods that began thousands of years ago with the simple process of artificial selection: humans, rather than nature, decided which wild plants would reproduce by preferentially planting certain seeds from the progenitors of today's crops. Analogous to evolution by natural selection, artificial selection preserves only certain of the genetic variants, which are derived from spontaneous mutation and nature's random mating processes, then discards others and, in the process, changes the gene frequencies in the population under selection.

[1] https://www.gpo.gov/fdsys/pkg/FR-2017-01-19/pdf/2017-00840.pdf

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Once scientists uncovered the basics of plant reproductive biology, thousands of years of selecting from an array of randomly generated genetic combinations gave way to controlled mating of plants with traits that were favored for various reasons. Combining the process of controlled mating with artificial selection, i.e., selective breeding, increased the prevalence of preferred characteristics in the breeding population much more rapidly.

Understanding even more details about plant reproductive biology then allowed breeders to overcome the many natural barriers to interspecific reproduction. Developing methods that short-circuited a plant's reproductive isolating mechanisms permitted crosses between different species (first recorded in 1800's) and even across genera (first recorded in early 1900's). This broadened the plant breeder's access to different genes, as they no longer were limited by the availability of genes in the species gene pool. The set of laboratory techniques that allowed "wide crosses" gave rise to the concept of a "breeder's gene pool."

Rediscovering Mendel's work at the beginning of the 20th century gave breeders insight into the hereditary mechanisms they were attempting to control and direct. The concept that discrete packages of information (genes) that separated during gamete production were the carriers of inherited traits allowed them to "see" the processes they were trying to guide down certain pathways.

Even though an understanding of Mendel's laws of segregation and independent assortment led to many new and improved plant varieties, that understanding did not allow breeders to "do" something new. They were still limited to combining existing genetic variants through sexual reproduction. The discovery of mutagenesis changed that. Using mutagens, such as chemicals and ionizing radiation, plant breeders were able to induce gene mutations and, in doing so, create new variants of existing genes (alleles), which were then cross bred into preferred varieties.

The serendipitous discovery of bacterial restriction endonucleases, combined with an improved understanding of the molecular mechanism of the plant pathogen, *Agrobacterium tumefaciens*, allowed the next leap in crop improvement. Recombinant DNA (rDNA) technologies, or genetic engineering, differs from breeding in that it removes all taxonomic barriers to gene exchange. A gene found in any organism can be inserted in a plant's genome. Breeders now have access to all of nature's genetic diversity, not just the variants in the breeder's gene pool.

Another difference between genetic engineering and traditional breeding relates to the number of genes that are transferred from one plant to another. Traditional breeding normally takes many generations of repeated cycles of selecting and discarding plants in order to obtain the best combination of characteristics. With rDNA techniques, a single gene of known function can be inserted into an elite variety, rather than combining two entire plant genomes. This decreases the number of generations of backcrossing. Perhaps more

importantly, breeders know the protein encoded by the inserted gene. As a result, they can better predict *a priori* the impact of the genetic change on the plant's biochemistry. However, insertion of the gene is a random process that can disrupt existing genes.

The evolution of genetic modification techniques has reached unprecedented precision with genome editing techniques. Rather than moving new genes into a plant genome through traditional breeding and genetic engineering, or inducing mutations randomly, by using the tools of genome editing breeders are able to improve on:

- genetic engineering techniques by inserting genetic material into specific positions in the genome, further decreasing the risk of potential unintended effects and, in many cases, the time needed to establish a new variety
- traditional breeding for multi-genic traits by rearranging the positions of the genes that contribute to the trait so that they are inherited together
- induced mutation techniques by making very precise, targeted changes to a plant's existing genetic material.

These developments are made possible not only by new molecular tools, but also as a result of the wealth of information provided by the many plant genome sequencing projects that have been carried out by scientists around the world over the past two decades.

It is worth stating that all of the genetic modification methods exploited by plant breeders occur in nature[2], and the molecular tools that allow for precise changes are molecules, designed by evolution, that naturally-occurring organisms use every day.

In summary, plant breeders have developed and continue to rely on a continuum of genetic modification techniques that have improved and broadened their capacity to make use of the two mechanisms that nature uses to generate variation in a population:

1. changing the makeup of an organism's existing genes through mutation, and
2. combining existing genes in different combinations.

The genetic modification continuum is characterized by an increased understanding of life at the cellular and molecular levels. As a result of that deeper understanding, the genetic modification methods used by breeders have become much more precise over time. That precision, informed by science, reaches a new high point with genome editing tools and techniques.

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[2] Arber, W. 2007. Genetic variation and molecular evolution. In Genomics and Genetics volume 1, pp. 385-406. R.E. Myers, editor. Wiley.
Arber, W. 2010. Genetic engineering compared to natural genetic variations. New Biotechnology. 27: 517-521

More specifics on the molecular mechanisms underlying the methods for creating genetic variation in nature and in plant breeding are provided below, because they are important for understanding the risks, or lack thereof, posed by the new genome editing tools.

OVERARCHING REGULATORY PRINCIPLES

In the 1980-90's, a number of countries recognized that a proliferation of regulations was creating obstacles to economic growth and innovation without providing the countervailing benefit of enhanced protection of the environment or human health. In response, each country began a systematic review of its regulatory structures and processes. These evaluations led to a number of principles of good regulation that countries increasingly use in guiding their revisions of existing and development of new regulations[3]. The U.S. government articulated these principles in a 1992 Executive Order[4]; each subsequent Administration, including the current one, has reaffirmed them[5]. The principles, as articulated by the Organization of Economic Cooperation and Development (OECD) in 1995, are provided in Appendix 1. We highlight key principles below.

- Regulate only when there is a significant problem that is best solved by regulation
- If the government decides regulation is warranted, it should first articulate the problem it is trying to solve to ensure the regulations it develops will solve the problem in a cost-effective manner and without impeding innovation unnecessarily
- The benefits of regulation should justify the costs, and the degree of regulation should be commensurate with the risk
- Base decisions on the best scientific and technical information concerning the need for and consequences of the intended regulation.
- Avoid development of regulations that are inconsistent, incompatible or duplicative.
- Select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety) and other advantages.

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[3] OECD. 1995. Recommendation of the Council on Improving the Quality of Government Regulation. http://acts.oecd.org/Public/Info.aspx?lang=en&infoRef=C(95)21/FINAL; OECD.1997. The OECD Report on Regulatory Reform: Synthesis. http://www.oecd.org/gov/regulatory-policy/2391768.pdf; APEC-OECD. 2005. Integrated Checklist on Regulatory Reform http://www.oecd.org/regreform/34989455.pdf OECD. 2005. Guiding Principles for Regulatory Quality and Performance. http://www.oecd.org/fr/reformereg/34976533.pdf; Middle East and North Africa-OECD.2009. Regional Charter for Regulatory Quality. http://www.oecd.org/mena/governance/45187832.pdf; OECD. 2012. Recommendation of the Council on Regulatory Policy and Governance http://www.oecd.org/regreform/regulatory-policy/49990817.pdf

[4] EO 12866 (Sept 1993) 3 CFR 638 *Regulatory Planning and Review*. http://www.archives.gov/federal-register/executive-orders/pdf/12866.pdf

[5] EO 13422 (January 2007) 72 CFR 2763. https://www.gpo.gov/fdsys/pkg/FR-2007-01-23/pdf/07-293.pdf; EO 13563 (January 18, 2011) Improving Regulation and Regulatory Review http://www.whitehouse.gov/the-press-office/2011/01/18/executive-order-13563-improving-regulation-and-regulatory-review; EO 13610 (May 10, 2012) Identifying and Reducing Regulatory Burdens http://www.whitehouse.gov/the-press-office/2012/05/10/executive-order-identifying-and-reducing-regulatory-burdens; https://www.whitehouse.gov/the-press-office/2017/02/24/presidential-executive-order-enforcing-regulatory-reform-agenda; https://www.whitehouse.gov/the-press-office/2017/05/08/memorandum-regulatory-reform-officers-and-regulatory-policy-officers

- Review regulations on a regular basis to ensure they still serve regulatory objectives in the least burdensome way.

REGULATORY PRINCIPLES AND THE COORDINATED FRAMEWORK

The Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework)[6], as articulated initially in 1986, clarified in 1992[7], and confirmed most recently in 2016-17[8], is consistent with the general principles of good regulation described above.

Adhering to the first principle of appropriate regulation – regulate only when there is a problem that needs to be solved - the Coordinated Framework said the agencies would focus on only those products that presented a potential risk, when compared to similar products that have a history of safe use and consumption. While the 1986 Coordinated Framework assigns regulatory responsibilities for specific biotechnology products to various agencies that have appropriate authorities, the 1992 Policy, *Exercise of Federal Oversight within the Scope of Statutory Authority*[9] (the Scope Policy) requires that the agencies show discretion in how they use those authorities:

> "Statutory provisions necessarily define the boundaries of the scope of discretion afforded to executive branch agencies to exercise oversight. Within the scope of authority provided by statute, federal agencies shall exercise oversight of planned introductions of biotechnology products into the environment only upon evidence that the risk posed by the introduction is unreasonable[10]."
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> "Exercise of oversight in the scope of discretion afforded by the statute should be based on evidence of unreasonable risk and should not turn on the fact that an organism has been modified by a particular technique, because such fact is not a sufficient indication of risk[11]."

