ABSTRACT. To be commercialized and grown in the US, genetically engineered (GE) crops typically go through an extensive food, feed, and environmental safety assessment process which, in certain instances, requires complex consultations with three different US regulatory agencies. Many small market, niche, and specialty crops have been genetically engineered using the modern tools of recombinant DNA but few have been commercialized due to real or perceived regulatory constraints. This workshop discussed the practical aspects of developing dossiers on GE specialty, niche, or small-market crops/products for submission to US regulatory agencies. This workshop focused on actual case studies, and provided an opportunity for public or private sector scientists and crop developers to spend time with regulatory officials to learn the specifics of compiling a dossier for regulatory approval. The objective of the workshop was to explain and demystify data requirements and regulatory dossier compilation by small companies, academics, and other developers.

KEYWORDS. regulatory dossiers; EPA; USDA APHIS BRS; FDA; small crop developers; transgenic crops
INTRODUCTION

This is a report on the second Nuts and Bolts Workshop held September 19–21, 2016, at the US Department of Agriculture (USDA) facility in Riverdale, Maryland, USA. The Organizing Committee was composed of: Kellye Eversole, IESCS (Infinite Eversole Strategic Crop Services), Elizabeth E. Hood, IESCS, Lori Leach, Eversole Associates, Mollie Hogan, Eversole Associates, Alan McHughen, University of California, Riverside, John Cordts, Cordts Consulting, Sally McCammon, USDA Animal and Plant Health Inspection Service (USDA-APHIS), Chris Wozniak, US Environmental Protection Agency (EPA), and Carrie McMahon, US Food and Drug Administration (FDA).

To be commercialized and grown in the US, genetically engineered (GE) crops typically go through an extensive food, feed, and environmental safety assessment process which, in certain instances, requires complex consultations with three different US regulatory agencies. Many small market, niche, and specialty crops have been genetically engineered using the modern tools of recombinant DNA over the past 25 years but few have been commercialized due to real or perceived regulatory constraints. This workshop, partially supported by the USDA National Institute of Food and Agriculture’s Biotechnology Risk Assessment Grants (BRAG) program, discussed the practical aspects of developing dossiers on GE specialty, niche, or small-market crops/products for submission to US regulatory agencies. While most workshops on the US regulatory system for biotech-derived crops provide general overviews of various aspects of the regulatory process, this workshop, focused on actual case studies, provided an opportunity for public sector scientists and public or private crop developers to spend two days with regulatory officials to learn the specifics of compiling an actual dossier for regulatory approval. The steering committee of the Specialty Crop Regulatory Assistance (SCRA) initiative (www.specialtycropassistance.org) organized the workshop as a follow-up to a highly successful “Nuts and Bolts” workshop in 2011. This workshop was held at USDA APHIS headquarters in Riverdale MD in September 2016.

The objective of the workshop was to explain and demystify data requirements and regulatory dossier compilation by small companies, academics, and other potential developers.

Approximately 40 workshop participants were able to network with regulatory officials from the 3 agencies with jurisdiction over GE products (USDA-APHIS, FDA, and EPA) and learn the specifics of putting together dossiers for regulatory approval. In the opening session, after an overview of the US Coordinated Framework for genetically engineered organisms, representatives from the USDA-APHIS, EPA, and FDA provided brief descriptions of the regulatory requirements of his/her specific agency. The rest of the workshop focused on case studies (i.e., dossiers of actual approved GE products and of GE products poised to enter or complete the US regulatory process) with an introduction by the product/crop developer followed by regulatory officials of the relevant agencies providing an explanation of how assessments were or would be conducted. Each case study discussed during the workshop differed from those covered in the 2011 workshop. To ensure the broadest exchange of information among participants, especially between crop developers and regulatory officials, workshop discussions were off-the-record. Presentations and case studies, however, are freely available on the SCRA website: www.specialtycropassistance.org.

THE COORDINATED FRAMEWORK AND USDA ANIMAL & PLANT HEALTH INSPECTION SERVICE (APHIS) BIOTECHNOLOGY REGULATORY SERVICE (BRS)

Drs. Sally McCammon (USDA-APHIS BRS) and Mike Mendelsohn (EPA) presented an overview of the Coordinated Framework for Regulation of Biotechnology (CF). The Executive Office of the President, Office of Science and Technology Policy, in 1986, declared in the CF that using genetic engineering
to produce new phenotypes in organisms was not substantially different from modifications produced using other types of technology (51 Federal Register (FR) 23302). The policy made clear that all regulations should be risk-based, using rational and scientific evaluation of products, on a case-by-case basis, and not on an a priori assumption about the processes used for modification. Laws (i.e., Statutes) existing at the time were deemed sufficient to regulate both the research and products of biotechnology.

The laws are implemented by the Executive Branch of government through regulations drafted by the agencies, which are posted for public comment, revised based on comment, and published in final form in the Code of Federal Regulations (CFR). In some cases, an agency issues a guidance document that elaborates its interpretation of the regulations to facilitate compliance. The following provides the statutory authority and implementing regulations for genetically engineered agricultural products:

- **Department of Agriculture (USDA-APHIS)** authority derives from the Plant Protection Act (PPA-2000), which is intended to protect agriculture against damage from plant pests and noxious weeds. It is implemented in 7 CFR Part 340- to prevent the introduction of genetically engineered organisms and products which are or may be plant pests;

- **Environmental Protection Agency (EPA)** authority derives from the (1) Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)- Regulating the safe use of pesticides, fungicides, and rodenticides (2) Federal Food Drug and Cosmetic (FD&C) Act - Tolerance actions for pesticide residues on food and feed products, implemented in 40 CFR Parts 152 and 174 related to pesticide registration and classification procedures, and 40 CFR Parts 172 related to experimental use permits, and (3) Toxic Substances Control Act (TSCA)- Reporting, record-keeping and testing requirements, as well as restrictions relating to chemical substances and/or mixtures implemented in 40 CFR Part 725 related to microorganisms; and

- **Food and Drug Administration (FDA)** authority derives from the Federal Food, Drug and Cosmetic (FD&C) Act - Ensuring the safety of food for humans and animals, sections 402 and 409. FDA’s Statement of Policy on Foods Derived from New Plant Varieties (57 FR 22984) describes the applicability of the FD&C Act to food from GE plants. FDA has issued Guidance on Consultation Procedures under FDA’s 1992 Statement of Policy - Foods Derived From New Plant Varieties and Guidance for Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use.

