CASE STUDY No. III

HERBICIDE-TOLERANT SOYBEAN

Overview

This case study examines the approved product glufosinate-tolerant soybeans. This variety of soybean was genetically engineered by AgrEvo (now Aventis) to be tolerant to the herbicide glufosinate ammonium (herein referred to as glufosinate), a chemical already in agricultural use. A modified bacterium gene was added to the soybean so that the plant produces an enzyme that breaks down the herbicide before it can harm the soybean plant. Genetic material from other sources was inserted to control the expression of the enzyme. As with many other genetically engineered products, glufosinate tolerant soybeans are regulated by various regulatory agencies as described in the "Coordinated Framework for Regulation of Biotechnology" (Office of Science and Technology Policy (OSTP), 1986).

The United States Department of Agriculture (USDA) assessed whether the growing of glufosinate-tolerant soybeans poses a direct or indirect plant pest risk to agriculture or the environment under its regulatory authority. The United States Environmental Protection Agency (EPA), being responsible for regulation of all pesticides, assessed the new use of glufosinate on soybeans and established the maximum residue levels ("tolerances") that were safe for human consumption. The safety and labeling of such soybeans for use as food or animal feed is regulated by the Food and Drug Administration (FDA). FDA enforces pesticide tolerances set by EPA. In addition, EPA has the authority under TSCA to address the potential for the development of herbicide-resistant relatives, or to otherwise regulate unreasonable risks to human health or the environment presented by herbicide tolerant plants.

1. Description of Proposed Organism and Its Use

Glufosinate-tolerant soybeans, comprised of multiple lines described in a petition submitted by AgrEvo (AgrEvo, 1996), have been genetically engineered to tolerate the herbicidal compound by producing phosphinothricin acetyl transferase (PAT), an enzyme that detoxifies the herbicide. The synthetic *pat* gene that was added was a modified version of the native *pat* gene from the soil bacterium *Streptomyces viridochromogenes*. Genetic lines described in the petition fell into two groups representing two different transformation vectors used to produce them. One group contained a promoter sequence designated as P-35S from cauliflower mosaic virus and a terminator sequence designated as T-*nos* from the plant pathogenic bacterium *Agrobacterium tumefaciens*. The other lines

contained both promoters (P-35S) and terminators (T-35S) derived from cauliflower mosaic virus. Each group also contained other sequences from varying sources that were not expressed in plants.

Soybeans were grown on over 72 million acres in the U.S. during 1999 and represent one of the most important crops in the U.S. for both export and internal consumption. The most important production area is the Midwest, but production also takes place in the southern Mississippi River valley, the southern coastal plains, and elsewhere along the eastern seaboard. Traditionally, pre-emergence herbicides have been the major tool used for weed control in conventional soybean production, in which entire fields are treated prior to or at planting before crops and weeds have emerged. Recently, due in part to the advent of effective post emergence herbicides, there has been a shift toward no-till production. No-till production systems involve planting crops into the stubble of previously-grown crops without plowing the soil, providing the advantages of decreased fuel use and less soil compaction due to reduced travel of heavy machinery through the fields, reduced soil erosion, and soil moisture conservation. Glufosinate-tolerant soybeans facilitates post emergence weed control which is critical to no-till agriculture. Under this production system, weeds can be more efficiently managed by applying herbicide when and where the weeds occur after planting, in contrast to a conventional soybean production system in which herbicides are applied as preventative measure prior to planting. The glufosinate herbicide is effective against a broad range of monocot and dicot plant species, and has low residual activity, low soil leaching and low toxicity to nontarget organisms.

It is anticipated that glufosinate tolerant soybeans might be grown in virtually all important soybean-growing areas of the U.S. The harvested product is used in a variety of domestic foods and feed products and is exported to numerous foreign countries. Most soybeans have been stored and marketed as bulk commodities such that transgenic soybeans are mixed with conventionally bred types.