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[6] OSTP. 1986. Coordinated Framework for Regulation of Biotechnology. 51 Fed. Reg. 23302, 23304

[7] OSTP. 1992. 57 FR 6753, February 27, 1992. Exercise of Federal Oversight within Scope of Statutory Authority: Planned Introductions of Biotechnology Products into the Environment. https://www.whitehouse.gov/sites/default/files/microsites/ostp/57_fed_reg_6753__1992.pdf

[8] The U.S. Government reaffirmed the scope guiding principles most recently in its 2017 update to the Coordinated Framework https://www.epa.gov/sites/production/files/2017-01/documents/2017_coordinated_framework_update.pdf and 2016 National Strategy https://www.epa.gov/sites/production/files/2016-12/documents/biotech_national_strategy_final.pdf

[9] OSTP. 1992. 57 FR 6753, February 27, 1992. Exercise of Federal Oversight within Scope of Statutory Authority: Planned Introductions of Biotechnology Products into the Environment. https://www.whitehouse.gov/sites/default/files/microsites/ostp/57_fed_reg_6753__1992.pdf

[10] Ibid., page 6756

[11] Ibid., page 6753

The regulatory principle of consistency underpins the fundamental structure of the Coordinated Framework. Because the potential uses and risks posed by products developed through modern biotechnology are the same, in kind, as existing products with similar traits that are developed with other methodologies, the Coordinated Framework relies on existing laws that were passed to regulate similar products in order to protect the public and the environment[12]. Thus, products with similar traits pose similar risks, and the Coordinated Framework paves the way for similar products to be regulated in similar ways.

The Coordinated Framework also recognized that, as more is learned, regulations should evolve and be refined. As biotechnology moved from contained laboratory research to the development and testing of potential products, the developers of the Coordinated Framework encouraged federal agencies to follow the pattern established by the National Institutes of Health (NIH) for biotechnology products. The NIH had successfully applied this regulatory principle in its oversight of genetic engineering laboratory research in the years that preceded the Coordinated Framework. The NIH guidelines for rDNA research, published initially in July 1976[13], were amended a number of times from 1977 - 1982[14], as scientists verified that the initial guidelines had been overly restrictive. Every revision was based on research findings that emerged from rDNA laboratory work. The body with oversight over rDNA research, the Recombinant DNA Advisory Committee (RAC), also evolved over time[15].

Finally, with respect to the regulatory principles above, the 1986 Coordinated Framework and 1992 Scope Policy set forth regulatory approaches that are science-based and risk-proportionate. Findings from laboratory work using rDNA techniques, which had allowed NIH to relax its guidelines, were reaffirmed by the U.S. National Academies in two reports. Their findings provided sound scientific footing for U.S. regulatory policy.

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[12] This approach was consistent with the findings of the OECD's Ad Hoc Group of Experts, which was convened in July 1983. They produced the report Recombinant DNA Safety Considerations. https://www.oecd.org/sti/biotech/40986855.pdf The report recommends the following, which was adopted by the OECD Council: "There is no scientific basis for specific legislation for the implementation of rDNA techniques and applications. Member countries should examine their existing oversight and review mechanisms to ensure that adequate review and control may be applied while avoiding any undue burdens that may hamper technological developments in this field."

[13] NIH. July 7, 1976. Recombinant DNA Research: Guidelines. 41. Fed.Reg. 27911-27943.

[14] Proposed Revised Guidelines for Research Involving Recombinant DNA Molecules FR 42:49596-49609 Sept 27, 1977; Proposed Revised Guidelines for Research Involving Recombinant DNA Molecules FR: 43:332042-33178. July 28 1978; Guidelines for Research Involving Recombinant DNA Molecules FR 43:60080-60131 December 22, 1978; Guidelines for Research Involving Recombinant DNA Molecules FR 45:6724-6749. January 29, 1980; Guidelines for Research Involving Recombinant DNA Molecules FR 45:77384-77409. November 28, 1980; Guidelines for Research Involving Recombinant DNA Molecules FR 47:17166-17198 April 21, 1982

[15] Frederickson, D.S. 2001. The Recombinant DNA Controversy: A Memoir. ASM Press. Washington, DC

Key findings of the 1987 National Academy of Sciences report[16] on rDNA organisms include the following.

- There is no evidence that unique hazards exist in the use of rDNA organisms or in the transfer of genes between unrelated organisms.

- The risks associated with rDNA organisms are the same in kind as those associated with unmodified organisms or organisms modified by other genetic techniques

These findings were completely consistent with the international scientific community's views, as expressed in the OECD report, *Recombinant DNA Safety Considerations* and in a number of statements released by interdisciplinary groups of scientists, brought together to specifically address questions related to the risks of rDNA organisms[17].

In 1989, the National Research Council (NRC)[18] reiterated these finding and concluded that "no conceptual distinction exists between the genetic modification of plants and microorganisms by classical methods or by molecular techniques that modify DNA and transfer genes." The NRC cited extensive experience with crops genetically modified through breeding and mutagenesis to support this statement.

In the intervening 30 years since the Coordinated Framework was established, significant experience and familiarity with new biotechnology products has accrued. Simultaneously, our understanding of molecular biology has grown by leaps and bounds. No event has occurred that would cause us to challenge the validity of the viewpoints expressed by the scientific community in the 1980's. In no fewer than 10 reports, including one in 2016, the NAS and NRC restate these principles unequivocally. So, too, has the European Commission (EC) verified the predicted safety of genetically engineered organisms. In two reports[19] that summarized over 25 years of EC-funded research specifically focused on identifying the risks of rDNA techniques and rDNA organisms, the EC makes the following statements:

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[16] National Academy of Sciences. 1987. Introduction of DNA-Engineered Organisms into the Environment: Key Issues

[17] International Council of Scientific Unions. 1987. Scientific Committee on Genetic Experimentation. Joint Statement. Bellagio, Italy. Fiskel and Covello (editors). 1988. Safety Assurance for Environmental Introductions of Genetically Engineered Organisms: Workshop Summary. NATO ASI Series. Springer-Verlag. Tiedje, J.M. et.al. 1989 The planned introductions of genetically engineered organisms: Ecological considerations and recommendations. Ecology. 70:298-315

[18] The National Research Council is the body that carries out the studies of the National Academies, which include the National Academy of Sciences, National Academy of Engineering and Institute of Medicine.

[19] Kessler, C. and I. Economidis (editors) 2001. EC-sponsored research on safety of genetically modified organisms: a review of results. Economidis,I., Danuta Cichoka and Jen Hogel (editors) 2010. A decade of EU-funded GMO research (2001-2010) http://ec.europa.eu/research/biosociety/library/brochures_reports_en.htm

> "The main conclusion to be drawn from the efforts of more than 130 research projects, covering a period of more than 25 years of research, and involving more than 500 independent research groups, is that biotechnology, and in particular GMOs, are not per se more risky than e.g. conventional plant breeding technologies."
>
> "According to the results of these projects, there is, as of today, no scientific evidence associating GMOs with higher risks for the environment or for food and feed safety than conventional plants and organisms."

However, regulatory agencies all over the world have ignored the scientific assessments of risks, years of real world experience, and the first principle of regulation – regulate when there is a problem that needs to be solved – and have developed unnecessarily burdensome regulatory processes for pre-market review and approval specifically focused on rDNA organisms.

THE 1992 FDA POLICY ON REGULATING NEW PLANT VARIETIES

The Food and Drug Administration (FDA) 1992 policy statement, *Foods Derived from New Plant Varieties*[20], adhered strictly to the principles set forth in both the Coordinated Framework and the Scope Policy. In doing so, it offered a clear and rational statement of a science-based, risk-proportionate approach to regulation of any new plant variety, irrespective of the techniques used in developing the variety. The regulatory process FDA described was, and still is, based on both deep scientific understanding of plant biochemistry and over a century of experience in modern plant breeding.

In its 1992 Policy, FDA lays out a comparative approach for risk assessment, first described by OECD (1986, 1992). Most countries continue to use this approach; international organizations, such as Codex Alimentarius[21], support the OECD/FDA framework as the most appropriate framework for reviewing any new plant variety, including those developed with genetic engineering. The approach is quite straightforward:

- There is a very long history of safe use of plants we grow and consume[22]
- This history has provided us with prior knowledge of
  - a crop's nutritional qualities, both the average value and normal variation of specific nutrients

[20] FDA. 1992. Statement of Policy: Foods Derived from New Plant Varieties. Federal Register. Vol 57. 104, 22984-23005

[21] Codex Alimentarius. 2003. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003).