In 1987, USDA-APHIS Biotechnology Regulatory Service (BRS) defined their scope as products produced through genetic engineering using a plant pest. In 1992, FDA defined their purview as the regulatory status of food independent of technique, and as dependent on the characteristics of the specific food or feed, and its intended use. In 2001, EPA defined their regulatory scope as a pesticidal substance intended to be produced in a plant through rDNA-manipulation in vitro using restriction enzymes or other enzymes and not introduced into the plant genome by conventional breeding. Thus, although the existing laws under the Coordinated Framework are designed to assure product safety regardless of technology, each agency implemented its regulations and guidance documents to specifically review biotechnology-produced products. Nevertheless, each agency’s goal is to focus on the characteristics of the plant phenotype (i.e., the product) and its intended use.

Dr. Mike Mendelsohn reported on the recent efforts to modernize the Coordinated Framework. On October 6, 2015, the OSTP issued in the Federal Register a notice of request for information to solicit data and information that could inform the development of a proposed update. The goals of the update would be to reduce unpredictability of the regulatory process, to make it
entirely science-based, and to promote public confidence in the system. The agencies are developing a long-term strategy for the future of biotechnology products. The update will also clarify the current roles and responsibilities of each of the agencies. It was open for public comment at the time of the workshop (September, 2016).

**USDA APHIS BRS**

Dr. John Turner, USDA APHIS, discussed the experience of APHIS in the regulation of biotechnology derived plants. In the nearly 30 years of looking at these products, the agency has authorized over 100,000 field releases and granted non-regulated status to 120 petitions representing 17 species. Triggers for regulatory oversight include:

- The organism has been altered or produced through genetic engineering (recombinant DNA techniques),

and

- The organism is produced using plant pests (i.e. donor, recipient, or vector is or is derived from a plant pest)

or

- There is otherwise a reason to believe that the organism is a plant pest.

Not all organisms produced using modern biotechnology techniques are regulated.

If a plant is regulated, a permit or notification is required for importation, movement or confined release (field trial). Field trials focus on confinement for the trial and do not require a full data package. Risk assessment for a field trial involves multiple questions about the plant and trait. Familiarity with the plant and trait helps with the overall assessment of the risk. Questions to be answered include: Is it outcrossing or self-pollinating? Is it weedy or invasive? Are there wild relatives in the growing area? Can the plant or offspring persist after the test is over? Would the trait be expected to change the plants’ weawdiness, invasiveness, or reproductive biology?

After multiple field trials and data collection in general, a petition can be filed with the APHIS BRS seeking non-regulated status. The two evaluations that APHIS BRS conduct are 1) the new variety as plant pest risk; and 2) an Environmental Assessment or Environmental Impact Statement to more broadly evaluate environmental impacts of an APHIS-BRS decision (National Environmental Policy Act; NEPA).

A petition is evaluated by a comprehensive team of APHIS scientists to determine: crop biology and taxonomy, genotypic or phenotypic differences from the control plant, field test reports for all releases in the US, relevant experimental data, and publications or external data that impinge on the decision. The first determination is whether this new plant variety will pose a risk through outcrossing or have a detrimental effect on non-target organisms, i.e., could it become a weed. The agency has often issued a FONSI (Finding of no significant impact) in response to environmental assessment. Not all plants granted non-regulated status have been commercialized presumably due to market constraints. Examples of approved plants are shown in Table 1.

The concept of “Extensions of Non-regulated Status” has been instituted by APHIS BRS allowing for a reduced data package and an expedited review for the de-regulation of GE plants which are sufficiently similar to other GE plants already de-regulated. These requests are called “extensions” because APHIS is extending non-regulated status from a previously de-regulated organism (called the antecedent) to a new organism. Extensions allow APHIS to leverage previous work and experience without compromising safety. Recent extensions have been completed in 5–7 months.

If a crop developer using recombinant DNA technology is not sure if they are regulated, they may ask APHIS “Am I Regulated” (AIR) through a web-based process. A letter of inquiry is submitted online and the agency will provide a response. All inquiries and the responses are made public on the web site.

APHIS has authorized numerous field trials (as many as 58 in 2015) for plants altered through the
CRISPR/Cas9, TALEN, or Zn-finger nuclease technology. Some plants edited through this technology are outside the scope of USDA APHIS regulation, such as simple deletions with any other added DNA removed through genetic segregation.

Additional information about USDA APHIS petitions and regulations can be obtained through the following websites:

General: [https://www.aphis.usda.gov/aphis/ourfocus/biotechnology](https://www.aphis.usda.gov/aphis/ourfocus/biotechnology)

Petitions – Guidance for new users: [https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/ct_new_users_petitions](https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/ct_new_users_petitions)

Extensions: [https://www.aphis.usda.gov/brs/aphisdocs/guidance_ext_nonreg.pdf](https://www.aphis.usda.gov/brs/aphisdocs/guidance_ext_nonreg.pdf)

### ENVIRONMENTAL PROTECTION AGENCY

Dr. Chris Wozniak from EPA presented the agency process for plant-incorporated protectants (PIPs). A PIP is a pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. It also includes “any inert ingredient contained in the plant, or produce thereof” (40 CFR Sec. 174.3). Developers are encouraged to consult early and often with EPA on new plant varieties with PIPs. Once a product is registered with EPA, maintenance fees are required for continued registration.

EPA implements regulations for 4 laws relative to pesticide oversight: the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for pesticides; Federal Food Drug and Cosmetic Act – (FFDCA) for food and feed safety; Food Quality Protection Act - (FQPA), which amends FIFRA and FFDCA to include sensitive groups; and the Endangered Species Act - (ESA) for any impact on threatened or endangered species. FIFRA is a risk: benefit act where risk equals hazard X exposure.

EPA analyzes data for PIPs at 3 levels: product characterization, effects on human health, and effects on the environment. For product characterization, data required include molecular characterization of the transgenic event, protein expression levels and analytical methods description. Parameters often examined include molecular weight and glycosylation status. For human health impacts, assessment of allergenicity status (through amino acid homology with known allergenic proteins and/or digestibility), acute oral toxicity and biochemical properties are examined. Environmental assessment includes effects on non-target species, gene flow impacts and environmental fate. Data required for ecological effects and non-target species include: Avian oral/dietary toxicity studies (Quail, acute/42-day poultry feeding); Freshwater fish oral/dietary toxicity studies (Rainbow trout or sunfish acute/catfish feeding); Freshwater invertebrate testing (Daphnia); Honey bee oral toxicity testing; Non-target arthropod testing; Wild mammal toxicity (acute oral for rat/mouse); Estuarine and marine animal testing; Non-target plant toxicity studies; Endangered species considerations—exposure determination. Data required for environmental fate include: Quantification of protein expression levels of the PIP in various plant tissues/organs over plant developmental growth stages and at multiple locations; Determination of fate of PIP residues in the environment through protein persistence and

| Species       | Traits                        |
|---------------|-------------------------------|
| Alfalfa       | Herbicide tolerance (HT), product quality (PQ) |
| Canola        | HT, Agronomic Properties (AP), PQ |
| Corn          | HT, Insect Resistance (IR), AP, PQ |
| Cotton        | HT, IR                        |
| Papaya        | Virus Resistance (VR)         |
| Soybean       | HT, IR, AP, PQ                |
| Sugar Beet    | HT                            |
| Rose          | PQ                            |
| Squash        | VR                            |
| Tobacco       | PQ                            |
| Apple         | PQ                            |
| Chicory       | AP                            |
| Flax          | AP                            |
| Plum          | VR                            |
| Potato        | IR, VR, PQ, FR                |
| Rice          | HT                            |
| Tomato        | PQ                            |

**TABLE 1. Species and traits that have been granted non-regulated status.**

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degradation in soil; and based on biology of the plant, a determination of Environmental Impact Assessment of Gene Flow.