2. Relevant Regulatory Agencies, Regulatory Authority and Legal Measures

USDA/APHIS

The USDA's Animal and Plant Health Inspection Service (APHIS) has the authority to regulate the importation, interstate movement, and release into the environment of plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants or plant products. APHIS regulates genetically engineered organisms under authority granted by the Plant Protection Act (PPA), (7 U.S.C. §§ 7701-7772) which states “it is the responsibility of the Secretary to facilitate exports, imports, and interstate commerce in agricultural products and other commodities that pose a risk of harboring plant pests or noxious weeds in ways that will reduce, to the extent practical, as determined by the Secretary, the risk of disseminating plant pests or noxious weeds.” A genetically engineered organism is deemed a “regulated article” if either the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in 7 CFR Part 340.2 of the regulations, or if it is not identified taxonomically. That is to say, the development of genetically engineered plants using

biological vectors or regulatory sequences derived from plant pathogenic sources serves as a regulatory trigger, initiating an evaluation process to assure that there is not a plant pest risk. Importantly, products of genetic engineering may still be regulated by APHIS, even if not developed using a plant pest, if there is a reason to believe that the product itself might pose a plant pest risk. Field testing is typically used to demonstrate that genetically engineered crops exhibit the expected biological properties and to demonstrate that, although they may be derived using components from plant pests, they do not possess plant pest characteristics.

The PPA, effective as of June 22, 2000, replaces the Federal Plant Pest Act (FPPA) and Plant Quarantine Act (PQA) as APHIS's regulatory authority for genetically engineered organisms. The present case study focuses on regulatory authority and activities at the time of de-regulation of the glufosinate tolerant soybean, i.e., authority granted by the FPPA and PQA. APHIS is presently analyzing whether there are changes in authorities or potential for change based on the new PPA.

Movement, importation, and field testing (introduction). Prior to the introduction of a regulated article, a person is required under §340.1 of the regulations to either (l) notify APHIS in accordance with 7 CFR 340.3 or (2) obtain a permit in accordance with 7 CFR 340.4. Prior to April 1993, the only regulatory option for the planned introduction of transgenic plants covered by APHIS regulations was the permit. Regulations stipulate that once a complete permit request has been submitted, APHIS has 120 days in which to reach a decision whether to issue or deny a permit.

The early 1990's were marked by a rapid increase in the number of field trials in the United States of transgenic plants and plant-associated microbes, and there was an associated rise in permit requests, as these organisms were subject to APHIS regulatory authority to control articles that posed a plant pest risk. After the first six years of evaluating permits and considering the results of field trials under permit, experience demonstrated that criteria and performance standards could be defined for certain field tests that do not present novel plant pest risks. This gave rise to a new option, the notification, effective in April of 1993. Transgenic plants which raised certain safety issues, for example pharmaceutical-producing plants, plants transformed with genes of unknown function, or plants expressing sequences from human or animal viruses, were not eligible for the new option. The notification option originally covered six major crops, including soybeans, and was modified in May of 1997 to cover nearly all plants. The notification option represents a simpler, streamlined application and review process for importation, interstate movement and field testing. Notifications are logged into the USDA database, reviewed by one of the scientific staff for qualification, completeness (see section 4 for Data Requirements), and then a recommendation is sent to the appropriate State department of agriculture for review. If the State concurs with an APHIS recommendation of approval, an acknowledgment is then issued to the applicant. The regulations stipulate that the entire process will take no longer than 30 days from receipt of the notification.

The notification option (7 CFR 340.3) requires that the introduction meet specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modification that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. These performance standards, compliance with which is subject to APHIS inspection, help to assure confinement of the regulated articles (see sections 5 and 7). Confinement is of central importance in APHIS's approach to the regulation of field testing. Confinement ensures that any environmental impact will be negligible because the article will not move beyond the field site and will not persist at the site beyond the intended duration of the test. All crop plants and most plants that are not listed as noxious weeds, as described in regulations at 7 CFR 360 under the Federal Noxious Weed Act at 7 U.S.C. § 2809, can be field tested under notification. Nearly 99 per cent of all field tests, importations, and interstate movements of engineered plants are performed under this system. The three major steps APHIS takes in this process are to: (1) evaluate relevant information (both that submitted by the permit applicant and that gathered by APHIS from other sources); (2) notify and consult with regulatory officials in States where the applicant proposes to field test; and (3) reach a decision as to whether to acknowledge or deny the notification.

In the particular case of soybean, performance standards were established that would maintain physical isolation of the plants and seeds.

Petition for determination of non-regulated status. As testing of one of these regulated articles proceeds, an applicant gathers information typically to establish for him/herself that the product has the new intended property, and also gathers information to demonstrate that the organism is not a plant pest risk. Evidence for safety relies in part on data that demonstrate that the engineered plant is biologically equivalent to a corresponding non-engineered line, with the exception of the intended new trait(s). When enough information is gathered, the applicant may petition APHIS for what is called a Determination of Non-regulated Status.