[22] Some crops, such as cassava and some legumes, need to be prepared in certain ways to be safe, but societies that use those crops appreciate their food preparation requirements.

- chemical hazards, such as endogenous toxins, possible allergens and anti-nutrient compounds, associated with a specific crop and the amounts of exposure that do not cause harm
- ecological attributes of cultivated crops (which, for purposes of this paper, will not be discussed)

- Existing crop varieties, which have been consumed safely, can be used a reference to establish acceptable amounts of nutrients and chemicals
- Amounts of nutrients and chemicals in new varieties can be compared to those established for existing varieties of the same crop. If the amounts are within the normal range of accepted variation, then the new variety is assumed to be as safe as the existing variety

In addition, the process of genetic engineering allows for additional tests, beyond the substantial equivalence assessment ("as safe as") described above. Because the molecular basis of the new phenotypic trait is known, additional tests for toxicity and allergenicity can be conducted when novel proteins are introduced in the crop.

In its 1992 policy statement, the FDA summarized the series of steps plant breeders follow in developing a new plant variety, acknowledged the long history of plant breeders safely introducing new varieties into the marketplace, touched on the necessary testing for establishing that the new variety is equal to or better than existing varieties[23] and noted that:

> "The established practices that plant breeders employ in selecting and developing new varieties of plants, such as chemical analyses, taste testing, and visual analyses, rely primarily on observations of quality, wholesomeness, and agronomic characteristics. Historically, these practices have proven to be reliable for ensuring food safety[24]."

Perhaps more importantly for the purpose at hand, FDA's 1992 policy offers an eloquent and lucid explanation of the rationale it used to formulate a science-based approach to regulation that remains valid today. As a testament to the document's soundness, BIO's responses to the questions that FDA has posed in this docket rely heavily on the 1992 policy statement; nothing that has occurred since it was published provides a reason to reconsider the validity of FDA's rationale, issues analysis or its conclusions.

Key statements from FDA's 1992 policy provide the conceptual/scientific grounding for BIO's responses to the questions posed and should define FDA's regulatory approach to plant products produced through any genetic modification technique, including genome editing:

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[23] ASTA's comments include a detailed description of the process

[24] FDA. 1992. Statement of Policy: Foods Derived from New Plant Varieties. FR 57:104. Page 22988

1. "Under this policy, foods…derived from plant varieties developed by new methods of genetic modification are regulated…utilizing an approach identical in principle to that applied to foods developed by traditional plant breeding"[25].

2. "The regulatory status of a food, irrespective of the method by which it is developed, is dependent upon objective characteristics of the food[26] and the intended use of the food (or its components)."

3. "The method by which food is produced or developed may in some cases help to understand the safety or nutritional characteristics of the finished food[27]. However, the key factors in reviewing safety concerns should be the characteristics of the food product, rather than the fact that the new methods are used"[28].

COMPARING THE SOURCES OF GENETIC VARIATION

Because FDA stresses the fact that the method of generating genetic change is not the most relevant factor for assessing risk, before answering the questions posed by FDA, it is important to go into some detail on the specific molecular mechanism that serve as the basis for the genetic variation found in nature and that is further leveraged in the techniques to genetically improve plants, whether through conventional breeding, genetic engineering or genome editing.

Natural Generation of Genetic Variation

As stated earlier, there are two basic sources of natural genetic variation that provide the phenotypic diversity on which evolution acts: mutation and recombination.

Mutation

Mutation is a major source of genetic variation in nature. Spontaneous mutations alter an organism's genetic material, and they occur continuously at low frequencies in all organisms[29]. Individual mutations differ in terms of size of the change in the genetic material. Some mutations are minor changes in the sequence of nucleotides in single

[25] *Ibid.,* p. 22984.
[26] FDA describes the objective characteristics of the food that may affect its regulatory status, in Section VII., 22991.
[27] Section VII also describes scientific considerations that are important in evaluating the safety and nutritional value of foods for humans or animals, regardless of the foods regulatory status.
[28] FDA. 1992. Statement of Policy: Foods Derived from New Plant Varieties. FR 57:104. Page 22988
[29] On average, more than 10 mutations/generation occur spontaneously in soybeans (Schnell, J. et al., 2015 A comparative analysis of insertional effects in genetically engineered plants: considerations for premarket assessments. Transgenic research 24: 1-17.)

genes[30] and can lead to new variants of existing genes (new alleles); others involve larger stretches of genetic material that change the amount of genetic material (duplications/deletions) or the positions of genes relative to one another (inversions/translocations).

Some mutations occur in somatic cells while others occur in germline cells. Those that occur in germ cells create genetic variation that is the raw material for evolution, whether driven by natural selection or artificial selection imposed by humans. Only recently, due to the development of plant cell and tissue culture techniques, have somatic cell mutations become a source of useful genetic variation for crop breeders.

The causes of mutation range from environmental and biological factors, such as exposure to pollutants chemicals and UV radiation; viral infections; transposons (also known as transposable elements, mobile genetic elements, or "jumping genes"), which are pieces of DNA that translocate to other sites within an organism's genome, sometimes leading to gene duplications[31]. Spontaneous mutations occur as part of normal functioning of cells, for example during cell division and DNA repair;[32] guiding crossing-over (homologous recombination) in meiosis; and even the presence of reactive oxygen species produced by normal plant metabolism[33].

Recent genomics investigations have also provided evidence of another type of spontaneous mutation that resembles transposition. Genetic material from organelles such as plastids and mitochondria, is increasingly being found in nuclear DNA. Sometimes the arrangement of the nucleotides in the nucleus is identical to that found in the organelle, but other times a rearrangement has preceded insertion. In rice, approximately 25% of the organelle DNA that is found in the plants chromosomes was inserted into an existing gene[34] and in some strains, the organelle DNA became a functional component of the gene's coding sequence[35].

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[30] SNP's occur every 48 – 2000 base pairs in wheat, soybean and maize, due to errors in either DNA replication or DNA repair or misalignment of homologous chromosome in meiosis (Weber et al. 2012. Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. Plant Physiol. 160: 1842-1853.)

[31] Federoff, N, Editor. 2013. Plant Transposons and Genome Dynamics in Evolution. Wiley-Blackwell

[32] Mistakes in repairing double-strand breaks in plant DNA occurs primarily by non-homologous end joining, which is an error-prone process that leads to deletions, insertions and rearrangements at the site of repair. Britt, A.B. 1999. Molecular genetics of DNA repair in higher plants. Trends Pl Sci 4:20-25; Gorbunova,V and A Levy. 1999. How plants make ends meet: DNA double strand break repair. Trends Plant Sci 4:263-269

[33] Clearly the various causes of mutation are interrelated. UV radiation causes double strand breaks which can be repaired by error-prone mechanisms.

[34] Richly, E and D Leister. 2004. NUPTs in sequenced eukaryotes and their genomic organization in relation to NUMTs. Mol. Biol. Evol. 21:1297-1980

[35] Noutsos, et.al. 2007. Nuclear insertions of organelle DNA can create novel patches of functional exon sequences. Trends Genet. 23:597-601

Associated mechanisms for changing genetic material that contribute to genome plasticity are discussed at length in Schnell, et.al. 2015[36]. The genomic impacts of each of these molecular mechanisms of mutation are discussed in more detail below.

Recombination

Recombination is a broad term that encompasses all forms of re-assorting preexisting genes, thus leading to new genetic combinations. Recombination occurs during segregation and independent assortment of chromosomes during gamete formation, chromosomal recombination, sexual reproduction and horizontal gene transfer.

Chromosomal recombination occurs during the formation of gametes when the arm(s) of chromosomes cross over each other and exchange genetic information during the meiotic pairing of homologous chromosomes. As described below, at times crossing over leads to mutations such as gene duplications, deletions and nucleotide substitutions.

For eukaryotes, sexual reproduction involves the fusion of gametes. For prokaryotes, such as bacteria that reproduce solely by dividing in two, recombination of genetic material within the same species occurs through direct contact (conjugation); a few species are also able to take up free, extracellular DNA from the environment under natural conditions (transformation). The efficiency of both mechanisms of gene transfer is greatest when the DNA donor and DNA recipient belong to the same species.

Horizontal gene transfer is defined as the transfer of genetic material across species rather than within species from one generation to the next. All organisms have evolved self-defense mechanisms that discourage HGT[37]. For example, reproductive isolating mechanisms in plants prevent pollination of an ovule by pollen from a different species and/or the development of viable embryos that become fertile adults.

Mechanisms for preventing HGT, however, are not foolproof. In fact, the discovery of *Agrobacterium* genes in plants provided the intellectual impetus for early research on plant genetic engineering[38]. As more is learned and the prevalence of HGT elucidated, scientists are beginning to uncover the importance of HGT in the evolution of life on

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[36] Schnell, et.al. 2015. A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Res. 24:1-17

[37] Many of these self-defense molecules have become indispensible research and product development tools: Bacteria produce restriction enzymes, now used in rDNA work, to cut up invading genetic material from other bacteria and viruses.