For a biotech plant producing a PIP, EPA sets tolerances (i.e., Maximum Residue Levels) in or on food and feed products as they do for all pesticides. A pesticide residue present on food or feed products, which is not covered by a tolerance or an exemption from the requirement of a tolerance results in that product being considered as ‘adulterated’ under the Federal Food Drug and Cosmetic Act (FFDCA). Food or Feed, which is considered adulterated, may be quarantined, seized or rejected at a port of entry. FDA carries out the enforcement aspects associated with EPA’s tolerance actions. Tolerance actions consider data from an acute oral toxicity test, sequence comparison to known toxins and allergens, in vitro digestibility and the source of the gene used for PIP or inert ingredient production. A food tolerance action is usually required prior to field-testing of a PIP-expressing plant. Submission of data to EPA in support of a tolerance or registration action is made with the understanding that the performing laboratory may be inspected to ensure compliance with good laboratory practices.

Data submitted to EPA under FIFRA section 3 and FFDCA sections 408 and 409 must be properly formatted. Data packages submitted to the Agency outside of this format (PRN 2011–3) will most likely be rejected and we may never see them for review. This is where a consultant comes in handy! (http://www.epa.gov/PR_Notices/pr86-5.html). For data submission, in Attachment 4: SUPPLEMENTAL STATEMENT OF DATA CONFIDENTIALITY CLAIMS, certain criteria must be met for confidential business information. For any portion of a submitted study that is not described by FIFRA section 10(d)(1)(A), (B), or (C), but for which you claim confidential treatment on another basis, the following information must be included within a Supplemental Statement of Data Confidentiality Claims: Identify specifically by page and line number(s) each portion of the study for which you claim confidentiality. Cite the reasons why the cited passage qualifies for confidential treatment. Indicate the length of time—until a specific date or event, or permanently, for which the information should be treated as Confidential.

At times, EPA uses scientific review panels. FIFRA authorizes the use of Scientific Advisory Panels (SAPs) for novel pesticides or new uses or for larger issues for which the Agency feels it needs a public forum and expertise from outside of EPA. Any questions posed, deliberations made and final reports from these panels are posted on the EPA website by year or in alphabetical order.

In summary: Early consultation before submission of an application is encouraged. Confidential pre-submission meetings are useful to determine the structure of an application for a PIP. In these meetings, a determination of applicable data requirements can be made early on. Formatting requirements are mandatory and a consultant is recommended for formal submissions to the agency to avoid errors. Contacts within the agency: Mike Mendelsohn, Branch Chief (Mendelsohn.Mike@epa.gov); and Chris Wozniak, Biotechnology Special Assistant (Wozniak.Chris@epa.gov)

Some useful EPA web sites: https://www.epa.gov/pesticides/biopesticides https://www.epa.gov/ingredients-used-pesticide-products/current-and-previously-registered-section-3-plant-incorporated https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra

**FOOD AND DRUG ADMINISTRATION**

Dr. Patrick Cournoyer, Center for Food Safety and Applied Nutrition and Office of Food Additive Safety, gave an overview of the FDA process for evaluating new plant varieties used in food. Within the coordinated framework, FDA enforces legal provisions pertaining to food safety and labeling. The safety provisions apply to endogenous substances already present in a plant and those added through biotechnology. Added substances must meet specific safety criteria. In addition, labeling must be truthful and not misleading (Fig. 1). Any developer of a biotechnology-derived plant product may
consult with the FDA to address any potential safety or other regulatory issues prior to marketing products from the plant.

Substances added to food are defined as food additives and must undergo FDA’s safety review and approval, unless their use is Generally Recognized as Safe (GRAS) among experts qualified by scientific training and experience to evaluate their safety. GRAS substances must be adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of their intended use. Safe or safety means that there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. (Code of Federal Regulations Title 21, 170.3(i)).

FDA offers a consultation program to evaluate developers’ safety assessments and resolve safety and regulatory questions before marketing. Safety assessments typically address endogenous substances, added substances, and labeling (Fig. 2). After a developer submits a safety assessment, an FDA team of experts will review the data and information and request additional information as needed. This process is repeated until the FDA is satisfied that the data set is complete, i.e., until all safety and regulatory questions are resolved. Then the FDA will summarize the evaluation in a memo and send a letter to the developer that states they have no more questions. The FDA encourages meeting early and often when developing a product that may enter the food supply. This can be in person or by teleconference. It is at no cost to the crop developer.

In a submission to the FDA, the basic information should include the crop uses, the trait description, a molecular characterization and evidence of trait stability. How many copies of the insert are there? Are sequences from the cloning vector present? Are new proteins present? What are the key nutrients in the engineered crop? Are the key nutrients one would expect still present? Are endogenous anti-nutritional compounds and toxicants at safe levels?

Compositional assessments typically use a comparative approach, comparing levels of...
key components in the GE crop to levels in an appropriate comparator. The ideal comparator is the non-engineered plant of similar genetic background, grown concurrently. Developers may also compare their results to reference varieties grown concurrently and to published data to demonstrate existing variation in composition. Field trials should represent the conditions under which the crop will be grown and should have sufficient power to detect meaningful differences.

If a crop is engineered to contain a new substance, the developer should evaluate it for safety. This would include describing the substance, determining how much is safe to consume, estimating how much would be consumed if the crop is commercialized, and assessing whether exposure would be within safe levels.

If a crop is engineered to contain a new substance, the developer should evaluate it for safety. This would include describing the substance, determining how much is safe to consume, estimating how much would be consumed if the crop is commercialized, and assessing whether exposure would be within safe levels. The developer should consider if the new variety differs enough from the progenitor to require different labeling. Is there a “material” difference? If so, the material difference would require disclosure, and a new common or usual name might be needed. The web site with information on consultations is at www.fda.gov/GEPlantFoods. An inventory of completed consultations is available on the web site: www.fda.gov/bioconinventory.