When APHIS gets a petition, the receipt of the application is announced in the Federal Register and copies are made available to the public (see Section 8, Public Involvement and Transparency). In 1996, when the glufosinate soybean petition was received and evaluated, the announcement marked the start of a 60-day public comment period on the petition and comments were then considered in the final determination and Environmental Assessment (EA). The EA was prepared pursuant to the National Environmental Policy Act (NEPA), 42 U.S.C. §§ 4321-4335. Since mid-1999, in addition to the 60-day comment period on the petition itself, notice of the availability of an environmental assessment (EA) is also published and public comments are solicited and accepted on the EA for a 30-day period. During the remaining 180 days, consultations are made as necessary with other agencies having expertise, the determination document is prepared, and the completed decision documents are subjected to legal review.

In general, the petitioner has to supply data and supporting information to indicate that the product does not present a plant pest risk at any time during the 180-day assessment process. The APHIS assessment relies on data and other information that demonstrates that, with the exception of the deliberately introduced trait, the genetically engineered line appears to be the same as a non-engineered parental line with respect to a suite of agronomic traits. If this is true, and if there is sufficient familiarity with the introduced trait, the recipient plant, and the environment, APHIS can determine with a high degree of confidence that the engineered plant is no more likely to be a plant pest than a traditionally bred plant. Issues and risks which are not science-based, such as consumer acceptance and marketability of genetically engineered products, are not a part of the APHIS analysis. Once a Determination of Non-regulated Status is issued, the new variety may be developed further through traditional breeding, produced, marketed, distributed, and grown without any other special oversight on the part of APHIS, however, before some plants can be used commercially, additional reviews may be necessary by the EPA and FDA. For example, the consultation process between FDA and AgrEvo for glufosinate tolerant soybeans was not completed until 1998, and so the product was not used for food or feed before that date.

Consideration by APHIS of a broad range of environmental issues is mandated under NEPA, which addresses the general decision making process for all government actions. In considering the broad range of possible impacts under NEPA, APHIS expertise overlaps with that of other federal agencies, namely with EPA for a host of environmental concerns such as nontarget effects, and worker exposure, and with NIH and FDA for potential negative impacts on animals and humans.

Glufosinate tolerant soybean, due to the presence of sequences derived from plant pests listed in 7 CFR Part 340.2, clearly meets the definition of a regulated article and is subject to APHIS regulation. All releases were conducted after APHIS approval in the form of 8 permits and 24 notifications issued from 1992 through 1996 when AgrEvo filed a petition for non-regulated status on March 8 of 1996. Following a review of the petition, a deficiency letter was sent to obtain additional information and clarification. Such letters are routine and are sent in response to virtually every petition, reflecting the thoroughness of the APHIS review. Upon receipt of the additional information the petition was announced in the Federal Register and made available for public reading and comments (see section on “Transparency and Public Involvement”). Eight comments were received from universities, extension centers, and a seed company. All supported the petition. A determination to deregulate under 7 CFR 340 and an environmental assessment to fulfill the NEPA obligation were prepared and the glufosinate tolerant lines were deregulated on August 16, 1996. Both of the decision documents are available at http://www.aphis.usda.gov/biotech (USDA, 1996).

EPA

Under Federal law, the EPA is responsible for regulating all pesticides, and setting the maximum levels ("tolerances") of pesticide chemical residues allowed in or on food and animal feed. In the case of

glufosinate-tolerant soybean, EPA does not regulate the soybean plants themselves, but rather regulates the use of the herbicide on those plants. EPA's authority, and the limits to that authority, are contained in two core statutes, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), 7 U.S.C. §§ 136-136y, and the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 408. In 1996, both statutes were amended by the Food Quality Protection Act (FQPA), Pub.L.104-170 (1996).

With minor exceptions, FIFRA requires that before anyone can sell or distribute a pesticide in the United States, they must obtain a registration, or license, from EPA. When making a registration decision, including those pertaining to the herbicides used on herbicide-tolerant crops, EPA must find that the pesticide, when used according to label directions, will not cause unreasonable adverse effects to human health or the environment. The registration of glusofinate on soybeans is considered to be a new use because heretofore the herbicide had not been used on soybeans. Registration decisions are based primarily on EPA's evaluation of the test data provided by applicants. FIFRA also requires a periodic reassessment of registrations to ensure they are meeting current scientific and regulatory standards, including with respect to the generation of data. FIFRA § 4.