[38]Chilton M-D, et al. (1977) Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of crown gall tumorigenesis. Cell 11:263–271. 11. Chilton M-D, et al. (1982) *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells. Nature 295:432–434. Gelvin SB. 2003. Agrobacterium-mediated plant transformation: The biology behind the “gene-jockeying” tool. Microbiol. Mol. Biol. Rev. 67:16–37

earth[39]. In prokaryotes, uptake of genetic material from a different species can occur through conjugation, transformation or transduction (genetic material exchange mediated by viruses). Genome sequencing of eukaryotes has revealed that HGT occurs much more often and across more widely divergent taxonomic division, including Kingdom level transfers, than previously assumed[40]. For example, DNA from plant viral pathogens has become integrated into the genetic material of many of our crop plants[41]. In addition, the amount of genetic material that is transferred can be quite substantial[42], and a recipient species may have received genetic material from many different species. Finally, some HGT events have been linked to phenotypic traits, some of which provide a selective advantage to the recipient organism[43].

Perhaps surprisingly, the movement of transposable elements between plant species has also been reported[44], as has the between-species movement of organelle genetic material into chromosomal genetic material[45].

Genetic Variation and Conventional Breeding

For decades the seed industry has been introducing hundreds of new crop plant varieties to the marketplace annually. To generate this continuous influx of new varieties, plant breeders make use of all of nature's mechanisms to create the genetic variation that will be funneled into the crop improvement process. They take advantage of any spontaneous mutation or natural recombination events that result in desirable traits:

- Spontaneous mutants were the source of the semi-dwarf varieties of wheat and rice that spawned the Green Revolution in the 1960's.
- Polyploidy, or whole genome duplication (WGD), has played a prominent role in the evolution of plants in nature, in crop domestication and in subsequent selection of preferred phenotypes by breeders[46]. WGD often trigger subsequent mutations such

[39] Nikoh N, et al. 2008. *Wolbachi*a genome integrated in an insect chromosome: Evolution and fate of laterally transferred endosymbiont genes. Genome Res. 18:272–280.

[40] Dunning Hotopp JC. 2011. Horizontal gene transfer between bacteria and animals. Trends Genet 27(4):157–163. Dunning Hotopp JC, et al. 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317(5845):1753–1756. 2.

[41] Staginns,C. et.al. 2007. Endogenous pararetroviral sequences in *Solanum lycopersicum* and related species. BMC Plant Biol. 7:24

[42] Gladyshev EA, Meselson M, Arkhipova IR. 2008. Massive horizontal gene transfer in bdelloid rotifers. Science 320:1210–1213

[43] Moran NA and Jarvik T (2010) Lateral transfer of genes from fungi underlies carotenoid production in aphids. Science 328:624–627. Li F-W, et al. (2014) Horizontal transfer of an adaptive chimeric photoreceptor from bryophytes to ferns. Proc Natl Acad Sci USA 111:6672–6677. Gasmi, L. 2015. Recurrent domestication by *Lepidoptera* of genes from their parasites mediated by bracoviruses. PLOS Genetiics. 11:e1005470

[44] El Baidouri, Moaine; et al. 2014. Widespread and Frequent Horizontal Transfers of Transposable Elements in Plants. Genome Research. 24: 831–838.

[45] Richardson, A.O. and J.D. Palmer. 2007. Horizontal gene transfer in plants. J.Exp.Bot.58:1-9.Bergthorsson, U., et.al. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature 424: 197-201

[46] Leitch A and I Leitch. 2008. Genomic plasticity and diversity of polyploid plants. Science. 320:481-83

as chromosomal rearrangements, gene loss, and problems with homologous recombination during meiosis[47]

- Early in its domestication the yield of bread wheat increased due to mutations at all levels from gene expression to chromosome rearrangements and WGD.
- Sequencing of crop genomes has revealed over 50 transposable elements that were important in converting wild plants into crops. The natural excision and insertion of transposons led to phenotypic characteristics, favored by early farmers, which resulted from the genetic changes, such as deletions, duplications or rearrangements that accompany transposition[48].

In addition to utilizing naturally occurring mutation and recombination events, plant breeders have developed many lab techniques that increase their capacity to capitalize on nature's molecular mechanisms for generating genetic variants and to push the boundaries of recombination. These include the following.

Induced Mutagenesis

Because spontaneous mutations occur at low frequencies, and those that lead to desirable traits are, thus, rare, in the 1920's breeders began inducing mutations with chemicals and irradiation. When beneficial traits appear, they are incorporated into breeding programs. At a molecular or cellular level, such mutations include point mutations in single genes, transpositions, chromosomal rearrangements, and polyploidy.

When breeders induce a mutation with ionizing radiation, they cause double-stranded breaks which are usually repaired by the error-prone NHEJ mechanisms[49]. Chromosomal analysis and genome sequencing has shown that many radiation-induced mutations have deletions that range from a few to over a million base pairs and many rearrangements (inversions and translocations)[50]

Chemical mutagens, such as ethyl methanesulfonate, are more likely to cause single base substitutions[51], rather than double stranded breaks, by changing which nucleotides pair with each other.

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[47] Adams, K and J Wendel. 2005 Polyploidy and genome evolution in plants. Curr. Opinion Plant Bio. 8:135-141; Lai, J. et al. 2004. Gene loss and movement in maize genome. Genome Res. 42:1027-31.

[48] Vitte, C. et.al. 2014. The bright side of transposons in crop evolution. Brief Func. Genomics:elu002; Lisch,D. 2013. How important are transposons for plant evolution? Nat. Rev.Genet. 14:49-61

[49] Friede, B. et. al. 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests. Euphytica. 91: 59-87

[50] Cecchini,E. et.al. 1998. Characterization of gamma radiation induced deletion mutations at a selectable locus in Arabidopsis. Mutat. Res. 401: 199-206; Morita,J. et.al. 2009. Molecular characterization of mutations induced by radiation in rice. Genes Genet. Sys. 84:361-370; Schuermann, D. et.al. 2005. The dual nature of homologous recombination in plants. Trends Genet. 21: 172-181; Shirley, B. et.al. 1992. Effects of ionizing radiation on a plant genome. Plant Cell 4: 333-347

[51] Greene, E. et.al. 2003. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. Genetics 164: 731-740.

According to the FAO[52] in 2009, induced mutations were responsible for more than 3100 new cultivars in at least 190 plant species. FAO notes that this is a "gross underestimate" of the actual number of varieties containing induced mutations due to FAO's limited information sources: breeders voluntarily provide the information to FAO, and FAO also collects information from publicly available databases[53]. In addition, once a mutant variety is reported, it becomes part of public and private breeding programs all over the world.

According to the FAO, "breeders are not particularly interested in the source of the variation and mutated lines are considered as basic, raw materials. Once a mutation for an important trait is captured and used over many years its novelty/ origin is often lost, ignored or forgotten[54]."

Capturing Somatic Mutations
When mutations occur in somatic cells, the ability of breeders to capture and use the genetic diversity for crop improvement depends on a variety of techniques that provide for plant propagation in the absence of seed production.

In the development of new horticultural varieties, a number of species, especially woody perennials, are often propagated vegetatively. The ability to capture somatic mutations in bud sports is responsible for 25% of the apple varieties grown in the U.S. and many other varieties of fruit trees, including nectarines (bud sports of a peach) and red Anjou pears. Colorless varieties of grapes and blood red oranges, both of which were developed through vegetative propagation, result from transposon-based mutations in the same metabolic pathway (anthocyanin pigment). In the case of the white grape, insertion of the transposon between the transcription factor and the coding sequence disrupted gene function; insertion of a transposon adjacent to a gene turned on anthocyanin synthesis in the orange.

Much more recently, breeders have developed ways to incorporate somatic mutations into the germlines of many crop plants, thus making the mutations heritable. Improvements in cell and tissue culture techniques allow non-reproductive cells, such as protoplasts, pieces of plant tissues, and single, differentiated cells, to germinate into an undifferentiated mass of cells (callus). When treated with certain hormones and grown under appropriate conditions the callus will complete the developmental process and

[52] FAO. 2012. Plant Mutation Breeding and Biotechnology. p 14 http://www.fao.org/3/a-i2388e.pdf. FAO database of mutant varieties can be found at http://mvgs.iaea.org
[53] Ibid, page 14
[54] Ibid, page 15

become whole plants capable of reproduction. Not only does this allow breeder to capture induced genetic changes (protoplast fusion/somatic cell hybridization) and spontaneous mutations in somatic cells, but the process of growing plant cells in culture induces mutations, which provides additional genetic diversity from which to choose (somaclonal variant selection). The mutations cause by cell and tissue culture range from single base changes, to loss and rearrangements of chromosomes. Tissue culture conditions also seem to activate excision and insertions of transposons[55].