Developers of food crops expressing new, non-pesticidal proteins are encouraged to consult with FDA early in development, using a process known as a “New Protein Consultation.” Through this process, FDA has evaluated the safety of proteins such as trypsin and phytase. This program gives FDA an efficient means of obtaining safety information about proteins used in crops that FDA has not yet evaluated in a traditional consultation.

THREE CASE STUDIES

1. Ultra-low Gossypol Cottonseed - Event TAM66274; Keerti S. Rathore; Institute for Plant Genomics & Biotechnology; Texas A&M AgriLife Research; College Station, TX

Cottonseed is a highly undervalued byproduct of lint production and the amount produced worldwide contains enough protein to meet the basic requirements of 500–600 million people per year at a rate of 50 g protein/day. However, gossypol, a noxious compound present in the seed glands, renders cottonseed unfit as food for
human consumption or even as feed for non-ruminant animals. The TAMU team used seed-specific RNAi to silence \( \delta \)-cadinene synthase gene(s), thus blocking the first committed step in the biosynthesis of gossypol. The gossypol levels in the seed have been reduced from \(~10,000\) ppm to well below \(450\) ppm, a level considered safe for human consumption. The rest of the plant maintains normal levels of this defensive terpenoid for protection against insects and diseases. This Ultra-low Gossypol Cottonseed (ULGCS) trait, when commercialized has the potential to make cottonseed as valuable as the lint, thus providing additional benefits to cotton producers.

Greenhouse- and field-testing of more than a dozen ULGCS events, over multiple generations, showed that the ULGCS trait is stable and heritable. A single ULGCS event was selected for deregulation in the U.S. Three years of multi-location field trials have been completed and agronomic data and seed composition data have been assembled and analyzed. In addition, biochemical, molecular, and genetic analyses on the chosen ULGCS event and its progeny have been completed. The regulatory dossier was submitted October 19, 2017. ULGCS is one of only five genetically engineered new plant varieties created by a public institution to seek pre-market approvals in the 25-year history of agricultural biotechnology.

USDA comments on this product: Ultra-low Gossypol Cotton is a Regulated Article because, it is a product of genetic engineering and the vector and the donor organism (\( \text{Agrobacterium tumefaciens} \)) belongs to a taxon designated in §340.2, and meets the definition of plant pest. GE cotton was field-tested using notification applications from 2009–2016, because it satisfies all 6 eligibility criteria and 6 performance standards. The Eligibility Criteria for notifications are 1) the regulated article is a plant species and not a noxious weed; 2) genetic sequence stably integrated; 3) the function of the integrated gene is known; 4) the engineered plant does not act like an infectious agent, no non-target impact, not a PMPI; 5) any plant virus sequence is non-coding or endemic; 6) it contains no plant or animal pathogen sequence. The Performance Criteria are 1) during shipment, no release during movement or at destination; 2) during the release, no mixing with non-regulated crops; 3) identity is known all the time and materials will be devitalized when it is no longer in use; 4) no viable vector sequences are present; 5) no persistence of the regulated article is expected; 6) volunteer management will be done post-harvest.

A pre-submission (of a petition) discussion with USDA APHIS BRS was requested by this developer. At this meeting, a brief presentation by the applicant included a discussion of the required molecular genetic data, crop composition data, and agronomic data. The APHIS BRS input included requests for pest and disease incidences, non-target impacts, and any changes in agricultural practices. When the petition is submitted to BRS, a team of 2–3 biotechnologists and a NEPA (National Environmental Policy Act) specialist will review it. The two parts of the review are 1) Whether the GE ULG cotton lines are likely to pose a plant pest risk (7 CFR 340 regulatory requirement); and 2) Whether the ULG cotton cultivation has (a) any significant impacts, individually or collectively, on the quality of the human environment (NEPA 7 CFR 372), and (b) any effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitats (ESA 16 U.S.C. §1531 et seq.) (NEPA). To address the plant pest risk, data should address the following: Whether the introduced plant pest sequences (introduced genes or sequences) cause or promote disease, damage or injury to plants (plant pest risk); whether the introduced genes are stably integrated (stable phenotype/not an infectious agent); whether the introduced genes produce any new enzymes or changes to plant metabolism (leading to plant pest risk); whether the introduced genes make the ULG cotton plant a weed (pest plant); whether gene flow from ULG cotton to any sexually compatible species imparts weediness to those taxa (creating a pest plant); whether ULG cotton impacts agricultural and cultivation practices (effects on disease and pest susceptibilities); whether ULG cotton affects non-target organisms (effects on beneficial organisms); whether ULG cotton has any indirect plant pest effects on other agricultural products (plant pest risk); and whether ULG cotton has the potential to transfer introduced
genes to organisms with which it cannot interbreed (horizontal gene transfer). Are any of these risks higher than the native cotton plant used to create ULGCS?

The petitioner will receive a letter within 30 days of submission from BRS personnel informing them if their submission is complete, needs clarification or needs more information.

As a Federal agency subject to compliance with the National Environmental Policy Act (NEPA) APHIS prepares an environmental assessment (EA) to consider the potential environmental effects of granting non-regulated status to the proposed cotton lines consistent with NEPA regulations and the USDA and APHIS NEPA implementing regulations and procedures. The EA is going to be prepared in order to specifically evaluate the effects on the quality of the human environment that may result from the deregulation of ULG cotton. APHIS may request additional information to assist in preparing the required NEPA documentation. The types of information requested are not specifically cited in the regulations. If the petitioner is unable to locate the requested information, please identify this lack of information and APHIS will attempt to secure the required information from other available sources. The environmental assessment could take up to 40 weeks.

For more information on BRS petitions, see the following web site: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/ct_submissions_home

Because the ULGCS does not Include a PIP, EPA did not Review This Product

FDA comments on the ULGCS: A submission to the FDA would comprise some elements that are similar to those for the APHIS BRS petition for non-regulated status, e.g., molecular characterization, the plant, the trait, and the intended use. Numerous cottonseed products are already on the market, particularly the “lint” that is removed from the seed after ginning. These are short cellulose fibers that have uses in processed foods such as shredded cheese and low fat ice cream. Cottonseed oil is used in baked products and can be added to margarine products. Cottonseed hulls can also be used for animal feed. The intended trait removes a substance that is toxic to monogastric animals, allowing increased use of cotton seed as human food as well as animal feed.

For the FDA, compositional analysis is important, which involves comparing levels of substances in the new variety to non-transgenic cottonseed. The developer can search databases as potential resources for “normal” cottonseed composition, e.g., the ILSI Crop Composition Database. A second resource is the USDA National Nutrient Database for Standard Reference release 28. An additional reference is the OECD Consensus Document on Compositional Considerations for New Varieties of Cotton. In this case when gossypol is being removed, it is important to quantify this toxicant and its related compounds—terpenoid phytoalexins.