Data requirements for pesticide registration are specified in the Code of Federal Regulations (40 CFR Part 158). Various types of data are required to assess the hazards and exposures for new chemicals prior to registration. Once a chemical is registered (which was the case here), there can be new data required to ascertain the additional exposure and ensure that the pesticide continues to meet the safety standard, i.e., that it "will not cause unreasonable adverse effects on the environment." In addition, a tolerance or a tolerance exemption may be required for the pesticide chemical residues resulting from this new use. Alternatively, the tolerance may be modified to change the existing tolerance level. What are usually required for herbicide-tolerant crops are data from field use on the herbicide-tolerant crop to provide an assurance about expected residues and any questions related to new metabolites or other products resulting from the activity of the newly introduced enzyme. EPA may also require other data relevant to determining whether the pesticide meets the safety standard. Specific reports submitted by the registrant in support of registration of the new use and a tolerance on herbicide-tolerant soybeans are cited in Appendix A.

EPA has established other requirements, such as the Good Laboratory Practice Standards, to ensure the quality and integrity of pesticide data. Depending on the type of pesticide, EPA's Office of Pesticide Programs can require more than 100 different tests. Testing is needed to determine whether a pesticide has the potential to cause adverse effects to humans, wildlife, fish, and plants, including endangered species. In addition to allowing the use of new pesticides, the Agency's Registration Program includes many activities related to the ongoing registration of existing pesticides. This may include, for example, label changes in where and how pesticides are used in order to reduce risks or in response to requests by registrants. These approved labels have the force of law, and any use, which is not in accordance with the label directions and precautions, may be subject to civil and/or criminal penalties.

Section 408 of the FFDCA, 21 U.S.C. § 346a, governs, among other things, the establishment of pesticide tolerances for food and feed products and gives the EPA authority to establish tolerances or exemptions from the requirement for a tolerance for pesticide chemical residues. A tolerance is the maximum level of pesticide chemical residues allowed in or on human food and animal feed.

The FFDCA makes unlawful the sale and distribution in interstate commerce of adulterated food. Food is defined broadly, and includes both food for humans and animals. Food is "adulterated" if it contains the residue of a "pesticide chemical" for which EPA has not established either a "tolerance" or an exemption from the requirement of a tolerance. (Almost all "pesticides" are "pesticide chemicals.")

The FFDCA authorizes EPA to establish a tolerance for a pesticide if the "residue in or on food is safe." Similarly, EPA may establish an exemption from the requirement of a tolerance if the Administrator determines that the exemption is "safe."

The Food Quality Protection Act (FQPA), signed into law on August 3, 1996, amended both FIFRA and FFDCA. The new FFDCA section 408 safety standard requires EPA to ensure that there is "a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." 21 U.S.C. § 346a(b)(2)(A)(ii). The new safety standard is measured by considering the aggregate risk from dietary exposure and other non-occupational sources of exposure, such as drinking water and residential lawn uses. In addition, to improve protection for all consumers, particularly the young, when setting new, or reassessing existing tolerances under the new standard, EPA must now focus explicitly on exposures and risks to infants and children. Decisions must consider whether tolerances are safe for children assuming, when appropriate, an additional safety factor to account for uncertainty in data. Any person may petition the EPA to establish a tolerance or an exemption from the requirement of a tolerance for a pesticide and its residues in food; the law authorizes EPA to require information in support of the petition to show that the tolerance or exemption would be safe.

In accordance with FIFRA and section 408 of the FFDCA, as amended by the FQPA, the information provided by the registrant (of glufosinate) allowed the EPA to amend the label for the currently registered use to include the use of glufosinate for use on glufosinate-tolerant soybeans in Spring of 1997. Since EPA is concerned with the risks associated with the uses of the herbicide, EPA does not necessarily reevaluate its analysis of the risks when a new herbicide-tolerant soybean variety with the same genetic transformation event, different events, or different constructs is created.

EPA also has authority under the Toxic Substances Control Act to regulate plants, including genetically engineered plants, when they are used for a purpose not excluded under section 3 of the Act. EPA's authority under TSCA extends from the research and development phase, through

commercial manufacture, use, and disposal. For example, EPA has authority to require pre-market notification and Agency review of a new chemical substances, as well as existing chemical substances whose uses EPA has determined (by rule) to be a significant new use. EPA is also authorized to regulate an existing chemical substances under TSCA section 4 (data generation), sections 6 and 7 (impose restrictions to prevent unreasonable risks of injury to human health or the environment), and section 8 (information collection). Further information on TSCA regulations and biotechnology products can be found in this report in the Bioremediation and Biosensing using Bacteria case study and the EPA website.