Recombination across Different Species

Plant breeding is based on the introgression of genes from other varieties or wild relatives into an elite variety that is the improvement target. Using genomics, Hajjar and Hodgkin (2007) demonstrated the introgression of genes from 60 wild relatives into 13 of our most important food crops. Disease resistance genes from over 10 different species in six different genera have been incorporated into wheat[56]. In every case it is correct to assume that some genetic material from the wild relative that is transferred along with the gene of interest is not eliminated through backcrossing.

Generation of double-stranded breaks followed by repair, primarily NHEJ in plants, is a feature of all cross breeding in plants. As taxonomic distance between the parental plants widens, generating viable offspring/seeds becomes more difficult. Breeders have developed an array of lab techniques that permit interspecific and intergeneric cross breeding. For each wide cross, they identify the point at which fertilization and subsequent developmental processes run aground, and then they flesh out the biochemical failure at the heart of the breakdown. Once the problem is elucidated, they develop solutions, which often involve mutagenic chemicals, to introgress the desired trait into the existing variety.

Chromosomal analysis has shown that translocations sometimes occur spontaneously when crosses are forced. More often than not, translocations or chromosome doubling are intentionally induced by breeders as a method for circumventing the plant's reproductive isolating mechanisms.

Most varieties of all major crop plants have a "wide cross" in their genealogy, which means that at some point in the development of most varieties, plant breeders used laboratory techniques, many of which are mutagenic.

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[55] Karp, A and S Maddox. 1984. Chromosome mutations in wheat plants regenerated from cultured immature embryos. Theor. Appl. Genet. 67: 249-255; Lee, M and R Phillips. 1987. Genomic rearrangements in maize induced by tissue culture. Genome 29: 122-128; Hirochika, H.et.al. 1996. Retrotransposons of rice involved in mutations induced by tissue culture. PNAS. 93: 7783-7788.

[56] Jones, S. et.al. 1995. The development of disease resistance in wheat. Ann. Rev. Phytopath. 33: 429-443

Recombinant DNA (rDNA) Technology

Of all natural mechanisms for creating genetic variation, rDNA technology conceptually is most similar to recombination via horizontal gene transfer. Existing genes discovered in any organism can be transferred into a recipient plant; the product is a transgenic plant. Like HGT, the insertion site is random. However, unlike the HGT that occurs in nature, in rDNA technology, the amount of genetic material that is inserted is controlled, the identity of the gene is known, and the gene's function is well understood, from genotype to phenotype.

At a molecular level, the mechanics of inserting the new DNA most resembles the movement of transposable elements, which are found naturally in the genomes of all plant, or the movement of organelle genetic material into the genetic material found in the nucleus. However, the intracellular and intragenomic movement of genetic material that occurs naturally is more disruptive to the plant's genome: in nature, double stranded breaks lead to excision and insertion of genetic material. In certain crop plants, a surprisingly large percent of the genome is composed of transposable elements (rice – 25%; corn – 57%)[57]. Naturally occurring transposons in plant genomes can be as large as thousands of base pairs, and some have their own open reading frames. According to Bennetzen (2000, 2007) and others[58], the molecular impact of intragenomic insertions of transposons ranges from no effect to 1) gene activation, 2) alteration of the gene's expression level, or 3) a change in the RNA/protein expressed by the gene.

To be effective, the rDNA construct needs to be inserted near an endogenous gene; the likelihood of that occurring varies with the density of structural and regulatory genes. For example, 24% of the rice genome consists of gene rich regions, which translates to one gene/4kb of DNA. The genetic density of rice is 10 times greater than that of maize (one gene/40 kb)[59].

Genome Editing

As described in the Introduction, there are two distinct types of genome editing approaches. The editing tools can be used to:

1. insert new DNA into the plant's genome at a very specific site; or

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[57]Yu, J. et.al. 2002 A draft sequence of the rice genome (*Oryza sativa*, ssp.i*ndica*). Science 296:79-92. Messing, J., et.al., 2004. Sequence composition and genome organization in maize. PNAS 101:14349-14354.

[58] Bennetzen, J. 2000. Transposable element contributions to plant gene and genome evolution. Plant Mol. Biol. 42:251-269. Bennetzen, J. 2005. Transposable elements, gene creation and genome arrangement in flowering plants. Curr. Opin.Genet.Develop. 15:621-627; Casacuberta, J and N. Santiago. 2003. Plant LTR-retrotransposons and MITEs: control of transposition and impact on the evolution of plant genes and genomes. Gene 311: 1-11. Greco,R. et.al.2003. Transposon insertional mutagenesis in rice. Plant Physiol. 125:1175-1177.

[59] Messing, J., et.al., 2004. Sequence composition and genome organization in maize. PNAS 101:14349-14354. Barakat,A. et.al. 1997.The distribution of genes in the genomes of Gramineae. PNAS.94: 6857-6861. Yu, J. 2002 A draft sequence of the rice genome (*Oryza sativa*, ssp.i*ndica*). Science 296:79-92.

2. make changes to the plant's endogenous genetic material. Those changes can range from single nucleotide changes to the deletion of an entire gene to rearranging the positions of existing genes so that they are inherited as a unit.

With respect to the first use above, if the new DNA that is inserted into the crop is from the same or a sexually compatible species, the transfer is termed "cisgenic"; if the DNA is from a species outside of the plant's accessible gene pool, the transfer is "transgenic". Transgenic plants developed with the aid of genome editing tools are therefore like rDNA plants developed with genetic engineering tools. The only difference is that with genome editing tools, plant breeders can control the site of insertion with greater precision.

Conceptually, the use of genome editing tools to introduce new genetic material to a crop is most like recombination through conventional breeding. If the two plants share the same breeder's gene pool, then the same genetic material could have been introduced into the recipient plant through conventional breeding techniques. The advantage of using genome editing rather than cross breeding is that the breeder is introducing only one or a few genes rather than the entire genome of the donor plant, thus avoiding the need for a number of backcrosses.

The second way of using genome editing tools is most akin to inducing mutations to achieve a certain phenotype. However, in the case of genome editing, specific biomolecular tools, rather than chemicals, radiation or tissue culture, are used to trigger changes in the plant's genes. Genome editing to induce mutations is much more targeted and specific than earlier induced mutagenesis methods of inducing mutations. Thus, with genome editing we use the term "targeted mutagenesis".

The molecular mechanics of both uses of genome editing tools are similar. Whether the breeder is introducing new genetic material or changing endogenous genes, the editing tool creates a double-stranded break at a selected and predefined location in the plant's genome. The introduced break serves as a trigger for the plant cell's DNA repair mechanisms, resulting in either non-homologous end-joining (NHEJ) or homologous recombination repair (HRR). NHEJ is error-prone, meaning that while the site of the mutation is defined, the outcome of the repair can include deletions, insertions and rearrangements similar to those observed at the site of DNA repair after spontaneous or induced mutations.

In summary, the main difference between

- targeted mutagenesis through genome editing and induced mutations with chemicals or radiation is specificity.

- genome editing that enables introduction of genetic material from within the breeder's gene pool and conventional cross breeding is specificity; it allows transfer of one to a few genes rather than the entire genome.

- genome editing that enables the introduction of genetic material from outside of the breeder's pool and rDNA technology is specificity. In both cases one to a few genes are transferred; in genome editing the insertion site is specific, but with rDNA techniques, the insertion of the genetic material into the genome is random.

RESPONSES TO QUESTIONS POSED BY FDA

Question: In what ways are the food safety risks associated with human and animal foods from genome edited plants the same as or different from those associated with other plant development methods?

Nature of the Food Safety Risks

In keeping with the NAS statements that the risks of organisms modified by any genetic modification technique are the same in kind as the risks associated with unmodified organisms or organisms modified by other genetic techniques, throughout its 1992 science-based, risk-proportionate policy statement[60], FDA reaffirms that food safety risks are related to the nature of the product and not the process used to create the product.

> "The key factors in reviewing safety concerns should be the characteristics of the food product, rather than the fact that the new methods are used"[61].
>
> "The regulatory status of a food, irrespective of the method by which it is developed, is dependent upon objective characteristics of the food[62] and the intended use of the food (or its components)[63]."

These statements also apply to the plant products developed with the new genome editing tools.

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[60] FDA. 1992. Foods Derived from New Plant Varieties; Statement of Policy. Federal Register 57:104, 22984-23005. May 29, 1992
[61] Ibid., page 22984-5
[62] FDA describes the objective characteristics of the food that may affect its regulatory status, in Section VII., 22991.
[63] Ibid., page 22984-5

Likelihood of Food Safety Risks

The 1992 policy statement notes that:

> "Virtually all breeding techniques have potential to create unexpected, including pleitropic, effects[64];" and
>
> "Any genetic modification technique has the potential to alter the composition of food in a manner related to food safety, although, based on experience, the likelihood of a safety hazard is typically very low[65]."