With respect to the new uses of the engineered cottonseed, questions concerning its nutritional value are important. What are predicted exposure levels (humans and animals) based on possible food uses and quantities? Would cottonseed differ from the grains it would replace in a way that would be consequential for human or animal nutrition? Would the estimated levels of exposure be safe considering the new uses?

When the FDA is satisfied that all their questions have been answered, they issue a letter to the developer letting them know that the agency has no more questions.

2. Late Blight-Protected Potatoes with Reduced Black Spot, Lower Reducing Sugars, and Low Acrylamide Potential

Tracy Rood and Susan Collinge; Simplot Plant Sciences; 5369 W. Irving St.; Boise, ID 83706

Simplot Plant Sciences, a private family-owned company, used Agrobacterium tumefaciens to transform the Russet Burbank potato variety to produce the W8 event. The W8 event is protected against late blight, which is caused by the oomycete Phytophthora infestans and is a devastating disease among cultivated Solanaceae species. If left untreated, late blight affects potato foliage and tubers causing rapid necrosis and crop loss. The Irish potato famine was the result of late blight and illustrates the catastrophic nature of the disease. In
addition to foliar protection against late blight, W8 addresses three critical potato quality issues:

1. High levels of reducing sugars in tubers, which increase during storage as starch is metabolized. Reducing sugars lead to dark colors in fries and chips and contribute to acrylamide formation in cooked potatoes.
2. High levels of free asparagine in tubers, a non-essential amino acid that is rapidly oxidized to form acrylamide during frying or baking; and
3. Susceptibility to enzymatic darkening and discoloration (black spot).

In W8, the inserted DNA comes primarily from cultivated potato or wild Solanum species, a group of related plant species that are sexually compatible with potato. Late blight protection in W8 is conferred by expression of an R-protein, VNT1, that recognizes the P. infestans Avr-vnt1 effector protein and initiates a hypersensitive response in the plant (Table 2). The death of infected cells stops the spread of infection, resulting in protection against disease. The other traits in W8 are due to down regulation of endogenous potato genes using RNA interference.

The W8 event is deregulated by USDA and has completed its FDA consultation. It is currently under review by EPA, where the VNT1 protein is regulated as a plant-incorporated protectant. In this presentation, Simplot shared their experiences and learning from taking W8 through the U.S. regulatory system. They discussed their registration strategy, how potato is different from other crops, and why the weight of evidence for the safety of the VNT1 protein is sufficient without the typical battery of toxicology studies. In these potatoes, the VNT1 protein is present at very low levels.

Potatoes have some unique aspects that complicate their improvement. The “seed” is a whole or cut tuber. Thus, they are vegetatively propagated. The genome is tetraploid and highly complex, and hence not fully sequenced to date. Potatoes are difficult to breed and backcross because they are highly heterozygous and subject to inbreeding depression. Thus, each variety must be transformed individually. The consequence is a low rate of introduction of new varieties.

Simplot consulted early with the regulatory agencies and was able to clear regulatory hurdles in 3 years (Fig. 3). The US regulatory strategy for the W8 event was built on the Gen1 success previously at USDA and FDA. For example, genes transformed in were native Solanum genes from sexually compatible species and no marker or herbicide genes were used. The potato industry and key stakeholders were engaged in the process of developing this variety. Simplot engaged in pre-submission consultations at all 3 agencies. EPA has the lead on VNT1 protein safety. Because the protein could not be expressed in a heterologous system, a typical data package was not feasible.

The safety rationale for the W8 potato event had numerous aspects. It was well characterized at the molecular level and the traits were shown to be stable during vegetative propagation. None of the open reading frames had significant homology to known toxins or allergens. Simplot down-regulated endogenous genes using RNAi, and RNAi is considered safe. R-proteins (resistance proteins) similar

| Construct | Gene | Method | Intended Trait |
|-----------|------|--------|----------------|
| pSIM1278  | Asparagine synthetase, Polyphenol oxidase, R1 water dikinase, Phosphorylase-L | RNAi | Lower free asparagine, Reduced black spot, Lower reducing sugars |
| pSIM1678  | Vacuolar invertase, \( R_{pi-vnt1} \) gene | Protein | Late blight protection |
to VNT1 have a history of safe use, and VNT1 has no significant homology to allergens or toxins. Nutritional composition of the W8 event is the same as conventional potatoes for key nutrients and toxins. Efficacy was demonstrated for lower reducing sugars and lower free asparagine—neither of which is nutritionally important for potatoes. The agronomic and phenotypic properties were the same as conventional potatoes and they showed no weediness or gene flow concerns.

If one focuses on Risk Assessment, one is concerned about hazard x exposure, which equals Risk. VNT1 and homologues have a history of safe consumption, thus pose a minimal hazard. The exposure is too low to measure. Together these factors indicate that VNT1 poses minimal risk. Simplot encourages trait developers to look at submissions from other developers and focus on what makes sense to do and what to disregard. For example, assessing the composition of above-ground potato vegetative material is not relevant for potato, since the tubers are the only portion of the plant that is consumed by humans and animals.

To evaluate the safety of a protein, typical steps include: Using bioinformatics, assess the protein for homology to known allergens and toxins; produce the protein in a heterologous system to establish equivalency between the heterologous protein and plant-produced protein; assess the protein lability in digestion and heat studies; conduct mouse acute oral toxicity; and quantify expressed protein in various tissues at multiple time points.

In this case, the VNT1 protein cassette contained a native promoter and terminator. Because protein amounts were so low, RT-qPCR was used to verify gene expression. Multiple approaches were used for antibody production against the VNT1 protein. The most sensitive polyclonal antibody generated was used for western blot studies. The limit of detection was 9 pg and the limit of quantitation was 30 ppb in tubers and 60 ppb in leaves. The antibody has high cross reactivity with other VNT1 homologues. However, even with this high sensitivity, VNT1 in W8 leaves and tubers was too low to quantify, indicating that it is approximately 1000X less abundant than Bt proteins in a typical genetically engineered crop.

Weight of evidence for safety: The VNT1 gene source has a history of safe use. The same gene is found in a European potato variety. Additionally, R-proteins such as VNT1 are ubiquitous in plants; in fact, it is one of the largest gene families known in plants, with...
over 435 genes found in potato and 900 in apple. VNT1 activates an endogenous immune response pathway comprising hypersensitive response as opposed to a toxic mode of action against the fungus. VNT1 lacks significant homology to known allergens and toxins. VNT1 has identity to proteins with a history of safe consumption in potato, tomato and pepper. Plus, because of its low expression levels, dietary exposure to VNT1 is extremely low—less than 100 ppb in W8 tubers. If children aged 1–2 (the highest consumption group for potatoes) ate 100% W8 potatoes, the amount of VNT1 intake compared to average daily protein intake is negligible at 0.000714 mg/kg bw/d or 0.00021% of daily protein consumption.