Implementing regulations

Experimental use permits. EPA's regulation of the pre-registration sale or distribution of a pesticide occurs primarily through its experimental use permit (EUP) process. EPA does not typically require an EUP if a field test is less than 10 acres. The agency approves testing only for the purpose of gathering data to support an application for registration, and only for an area sufficient to collect reliable information. Typically, EPA does not approve field testing for more than 5,000 acres.

In addition, if the experimental design involves the production of food for distribution in commerce, a tolerance, temporary tolerance, or exemption must be established. A person may avoid the need for a tolerance by destroying the crop treated with the unregistered pesticide; a "crop-destruct" provision would then be imposed on the EUP. Granting of an EUP is contingent on satisfactory data to support a risk assessment and a finding that the proposed experimental use will not result in unreasonable adverse effects on the environment. The data required to support a request for an EUP are detailed in 40 CFR Part 158. Specific reports submitted by the registrant in support of the EUP and temporary tolerance on herbicide-tolerant soybeans are cited in Appendix B.

Registration. An application for registration typically requires substantially more data than an EUP. The data requirements depend on the type of product for which registration is sought. See 40 CFR Part 158. EPA regulations describe labeling and packaging requirements for pesticide products. See 40 CFR Parts 156 and 157. On a case-by-case basis, EPA may impose additional requirements or conditions on registrations for individual products. For example, EPA may issue a "seed increase registration" for an herbicide that allows a registrant to apply the herbicide to the GEOP breeding stock for the purpose of producing seed for propagation and future sale. The genetically altered seeds, however, could not be sold pursuant to the terms and conditions of the "seed increase registration" until a full-scale registration was approved for the use of the herbicide on the crop for which the seed was produced. Specific reports submitted by the registrant in support of registration of the new use and tolerance of herbicide-tolerant soybeans are cited in Appendix A.

Tolerances. The tolerance process starts with the submission of a petition to establish a tolerance or an exemption from the requirement of a tolerance. The petitioner must provide data to

support the tolerance. When EPA receives a petition, the agency publishes in the Federal Register a notice of receipt of the petition, together with a summary of the petition's contents. 21 U.S.C. § 346a(d)(3). For the notice of receipt for the glufosinate residues on soybeans, see 60 Fed. Reg. 54689. In order to set a new tolerance, the Agency also reevaluates all existing tolerances for a chemical. Following review of the petition and any comments from the public (no comments were received in response to this filing), EPA may publish a final rule establishing the tolerance or exemption, provided that the available information demonstrates that the action would comply with the statutory standard. The final rule announcing the new soybean tolerances for glufosinate was published in the Federal Register on February 5, 1997 (62 Fed. Reg. 5333 – 5338.

All Federal Agencies

The Endangered Species Act (ESA), as amended, jointly administered by the Secretaries of the Interior and Commerce, could also affect the use and dispersal of transgenic plants.

The ESA requires importers of plants to file declarations, and limits importation to designated ports. Id. §§ 1538(d), (f). Section 7 of the ESA requires any federal agency to ensure that any action authorized, funded, or carried out by the agency not jeopardize the continued existence of any endangered or threatened species or adversely modify any critical habitat of such species. Id. § 1536(a)(2). Thus, each federal agency must consult with the U.S. Fish and Wildlife Service or the National Marine Fisheries Service, depending on the species, for any action that "may affect" a listed species. If the action is likely to adversely affect a listed species, the appropriate Service issues a Biological Opinion, which may authorize take of fish or wildlife species that is incidental to the action or, if the federal action would otherwise jeopardize the continued existence of the species, offers alternatives to the federal action that will avoid such jeopardy. Id. § 1536(b). Any take of an endangered or threatened fish or wildlife species unless otherwise authorized is unlawful under the statute. Id. § 1538. If the action is likely to adversely affect a listed plant, the situation is somewhat different. Section 9 prohibitions on take do not apply to plants, see *id*. at § 1538(a)(2), but cautions can be provided in the Biological Opinion on prohibitions against removal or disturbance of plants. Thus, a federal agency will be held responsible for prohibited acts affecting both wildlife and plants that result from authorization, funding, or other federal action associated with a genetically engineered organism or product (GEOP).

Additionally, the Federal Migratory Bird Treaty Act (MBTA), administered by the Department of the Interior, also requires that any federal action that might impact migratory avian species be minimized or excluded so as not to harm populations.