The likelihood of a safety hazard is low because "producers of new foods have an obligation to ensure that the foods they offer to consumers are safe and in compliance with applicable legal requirements[66]." Therefore, "plant breeders, using well established practices have successfully identified and eliminated plants that exhibit unexpected, adverse traits prior to commercial use[67]."

The 1992 policy statement summarizes the series of steps plant breeders follow in developing a new plant variety, irrespective of the source of the genetic variability being incorporated into the new variety. The policy touches briefly on the necessary testing for establishing that the new variety is equal to or better than existing varieties[68] and notes that "in the course of this intensive assessment, individual plants exhibiting undesirable traits are eliminated[69]."

FDA's observation of the long history of safe introductions of new plant varieties is confirmed by the extensive literature reviews conducted by the National Academy of Sciences in 1973[70], food scientists[71], and the International Food Biotechnology Council (IFBC) in 1990[72].These comprehensive reviews of a number of data sources in addition to peer-reviewed publications have revealed one case in which breeding led to increase in natural toxins, and another in which it might have.

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[64] Ibid., 22986
[65] Ibid., 22986
[66] Ibid., 22985
[67] Ibid., 22986
[68] ASTA's comments include a detailed description of the process
[69] Ibid., 22986
[70] National Academy of Sciences. 1973. Toxicants Occurring naturally in Foods. NAS, Washington, D.C. https://www.nap.edu/catalog/21278/toxicants-occurring-naturally-in-foods ; Leiner, I.E. (Ed.) 1980. Toxic constituents of Plant Foodstuffs. Academic Press, New York.
[71] Leiner, I.E. (Ed.) 1980. Toxic constituents of Plant Foodstuffs. Academic Press, New York.
[72] IFBC. 1990. Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification. Reg Toxicol Pharmacol 12: SI-SI96. http://ilsirf.org/wp-content/uploads/sites/5/2016/06/01_1990RegToxPharm-CSAFF.pdf

In the 1970's USDA scientists developed a potato variety (Lenape) having a high solid content, which is useful in food processing, and resistance to late blight provided by a wild ancestor of potato (*Solanum demissum*)[73]. Routine monitoring by a food company found high levels of the endogenous toxin, solanine. The company notified both USDA and FDA, and the variety was immediately withdrawn[74]. More recently (1990's) Swedish officials noticed high solanine levels in an heirloom potato variety that had become popular with some Swedish consumers. However, this variety, which was developed in England in the 1800's, had been replaced with modern varieties long ago[75]. The reintroduced heirloom variety, given the name *Magnum Bonum*, was withdrawn from the marketplace.

Celery naturally produces toxins, known as psoralens, as a defense against insect pests and plant pathogens. Psoralens temporarily increase the photosensitivity of human skin to UV light and are used by dermatologists to treat certain acute skin diseases. Photodermatitis, caused by increased psoralen levels, has been observed in field workers who harvest celery and produce handlers in grocery stores who frequent tanning salons. However, it is not clear if breeding was responsible for higher psoralen levels. There is a strong correlation between incidences of photodermatitis and the levels of plant pathogens on the celery. Therefore infection could have triggered the plants to increase psoralen synthesis. Therefore, two environmental factors– UV light and plant pathogens – play key roles in determining whether psoralen causes contact photodermatis, making it difficult to determine the role played by breeding, if any.

Consistent with the first principle of regulation – regulate when there is a problem that needs to be solved and regulation is the best way to solve it --- FDA states:

> "Based on this record of safe development of new varieties of plants, FDA has not found it necessary to conduct, prior to marketing, routine safety review of whole foods derived from plants[76]". Instead FDA relies on its post market authority provided in section 402(a)(1)[77] to ensure the safety of food crops and their components and reserves premarket review and approval, provided for in section

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[73] Akeley, R.V. et.al., 1968. A new potato variety very high in solids and good chipping qualities. Am Potat J 45:142-45
[74] Zitnak, A. and G.R. Johnston. 1970. Glycoalkaloid content of BF 5141-6 potatoes. Am Potat. J. 4.7:256-260
[75] National Academy of Sciences. 1973. Toxicants Occurring naturally in Foods. NAS, Washington, D.C. https://www.nap.edu/catalog/21278/toxicants-occurring-naturally-in-foods ; Leiner, I.E. (Ed.) 1980. Toxic constituents of Plant Foodstuffs. Academic Press, New York.

[76] FDA. 1992. Foods Derived from New Plant Varieties; Statement of Policy. Federal Register 57:104, 22984-23005. May 29, 1992. 22988
[77] 21U.S.C. 342(a)(1)

409[78], for those cases when the objective characteristics of the substance raise questions of safety.

FDA should follow this approach for products of genome edited crop plants.

If FDA has not found it necessary to conduct pre-market review of foods derived from whole plants developed through conventional breeding, and if FDA adheres to the regulatory principles articulated above, both by FDA and others, it is virtually impossible to justify a different approach for genome edited crop plants developed with more precise techniques, based solely on the methods used in developing the crop.

Question: To what extent is the scientific knowledge of and experience with current new plant varieties (such as those developed with in vitro recombinant DNA technologies that have gone through the voluntary consultation process) relevant to the safety assessment and regulatory status of food from new plant varieties produced using genome editing?

The scientific knowledge and experience with ALL new plant varieties, not just those developed with rDNA techniques, is relevant to the safety assessment and regulatory status of food from new plant varieties using genome editing. Most applications of genome editing techniques are more similar to traditional breeding than to rDNA modifications.

In its 1992 policy statement[79], FDA acknowledged that both spontaneous mutations or those induced by breeders can range from single-gene changes to chromosomal rearrangements and that induced mutations are limited by their inability to precisely target a desired trait. In spite of the inability to precisely target a desired trait, FDA also noted:

> "The established practices that plant breeders employ in selecting and developing new varieties of plants such as chemical analyses, taste testing, and visual analyses, rely primarily on observations, of quality wholesomeness and agronomic characteristics. Historically these practices have been proven to be reliable for ensuring food safety. The knowledge from this experience coupled with safe practices in plant breeding has contributed to continuous improvements

[78] 21 U.S.C. 348

[79] FDA.1992. Statement of Policy: Foods Derived from New Plant Varieties. Federal Register. Vol 57. 104, 22984-23005

in the quality variety nutritional value and safety of foods modified by a range of traditional and increasingly sophisticated techniques.[80] " and

"Based on this record of development of new varieties of plant, FDA has not found it necessary to conduct, prior to marketing, routine safety reviews of whole foods derived from plants."

No new developments have occurred since 1992 that would justify FDA changing this policy, given that governments should regulate only when there is a problem that needs to be solved. The principle remains valid. Scientific understanding and experience accrued only serve to buttress FDA's policy as articulated in 1992.

Quoting from ASTA's comments to this docket: "Targeted mutations through genome editing should not be treated any differently with respect to the FDA consultation process as are spontaneous or induced mutations. Likewise, new varieties developed using genome editing to precisely change endogenous DNA sequences should not be treated differently than new varieties developed using cross-breeding to change endogenous DNA sequences. As virtually all products of the plant breeding process using mutation and crossing methods have not exhibited safety concerns to date, there is no reason to believe that reproducing those genetic changes using gene editing would raise any additional concerns."

With respect, specifically, to rDNA crops, after over 20 years of conducting safety assessment of applications of rDNA, no unintended effects or safety issues have ever been identified. The findings of these safety assessment are consistent with empirical evidence:

- Annually, billions of animals consume feed derived from plants developed with rDNA techniques with no evidence of harm[81].
- People all over the world have consumed food derived from transgenic crops for decades, and no adverse effects have been observed.

Unlike the products of rDNA technology, the phenotypic alterations that will result from most genome editing applications will not involve the introduction of new proteins to a familiar crop. Most will lead to either the removal of a protein from the food derived from the crop or minor changes to a protein currently in the food supply.

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[80] Ibid. p. 22988

[81] Van Eenennaam, A. and A. Young. 2014. Prevalence and impacts of genetically engineered feedstuffs on livestock populations. Journal of Animal Science. 92:4255-4278.

Question: Is there additional scientific knowledge that would be relevant specifically to the safety assessment and regulatory status of new plant varieties produced using genome editing?

BIO included the extensive literature review on the dynamic nature of the plant genome, above, largely in response to this question. Our understanding of the mechanisms, impacts and prevalence of genetic variation has grown exponentially in the past 20 years. High throughput genomic analysis, in conjunction with gene expression profiling, has provided scientists with an unprecedented opportunity to

- assess the frequency and magnitude of naturally occurring genetic changes that resemble those that breeders induce using the many genetic modification techniques available to them, and

- determine the phenotypic effects of those changes at every organizational level from the gene to the whole organism.

To implement a science-based, risk-proportionate regulatory approach to genome editing, FDA and other regulatory bodies should view the concept of risk through the lens provided by nature. Clearly a genetic modification event does not, in and of itself, equate to risk.