Simplot generated a number of self-reported lessons learned along the way (Table 3). Customize the submission for each agency. Weave the safety data into a simple, compelling story. Focus on readability with simple tables and graphics interspersed with bullet points and white space. The regulatory process is not easy for first timers. Refer to the online resources: USDA petitions; FDA Notes to File. The EPA process is the most opaque because of complicated PRIA categories, forms, data requirements, and EPA-specific processes. A regulatory package that is customized to your product that may look different from the standard package may be the right approach. As always, ask for help from the agencies to understand the process and next steps. Regulators are there to help, so formal and informal consultations are key to a successful process. Say thank you! Keep industry stakeholders in the loop with information about your commercial intentions and timelines as they can be valuable advocates.

With reference to EPA, plan for your Experimental Use Permit (EUP) long before anticipating a 10-acre field trial. The Pesticide Registration Improvement Act (PRIA) timeline is ~7–11 months to approval. Because this was the first new late blight trait and the first R-protein, Simplot worked with EPA to determine the most efficient PRIA strategy. Careful consideration should be given to stewardship post approval. A product use guide generally will need to be constructed, and the detection method for the product needs to be included. An IPM strategy should be considered as well as trait durability in order to investigate unexpected damage or pathogen resistance in the field.

**USDA comments on the W8 potato:** Simplot submitted a petition to the USDA for non-regulated status of the Russet Burbank Event W8 potato. The lead reviewer at USDA was Kate Rappaport. The process included authorizations of regulated activities for field-testing, inspections and compliance of those tests, and ultimately, determination of non-regulated status. Activities were conducted under authority of the Plant Protection Act of 2000. Non-regulated status means the GE organism would no longer be subject to regulation. Petition information should support the conclusion that the regulated article is unlikely to pose a greater plant pest risk than the non-GE organism.

The W8 event was determined to be under the purview of the USDA because it was generated using *A. tumefaciens* and contained

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**TABLE 3.** Self-reported lessons learned along the way by Simplot: customize the submission for each agency.

|                             | USDA | FDA | EPA |
|-----------------------------|------|-----|-----|
| Molecular: structure, backbone, stability | Yes  | Yes | Yes |
| Allergen and toxin homology of novel proteins | Yes  | Yes | Yes |
| Open reading frame analysis  | No   | Yes | No  |
| Agronomic field data: responses to biotic stress | Yes  | No  | No  |
| Nutritional composition data | Yes  | Yes | No  |
| Detection method            | No   | No  | Yes |
| Efficacy information        | Ask  | Ask | Yes for PIP |
border sequences derived from *A. tumefaciens*, a plant pest. The petition was received and given a document control officer stamp on April 3, 2014. The petition was reviewed and Simplot was issued a request for clarification/more information on May 8, 2014. The revised petition was submitted on June 5, 2014 and deemed complete within 69 days after initial submission. A notice concerning this petition was first placed in the Federal Register for public comment on November 10, 2014 as Docket No. APHIS 2014–0076. With the review and public comments, it was assessed for its potential to pose a plant pest risk under the Plant Pest Risk Assessment Act. It was also reviewed for Environmental Impacts under NEPA, the National Environmental Policy Act. The conclusion of APHIS reviewers after careful consideration of the molecular data was that the W8 potato poses no more of a plant pest risk from the introduction of the six genes, changes to plant metabolism or composition than its respective parental variety or other conventional potato varieties.

Field studies were conducted in multiple locations under release authorizations from USDA to assess agronomic and disease susceptibility (Table 4). Data collected from 11 field trials led to the conclusion that the W8 potato does not exhibit plant pest or disease characteristics and that its use will not lead to new plant pests or to the dissemination of plant pests. Additional field observations led to the conclusion that exposure to and/or consumption of W8 tubers or other plant parts is unlikely to have adverse impacts on organisms beneficial to agriculture.

Potential weediness of the W8 potato was also assessed and the data showed no evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor (other than late blight resistance), increased tuber set or delayed senescence. Thus the conclusion was that the W8 potato will not become a weed. Additionally, use of the W8 potato will not cause sexually compatible relatives to become weeds because it produces very little pollen and is male sterile. No significant changes in agronomic practices are expected with the exception of potentially less fungicide use. Therefore, no impact on other plant diseases or plant pests or their management is likely to occur. Horizontal gene transfer (HGT) of the new genetic material inserted into W8 potatoes to other organisms is highly unlikely and is an extremely low risk.

Federal register notices 2 and 3 for public comment were published May 5, 2015 and September 5, 2015. The potato was deregulated after 517 days.

A new process within USDA APHIS is the Extension. This is a process that takes advantage of data and reviews that were done on previous submissions and can substantially reduce the time to deregulation. Some examples for potato are shown in Table 5.

In summary, the USDA Protection goals are aimed at protecting plant health and the environment. Late Blight is a devastating plant disease among cultivated potato cultivars. It was responsible for the Irish Potato famine. BRS regulates GE crop plants if they pose plant pest and weed risk. BRS reviewed the W8 potato petition and conducted risk assessments based on the scientific data provided by Simplot, LLC. It was determined that W8 potato does not pose a plant pest or weed risk.

**TABLE 4.** Field study release authorizations issued to Simplot by the USDA to assess agronomic and disease susceptibility.

| Petition # | Type       | # Events | Traits                          | # Days |
|------------|------------|----------|---------------------------------|--------|
| 13–022-01p | Original   | 10       | Acrylamide, Black Spot          | 661    |
| 14–093-01p | Original   | 1        | Late Blight, Acrylamide, Black Spot, Reducing Sugars | 528    |
| 15–140-01p | Extension  | 1        | Acrylamide, Black Spot          | 238    |
| 16–064-01p | Extension  | 2        | Late Blight, Acrylamide, Black Spot, Reducing Sugars | 238    |
FDA comments from Robert Merker on the Simplot W8 potato: Policy was established in 1992 to define the risks related to new food products:

Category 1: New substances

- Likelihood of toxicity (is the source toxic or is the structure similar to known toxins)
- Likelihood of allergenicity (allergenic source or similar structure to known allergens)

Category 2: Existing (endogenous) substances

- Plants produce toxins and antinutrients, these should be at levels that are safe for consumption

Category 3: Adequate nutrition

- Do the changes in the plant affect nutritional value?