3. Hazard Identification and Risk

USDA

In many respects, the main elements of hazard identification are embodied in the statutory authorities of USDA, EPA, and FDA that were summarized when the Coordinated Framework for the Regulation of Biotechnology was published by the Office of Science and Technology Policy in 1986 (OSTP, 1986). These legal authorities address risks that may be associated with organisms that harm plants (plant pests), pesticides which may be toxic to humans or other nontarget organisms, and foods and feeds that are adulterated, improperly labeled, or have significantly altered nutritional qualities. As described in Section 2, USDA now derives authority from the Plant Protection Act, enacted in 2000, but the elements of hazard identification remain essentially unchanged.

To perform risk assessments, APHIS has recognized that it is necessary to identify and focus on specific hazards that are potential components of risk based on the particular organism in question and its use. Here, the organisms in question are crop plants intended for use in agriculture, or to be eaten as food, or used to make ingredients in food. To identify these hazards, it is necessary to start with a good understanding of the existing traditional knowledge base and of the procedures that are routinely carried out in the course of developing any new crop variety that is released for commercial use. This knowledge serves as a baseline to decide whether the risk posed by a specific hazard is significantly changed in potential magnitude from any well-known one that is part of established practice. It also enables the hazard identification.

The use of knowledge and experience gained from traditional breeding as a basis for establishing parallel risk associations for newly developed crops is referred to as familiarity. The concept of familiarity is based on the philosophy that the types of safety issues raised by genetically engineered plants are no different from those for traditional breeding when similar traits are being conferred, though the magnitude of any particular risk may differ (NRC, 1989, NRC 2000). Thus, the extensive record provided by experience with traditional plant breeding provides useful information for evaluation of genetically engineered crops with similar alterations and, as with traditionally bred crops, such alterations are likely to pose few ecological problems. (Tiedje et al., 1989). Familiarity is not a risk/safety assessment in itself (NRC, 1989). However, the concept facilitates risk/safety assessments, because to be familiar, means having enough information to be able to make a judgment of safety or risk (U.S. NRC, 1989). Familiarity can also be used to indicate appropriate management practices including whether standard agricultural practices are adequate or whether other management practices are needed to manage the risk (OECD, 1993). As familiarity depends also on the knowledge about the environment and its interaction with introduced organisms, the risk/safety assessment in one country may not be applicable in another country. However, as field tests are performed in different locations, information will accumulate about the organisms involved and their interactions with other organisms in these varied environments.

Familiarity comes from the knowledge and experience available for conducting a risk/safety analysis prior to large -scale introduction of any new plant line or crop cultivar in a particular environment. For plants, for example, familiarity takes account of, but need not be restricted to, knowledge and experience with:

- the crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences;
- the agricultural and surrounding environment of the trial site;
- specific trait(s) transferred to the plant line(s);
- results from previous basic research including greenhouse/glasshouse and small-scale field research with the new plant line or with other plant lines having the same trait;
- the scale-up of lines of the plant crop varieties developed by more traditional techniques of plant breeding;
- the scale-up of other plant lines developed by the same technique;
- the presence of related (and sexually compatible) plants in the surrounding natural environment, and knowledge of the potential for gene transfer between crop plant and the relative; and
- interactions between/among the crop plant, environment and trait. (Organization for Economic Cooperation and Development (OECD), 1993)

With respect to the above factors, familiarity can range from very high to very low. For genetically engineered crop plants commercialized to date in the U.S., there has been a high degree of familiarity. This is certainly the case for soybeans. The degree of familiarity is important to the assessment, and could affect the type of data required to perform the assessment.

APHIS environmental assessments are consistent with Annex 3 of the United Nations Environment Program (UNEP) Guidelines for Safety in Biotechnology, which lays out the broad steps in biosafety review. These can be paraphrased as (1) identifying hazards; (2) assessing actual risks that may arise from the identified hazard; (3) determining how identified risks can be managed and whether to proceed with proposed action; (4) comparing the assessed risks with those posed by actions with comparable organisms. These steps are relevant to both APHIS's authority to regulate under the Plant Protection Act and to its obligations under NEPA. The APHIS assessments are based on the principle that the environmental risks that may be posed by a certain use of a particular organism will depend on: the properties of the organism, the way the organism is to be used (including whether the organism is to be used under confinement or in the context of an environmental release), and safeguards that are built into experimental design or conditions of use.

APHIS has worked closely with member countries of the OECD, and in other fora, to bring about international consensus on the safe development, testing, and use of genetically modified plants and microorganisms. In 1986, OECD published its first safety considerations for genetically engineered organisms (OECD, 1986). These included the issues (relevant to human health, the environment and

agriculture) that might be considered in a risk/safety assessment. These issues were re-iterated in a recent report on harmonization of regulatory oversight in biotechnology published in 2000 (OECD, 2000). OECD has also published several [1]consensus documents that are useful in risk assessments.