The risks of any conceivable genetic change created with the genetic modification techniques that breeders use are dwarfed by the magnitude, frequency and remarkable creativity of nature's mechanisms for creating genetic variation. Investigations into the genomics, functional genomics and proteomics of a wide array of organisms demonstrate nature's propensity for generating variation, which will provide a rich source for future innovation and crop improvement.

Question: Are there categories of genome edited plant varieties for which there are scientific bases to conclude that foods from such categories are unlikely to present food safety risks different from or greater than those for traditional plant breeding? Similarly, are there categories of genome edited plant varieties for which the regulatory status of the food derived from such plant varieties can be said to be no different from that of traditionally-bred plants? If there are such categories, is there a basis upon which to determine that there would be no reason to include them in any voluntary premarket consultation process?

In 1992, FDA's policy on new plant varieties[82] provided answers to these questions, and those answers remain valid 25 years later.

> "In most cases the substances expected to become components of food as a result of genetic modification of a plant will be similar to substances commonly found in food, such as proteins, fats and oils, and carbohydrates[83]….When the substance present in the food is one that is already present at generally comparable or greater levels, in currently consumed food, there is unlikely to be a safety question sufficient to call into question the presumed GRAS status of the of such naturally occurring substances and thus warrant formal premarket review and approval by FDA. Likewise, minor variations in molecular structure that do not affect safety would not ordinarily affect the GRAS status of substances and, thus, would not ordinarily require regulation of the substance as a food additive."

Therefore, those uses of genome editing that make minor changes in a plant's endogenous genetic material 1) are unlikely to raise questions sufficient to call into question the presumed GRAS status, and 2) some may result in minor changes to a plant's endogenous proteins and should not require regulation as a food additive.

New plant varieties developed using genome editing applications that are essentially a more precise way of cross-breeding or inducing mutagenesis should not be treated differently from a regulatory perspective than those new plant varieties developed through these more traditional breeding methods.

Question: Are there categories of genome edited plant varieties for which there are scientific bases to conclude that foods from these categories are more likely than traditionally-bred plants to present food safety risks? If so, please describe the characteristics of these categories (including, for example, information about the types of phenotypes and modifications (insertions, deletions or substitutions) achieved through genome editing) and provide data and/or information to support why plant varieties in these categories are more likely to present food safety risks than traditionally-bred plants.

Again we refer FDA to statements made in the 1992 policy.

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[82] FDA. 1992. Foods Derived from New Plant Varieties; Statement of Policy. Federal Register 57:104, 22984-23005.
[83] Ibid., page 22985

> "New methods of genetically modifying plants …enable developers to make genetic modifications that would not be possible with traditional breeding methods[84]….New genetic modification techniques may develop plants that produce non-food chemicals, such as polymers and pharmaceuticals. .. If plants used to make non-food chemicals are also intended to be used for food, *producers should consult with FDA.* If the intended expression product in a food [is] "a protein, carbohydrate fat or oil, or other substance that differs significantly in structure, function or composition from substances currently in food, *such substance may not be GRAS and may require regulation as a food additive.*

Therefore, if genome editing tools are used to 1) introduce novel or unfamiliar genetic material, in a manner similar to rDNA, or 2) to make *significant* changes to the molecules that are already present in food, the developer should assume the new genome edited variety is subject to pre-market review by FDA and therefore should consult with FDA.

If the intent of the genome edit is to change the nutrient level of the food, the food will need to be labeled accordingly. Therefore, the developer should contact FDA early in the development process.

With respect to unintended changes, FDA noted that plant breeding may induce unexpected changes, but, with the exception of one product in tens of thousands[85], the standard practices followed by plant breeders has kept plants with higher levels of toxicant or anti-nutrients out of the marketplace. The one instance of unexpected food allergenicity in a large number of people occurred in the 1960's when kiwi fruit first came to the U.S. It was not caused by unintentionally increasing the allergenic potential of a familiar food through breeding, but was a result of introducing a novel, whole food to a public that had no prior experience with it.

Finally, there is no evidence that either conventional breeding or genetic engineering has ever created a new toxin or new anti-nutrient[86], or activated a vestigial metabolic pathway that produced an unknown toxin in a crop with a long history of safe use.

If conventional breeding has not introduced the risks described above, then the likelihood of genome editing causing such a problem are miniscule due to the greater specificity of these techniques.

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[84] Ibid., page 22984
[85] The Lenape potato described earlier
[86] Steiner et.al. 2013

In its 1992 policy, FDA included a number of decision trees to help crop developers know if and when they needed to consult with FDA. Because these decision-trees are not available on-line, we have attached them as Appendix 2.

BIO appreciates the opportunity to respond to the questions posed by FDA, and we look forward to continuing to work with FDA in the future.

Sincerely,

Dana O'Brien
Executive Vice President

cc. Jason Dietz, Center for Food Safety and Applied Nutrition
Kathleen Jones, Center for Veterinary Medicine

APPENDIX 1. OECD PRINCIPLES FOR BETTER REGULATION

1. There is a problem that needs to be addressed.
The problem to be solved should be precisely stated, giving clear evidence of its nature and magnitude, and explaining why it has arisen.

2. Government action is justified.
Intervention should be based on clear evidence that government action is justified, given the nature of the problem, the likely benefits and costs of action, and alternative mechanisms for addressing the problem.

3. Regulation is the best form of government action for addressing the problem.
Regulators should carry out, early in the regulatory process, an informed comparison of a variety of regulatory and non-regulatory policy instruments, considering relevant issues such as costs, benefits, distributional effects, and administrative requirements.

4. There is a legal basis for regulation
Regulatory processes should be structured so that all regulatory decisions rigorously respect the "rule of law", i.e., responsibility should be explicit for ensuring that all regulations are authorised by higher level regulations/laws, are consistent with treaty obligations, and comply with legal principles such as certainty and proportionality.

5. Decide on the appropriate level (or levels) of government for this action
Regulators should choose the most appropriate level of government to take action, or, if multiple levels are involved, should design effective systems of coordination between levels of government.

6. The benefits of regulation justify the costs
Regulators should estimate the total expected costs and benefits of each regulatory proposal and of feasible alternatives, and should make the estimates available in accessible format to decision-makers. The costs of government action should be justified by its benefits before action is taken.

7. The distribution of effects across society is transparent
To the extent that distributive and equity values are affected by government intervention, regulators should make the distribution of regulatory costs and benefits across social groups clear to all.

8. The regulation is clear, consistent, comprehensible, and accessible to users
Regulators should determine if rules will be understood by likely users, and to that end should take steps to ensure that the text and structure of rules are as clear as possible.

9. All interested parties should have the opportunity to present their views
Regulations should be developed in an open and transparent fashion, with appropriate procedures for
effective and timely input from interested parties, such as affected businesses and trade unions, other interest groups, or other levels of government.

10. Compliance must be achievable
Regulators should assess the incentives and institutions through which the regulation will take effect and design responsive implementation strategies that make the best use of them.