These W8 potatoes bruise less and are less likely to produce acrylamide. FDA had evaluated 6 potato events from Simplot previously. W8 potatoes were similar, but had additional traits from a second plasmid. One of the additional traits is a PIP under EPA purview. FDA received the submission on April 15, 2014. New information was received from Simplot on several additional dates: March 10, June 11, August 6 & 17, and December 9, 2015. FDA sent a letter to Simplot to close the consultation on January 12, 2016.

Molecular and compositional analysis information provided to FDA was the same as for USDA.

From the pSIM1278 vector, 2 expression cassettes were inserted: The potato Asn1 Ppo5 partial sequences followed by complementary sequences in reverse orientation that were driven by potato promoters. Also, complex insertion- multiple copies of silencing cassettes but at a single locus.

From the pSIM1678 vector, 2 additional expression cassettes were inserted: the Potato Vinv partial sequence followed by complementary sequence in reverse orientation (the EPA regulates this as a PIP:Rpi-vnt1 gene from S. venturii), as well as its native promoter and terminator; the event is a single locus and single copy with a slight deletion at the left border; No vector backbone sequences were observed.

The intended effects in tubers of each insertion were observed; that is, reduced transcription through RNAi resulting in reduced levels of enzymes and consequent lower levels of asparagine and reducing sugars, and lower levels of bruising. Lower levels of acrylamide were observed in fried potatoes. Minor though significant differences were also observed in levels of some amino acids and vitamins B6 and C, but were not considered to be biologically meaningful. The new traits in W8 may constitute material differences and, depending on the nature of the product to be marketed, could subject the product to labeling that would describe material differences. Simplot should contact FDA’s Food Labeling and Standards Staff.

FDA Assessments:

W8 Potatoes from GE plants are as safe as their traditionally bred counterparts. The Developers engaged with FDA prior to marketing. Results of this consultation and others are on

| Petition # | Type     | # Events | Traits                                      | # Days     |
|------------|----------|----------|---------------------------------------------|------------|
| 13–022-01p | Original | 10       | Acrylamide, Black Spot                      | 459        |
| 14–093-01p | Original | 1        | Late Blight, Acrylamide,Black Spot, Reducing Sugars | 517        |
| 15–140-01p | Extension| 1        | Acrylamide, BlackSpot                       | 419        |
| 16–064-01p | Extension| 2        | Late Blight, Acrylamide,Black Spot, Reducing Sugars | Under Review |

TABLE 5. Extensions of petitions to gain approval for new engineered crops that are highly similar to previously approved crops.
FDA’s website at: http://www.fda.gov/biocoinventory

EPA comments from Milutin Dijurickovic and Shannon Borges on the Simplot W8 potato: An Experimental Use Permit was issued for the Simplot W8 potato for field trials across ~95 acres across 10 states. All data submitted were sufficient for the EUP but more were needed for a Section 3 registration. Data needed included: a bioinformatics review of genes homologous to Rpi-Vnt1; a natural history of wild Solanum species; information on how wild Solanum has been used as a genetic resource of currently consumed potatoes. Bioassay data compared differences in expression and how this related to efficacy. Information on how tubers collected from experimental plots were stored and kept out of the food supply were also needed. Data waivers were requested for the EUP, since the VNT1 protein is below detectable levels and the field test crop will be destroyed after the EUP. Endpoints for pepsin stability and toxicity testing were requested due to lack of available protein from a heterologous system. Developers conducted in silico analysis of putative open reading frame sequences at the site of insertion to identify possible allergens and toxins. An evaluation of potential newly created ORF’s that span 5’ and 3’ junctions of the transgenic insert or the two internal junctions created when T-DNA inserts itself during transformation were conducted to eliminate the potential for new toxins.

3. Use of Spinach Defensins to Manage Citrus Greening Disease in Citrus

Developers: Southern Gardens Citrus; Texas A & M; and the University of Florida

Citrus huanglongbing (HLB), also called citrus greening disease, is considered to be the most serious disease of citrus worldwide. HLB is a phloem-limited, systemic, bacterial disease caused by one of three species of Candidatus liberibacter. The disease was found for the first time in the United States in 2005 in Florida and is now found in virtually 100% of the groves in Florida. HLB is also present in the commercial industry in Texas and in dooryard trees in California. In the United States, HLB is vectored by the Asian citrus psyllid insect, which is endemic in both Florida and Texas and is being found with increasing frequency in California. Nothing about working with HLB is easy. The bacterium cannot be cultured, the insect is endemic and occurs with high frequency in Florida and Texas, and commercial cultivars do not have adequate levels of resistance to the disease. Losses due to HLB have been widespread and large, approaching 30–40% in Florida. Attempts to manage the disease by controlling the vector have not been successful, and even the limited success that has been achieved is not considered sustainable due to economic and environmental factors. Most growers and researchers consider plant disease resistance to be the only long-term solution that will be successful. However, there is no good source of resistance in most commercial lines of citrus and the breeding cycle in citrus is long, thus conventional breeding efforts that result in truly resistant plants (vs. somewhat tolerant plants) will likely take decades if at all due to the many varieties and cultivars that will be needed. Thus many scientists are turning to biotechnology to provide resistant commercial cultivars in a shorter time frame. Southern Gardens Citrus in conjunction with Texas A & M and the University of Florida have been working on a biotechnology-based approach by producing transgenic trees expressing antimicrobial spinach defensins. As mentioned earlier, working with HLB is not easy and there have been many challenges in this project. Among them are the regulatory requirements for a crop relatively new to the system (citrus), the development of an effective screening procedure, dealing with juvenility, the time frame needed for success (due to the rapid decline of the industry), the costs associated with bringing a trait to market for a minor crop, the need for regulatory approval for a number of products and byproducts (juice, fresh fruit, pulp, pulp wash, animal feed, etc.), and the means of value capture for a vegetatively propagated perennial crop. After a brief introduction of the partners and the citrus industry, the focus turned to Huanglongbing (HLB), or Citrus Greening. Citrus is amenable to A. tumefaciens transformation, and spinach defensin constructs seemed to offer a reasonable opportunity, so research embarked on the potential for spinach defensin to counteract HLB in Citrus.
Several defensin constructs were available and inserted into several Citrus lines in Texas. Regulatory constraints delayed the initial field trials in Florida, and ongoing regulatory requirements (e.g., trial isolation distances from other citrus) are being met. Additional field trials are being conducted with additional transgenic lines, all with regular interactions with USDA-APHIS personnel to assure regulatory compliance. EPA is also involved for larger (>10 acre) trials, and EPA has different requirements. Regular interaction with both USDA and EPA are keys to success. Interactions with FDA, concerned with food safety, will come later.