In specific terms, the following represent the major hazards that have been identified by APHIS and for which risks are assessed:

- Plant pathogenic potential of the transgenic plant (i.e., either symptomology in the transgenic
- crop plant or the ability of the transgenic crop to harm other plants)
- Potential to affect handling, processing, or storage of commodities containing the genetically engineered plant.
- Changes in cultivation that might accompany adoption of the transgenic variety
- Potential to harm nontarget organisms
- Changes in the potential of the genetically engineered crop plant to become a weed
- Potential to affect "weediness" of sexually compatible plants
- Potential impacts on biodiversity

Based on the data provided by AgrEvo, available information about the crop (soybean), and the engineered genes, APHIS assessed the risks of introduction of glufosinate tolerant soybeans. The assessment can be summarized as follows:

_ Plant pathogenic potential of the transgenic plant - Though the transgenic plant contains certain sequences from plant pathogens, specifically, the promoter and terminator sequence from 35*S* CaMV and the *nos* terminator from *Agrobacterium tumefaciens*, APHIS concluded that these did not pose a significant risk of imparting plant pathogenicity. All of the sequences are well-characterized regulatory sequences that are not transcribed or translated to protein and all have a history of safe use in transformed plants. Evaluation of data from field tests did not identify plant pathogenic effects due to the introduced sequences.

_ Potential to cause harm to commodities - Because the harvested products are the same for the glufosinate tolerant varieties as for traditional varieties with respect to the required methods for handling, processing, and storage, APHIS did not identify a risk to raw or processed commodities.

_ Changes in cultivation that might accompany adoption of the transgenic variety - Due to the nature of the product, use of transgenic soybeans will likely be accompanied by a shift in

[1]Relevant to this case study, OECD has published a consensus document specifically on the genes responsible for glufosinate (syn.=phosphinothricin) tolerance and their enzyme products in plants (OECD, 1999), but this report was not available at the time glufosinate tolerant soybeans were de-regulated.

herbicide usage patterns. Traditionally, soybean weeds have been managed on a field-wide basis as a preventative strategy, but the availability of an effective broad-spectrum post-emergence weed control option encourages farmers to treat weeds when and where they emerge on an "as needed" basis. The advent of effective post-emergence herbicides has also facilitated a move toward no-till production systems in soybeans and glufosinate tolerant soybeans are likely to be cultivated in this way. APHIS does not find any negative impacts associated with these changes in cultivation.

– Potential to harm nontarget organisms - APHIS considered the mode of action of the PAT enzyme, the lack of any known toxicity associated with the enzyme, as well as data supplied by the company that showed that the protein shares no homology with proteins known to be toxic, that the protein has no characteristics of a toxin or allergen, and field observations from numerous sites revealing no negative effects on insects, birds, and other species. Because information developed by the company and the scientific literature show no toxicity, no specific monitoring protocol could be developed. In this case, APHIS depended on adverse effects reports noted by the company for this information. Based on the scientific literature and the information from the company described above, APHIS concluded that glufosinate tolerant soybeans pose an insignificant threat to nontarget organisms, including endangered species.

– Potential of the crop plant to become a weed - Central to the conclusion that soybean is not likely to become a weed is the substantial evidence that soybean does not possess weedy tendencies, based on it having not established populations outside of agriculture despite years of wide-spread cultivation, and that fact that it is not listed in standard texts or references as a weed. The introduced characteristic of glufosinate tolerance is not expected to add any characteristics of weediness to soybean. In the highly unlikely event that there was a need to control soybean as a weed, for example volunteer soybeans in fields that were converted to another crop, chemical options other than glufosinate are available.

– Potential to affect "weediness" of sexually compatible plants - There are no wild relatives of soybean nor any other plants sexually compatible with soybean in the continental United States, though some occur in U.S. territories in the South Pacific. In addition, there are significant barriers to outcrossing. Soybeans are nearly exclusively self-pollinating. Hybrid crosses between cultivated soybean (*Glycine max*) and other members of the subgenus *Glycine* as are found in the Pacific territories, have been achieved only through seed culture. These hybrids are generally sterile with further offspring only being obtained with extreme difficulty.