Appendix 2. FDA Decision Trees from the 1992 Policy on New Plant Varieties

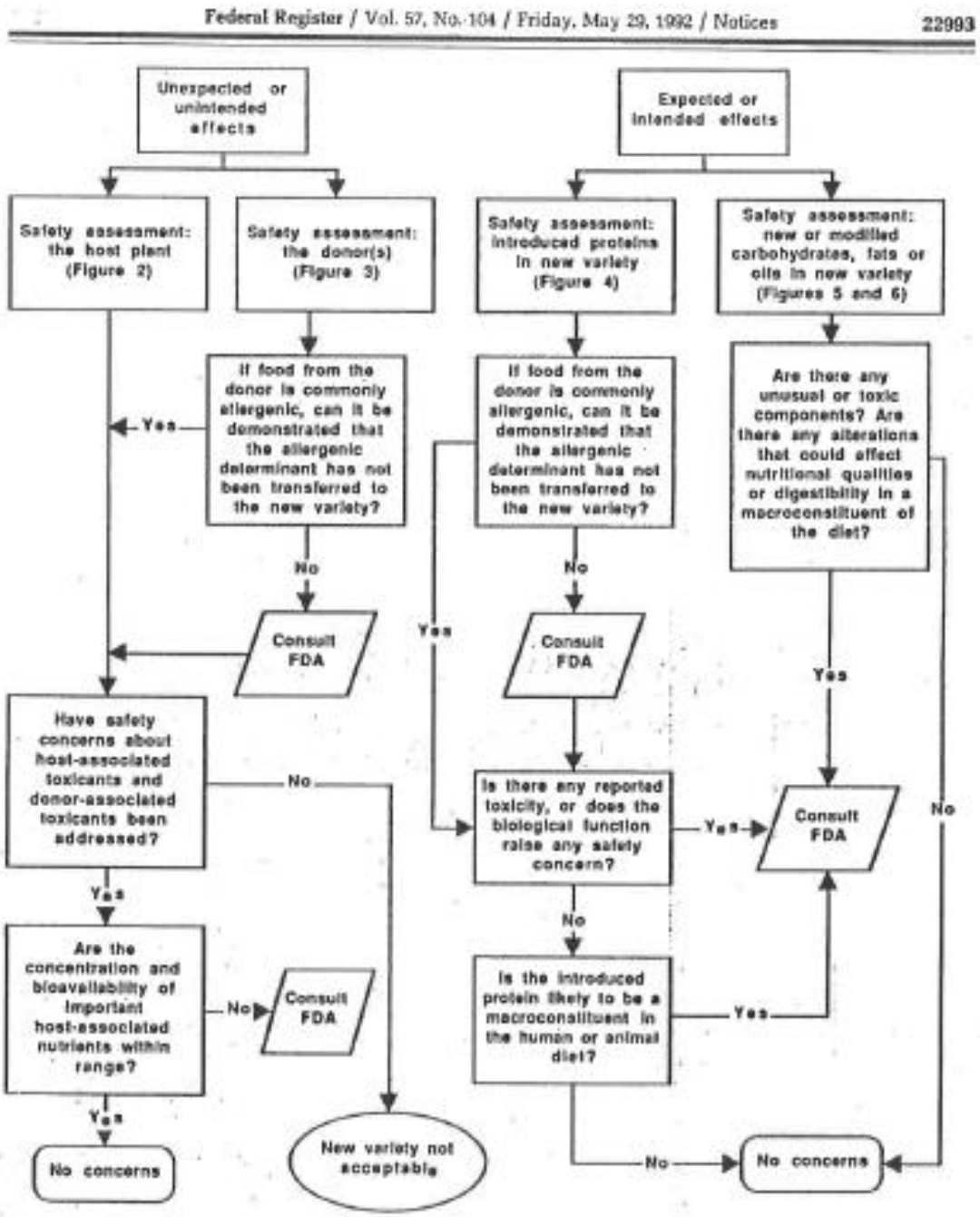

Figure 1. Safety Assessment of New Varieties: Summary

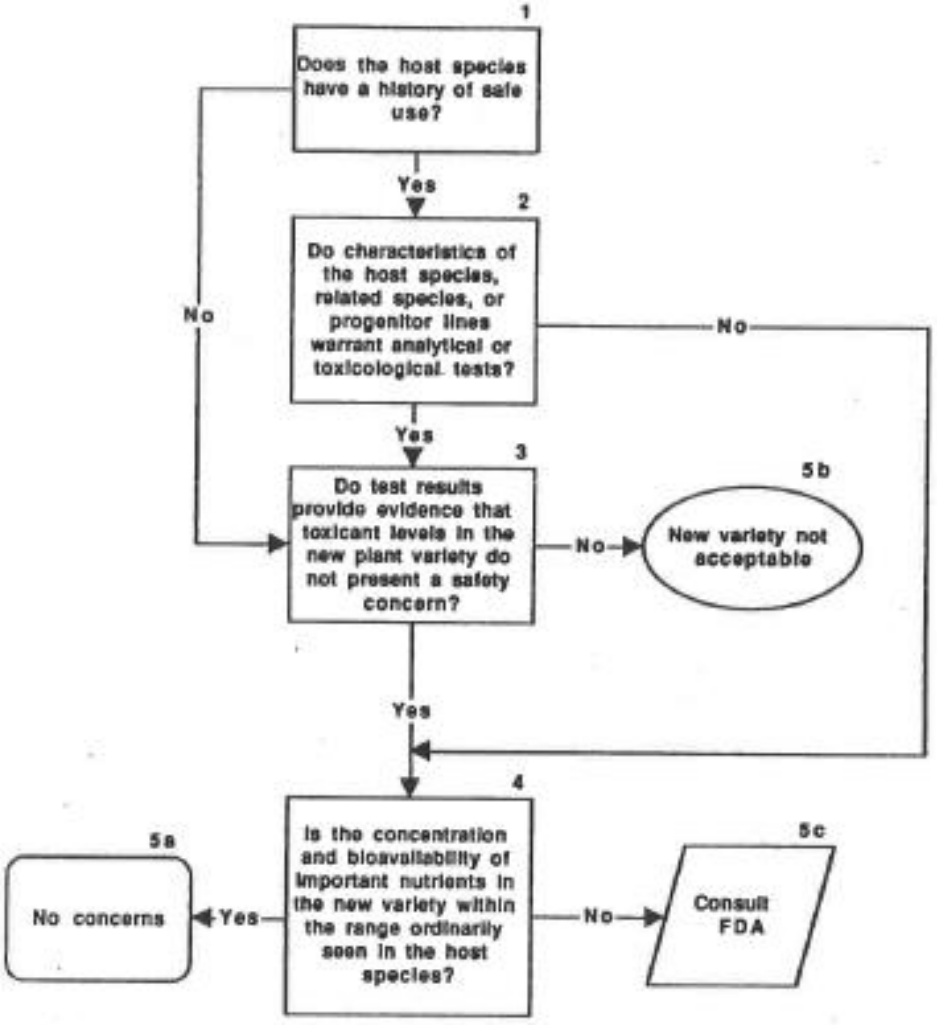

Figure 2. Safety Assessment of New Varieties: The Host Plant

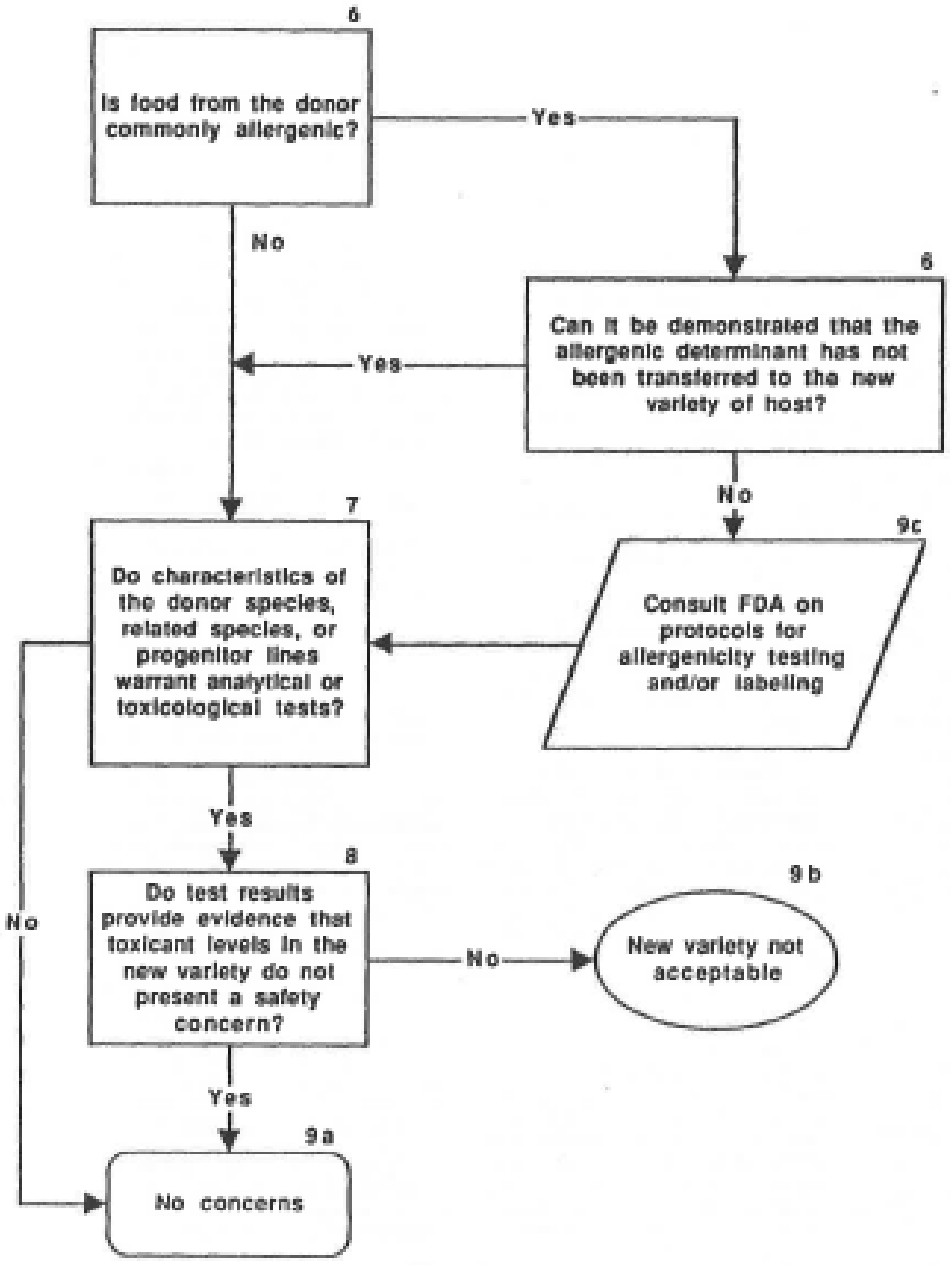

Figure 3. Safety Assessment of New Varieties: The Donor(s)