USDA-APHIS comments from Margaret Jones on the Citrus Greening Case study: USDA-APHIS is primarily concerned with the genetically engineered citrus as ‘regulated articles’. The citrus genus offers some unique challenges in that it is a perennial tree, produces few to many seeds, is usually propagated by vegetative means, is bee pollinated, has either zygotic or nucellar seeds, can be self-incompatible and has a very long juvenile period before flowering. Several field trials have been permitted to date in specific locations.

Citrus Defensin was developed by US Sugar/Southern Gardens Citrus using *A. tumefaciens* transformation, causing it to be a regulated article. The vector was engineered with several defensin genes from spinach: SoD2, SoD7, SoD2/SoD7 in tandem with the NPT II selectable marker. Constitutive promoters, which are already present in deregulated products were used to express the genes. Defensin is an antimicrobial protein expressed in spinach that has been consumed for years by humans with no report of harm. When authorized under 340 permit, movement and release are done in such a way as to minimize the potential for the regulated organism to escape and disseminate in the environment; persist in the environment; produce offspring that will persist; or significantly impact non-target organisms. Citrus may be moved under notification to USDA APHIS, but requires a permit for environmental release. The workflow for permit approval is shown in Fig. 4. It can take up to 120 days from submission until the permit is issued.
In a controlled field test, GE and Non-GE plants must remain separated and GE plants must not persist. Border rows of citrus trees must surround the trial, they must be separated by at least 1500 feet from sexually compatible species, located 1 mile from uncontrolled breeding operations and beehives must not be near the field trial in order to control spread of GE pollen.

In a petition for non-regulated status, citrus under 7 CFR 340.6(c) must include a description of the biology of the species and events; relevant experimental data and publications; genotype difference between the regulated article and the non-modified recipient organism and where the event was developed. Field test reports for all trials conducted under permit or notification must be summarized. This standard petition information may also be accompanied by any other information, which the Administrator believes to be relevant to a determination of plant pest risk as well as any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

In the context of plant pest risk assessment, the potential for defensin citrus to increase the weediness of sexually compatible plants must be determined. What citrus relatives are present in the U.S. that could interbreed? If hybrids can be produced, would they survive in the wild? Are there indications that inclusion of defensin resistance would affect fitness of any resulting hybrid progeny?

APHIS reviews a petition for deregulated status for each GE citrus ‘event’, and acts in accordance with the PPA and NEPA. In the context of NEPA, the following options are considered: to take no action; to determine a preferred alternative; to consider alternatives but rejected from further consideration; and comparison of alternatives. The scope of the NEPA analysis is agricultural, physical and biological. Agricultural analysis includes consideration of Areas of Citrus Production, Agronomic Practices, and Organic and Specialty Citrus. Physical environment considerations include soil, water, and air. Biological Resources to consider include Climate Change, Animal & Plant Communities, Soil Microorganisms, Biological Diversity, and Gene Movement potential. The steps from petition submission to a final decision are shown in Fig. 5.

Guidance documents can be found here:
Notification https://www.aphis.usda.gov/biotechnology/downloads/notificationguidance_0311.pdf
Permit https://www.aphis.usda.gov/biotechnology/downloads/permit_guidance.pdf
Petition

FIGURE 5. The steps from petition submission to a final decision.
Comments from Milutin S. Djurickovic of EPA on the Citrus Defensin Case Study: EPA issued an Experimental Use Permit (EUP) approval for the pesticidal properties of the GE citrus trees in the field trials, and may issue a tolerance exemption for the pesticides. Data required include molecular characterization; description of disease resistant citrus; plasmid maps and transformation method, as well as molecular characterization of disease resistance events. The genes for SoD2 and SoD7 derived from spinach were expressed in sweet orange (Hamlin), grapefruit (Ruby Red), and lemon (Frost Lisbon) using a disarmed A. tumefaciens and driven by the CaMV 35S promoter in pBINplus. Required allergenicity and toxicity data include: acute oral toxicity; thermolability; stability in gastric fluid; stability in fresh and pasteurized juices; physicochemical characterization of SoD proteins; and bioinformatics of potential allergenicity. Northern and Southern blots characterized the single insert and low expression levels. A western blot confirmed low levels of SoD protein in the transgenic events. Acute oral toxicity tests in rats showed no toxicity and all other toxicity tests were waived because no exposure would be found. Allergenicity was low based on digestibility and lack of homology with known allergens. SoD2 and SoD7 were degraded in fresh orange and grapefruit juice, but stable in pasteurized juice. SoD2 was also stable in fresh and pasteurized lemon juice. However, SoD7 was degraded in fresh lemon juice, but stable in pasteurized lemon juice. Researchers could not extract adequate amounts of defensin proteins from citrus for comparison to microbially produced protein.

FDA is not yet Involved, but that Will Come in Due Course

Status of the Product from Commercial and Regulatory Aspects

GE Citrus with spinach defensin genes conferring improved resistance to HLB (Citrus Greening) still requires agronomic and efficacy evaluation and regulatory approvals prior to commercial release.

WORKSHOP SUMMARY

The purpose of this document was to inform the public of the occurrence of the workshop and to introduce stakeholders to the regulatory agencies and their processes. The document is not focused on presenting a formula or pattern for producing a dossier to be presented to the regulatory agencies. In fact, the agencies specifically do not want to provide a pattern or formula because every plant/event/case is different and will have different data requirements. Moreover, the definition of words in the guidelines remain soft because their meaning varies with relation to the crop/trait combination. For example, a “meaningful difference” for GE potatoes having less asparagine compared to non-GE potatoes is not relevant; but this difference could be quite important when considering it for a major protein source such as a grain.

Another consideration for presenting petition dossiers to the agencies is that each case requires a unique data set and may have unique qualities that require a waiver of expected data. On the other hand, the inserted trait may trigger the request for additional data to answer questions unique to the crop/trait interaction. One result from the unscripted petitioning process is the growth of a group of consultants with broad
expertise in answering the questions of developers. In any case, the preparation, submission and approval of a petition for non-regulated status is a complex interactive process.

Presentations from regulatory agencies involved in genetically engineered crop regulation were offered to workshop participants. The status of three genetically engineered crops in review by these agencies was presented along with the issues addressed for each of them by the agencies. The reviews allow participants to understand the process of petitioning the regulatory agencies, the data requirements and the people within the agencies to contact for assistance.

ACKNOWLEDGMENTS

We thank Sally McCammon, John Turner, Kate Rappaport, and Margaret Jones (USDA APHIS, BRS); Robert Merker and Patrick Cournoyer (US FDA); and Chris Wozniak and Milutin Djurickovic (US EPA) for reviewing the manuscript.

FUNDING

This work was supported by the National Institute of Food and Agriculture Biotechnology Risk Assessment [2015-33522-24108].

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