– Potential impacts on biodiversity - APHIS concluded that glufosinate tolerant soybeans do not pose a threat to biodiversity based on: 1) soybeans will not become a weed and do not significantly hybridize with related species; 2) the high specificity and lack of toxicity of the PAT enzyme result in an insignificant threat to nontarget species; 3) APHIS can envision no threat to

biodiversity for glufosinate tolerant soybeans that will not apply to traditionally bred soybeans.

EPA

In evaluating a pesticide registration application, EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices. The Agency also assesses a wide variety of potential human health and environmental effects associated with use of the pesticide. The registrant (typically the manufacturer of the pesticide) must provide data from specific, required studies (tests) conducted according to EPA guidelines. These tests are needed to allow the Agency to determine whether a pesticide has the potential to cause adverse effects on humans, wildlife, fish, invertebrates, and plants, including endangered species and other non-target organisms, as well as possible contamination of surface water or groundwater from leaching, runoff, and spray drift.

As the Agency considers a use for an herbicide on an herbicide-tolerant crop, it applies the same standard risk assessment methodologies to assess the environmental safety issues associated with the use of the herbicide, as would be applied to any herbicide use prior to its registration. For chemical pesticides, the Agency relies on the data generated under Good Laboratory Practices (see 40 CFR Part 160) to assess hazard and exposure. Glufosinate-ammonium was first registered by the EPA in 1993 as a non-selective, water soluble herbicide for application as a foliar spray for the control of emerged annual and perennial grasses and broadleaf weeds. Its original end-use products included home owner uses for weed control around trees, shrubs, fences, walks, patios, driveways, sidewalks, in flower beds, and to spot kill weeds in lawns. Another product (Ignite 1SC) targeted light industrial non-food uses such as trimming and edging of landscape areas, recreation and public areas, nursery uses such as field grown and container stock weed control, and non-food use around farmsteads.

EPA's review of the environmental studies concluded that glufosinate-ammonium was practically nontoxic to birds and aquatic species. Laboratory studies indicated that the chemical was mobile and persistent, which resulted in a groundwater advisory statement on the product label that contained the light industrial non-food uses.

Avian studies using the technical grade product (i.e., a product that is solely or primarily intended to be used for the manufacture of pesticide end-use products) indicated that the avian acute oral LD50s were greater than 2000mg/kg (mallard and bobwhite) and the dietary LC50s of 5000 ppm for both mallard duck and bobwhite quail indicated that glufosinate-ammonium is practically nontoxic to birds. The avian reproductive NOEL value for both mallard and bobwhite appears to be greater than 400 ppm based on statistical analysis.

Aquatic studies using the technical grade product indicate that the fish LC50s for both rainbow trout and pumpkinseed were greater than 320 ppm, indicating that glufosinate-ammonium is practically

nontoxic to both warm water and cold water fish species. The LC50 for *Daphnia magna* was 667 ppm that indicates that glufosinate-ammonium is practically nontoxic to aquatic invertebrates.

Toxicity studies using the formulated product (i.e., an end-use pesticide product, here Ignite) indicate that the LC50s ranged from 26.7 ppm for rainbow trout to 65 ppm for bluegill sunfish; Ignite can be classified as being slightly toxic to fish. The LC50s for *Daphnia magna* ranged from 15.0 to 79.5 ppm indicating that Ignite is slightly toxic to aquatic invertebrates. The available honeybee toxicity data for Ignite (LC50 greater than 100 ug/bee for the 20% active ingredient product; LC50 = 345.5 ug/bee for the 95.3% active ingredient product) indicate that this chemical is practically nontoxic to bees.

A summary of the environmental fate characteristics for glufosinate-ammonium indicates a hydrolysis half-life greater than 300 days at pH 5, 7, and 9; an aerobic soil metabolism half-life greater than 120 days (sandy loam soil); an aerobic aquatic metabolism half-life greater than 64 days; high mobility; photodegradation in soil declined to 87.5% of applied during 45 hours irradiation; photodegradation in water showing no degradation at pH 5, 7, and 9; anaerobic soil metabolism of 45 to 60 days; terrestrial field dissipation less than 3 to 4 days (loamy sand); and no accumulation in fish (bluegill).

Laboratory studies indicated that glufosinate-ammonium and its degradates were mobile and persistent. Thus, the potential for groundwater contamination did exist. However, the use of the homeowner products for spot treatments on turf was not expected to present a risk for groundwater contamination. A groundwater advisory statement was required for the Ignite label with light industrial non-food uses that read as follows: "Glufosinate-ammonium and its degradates have those properties normally associated with pesticides that have been detected in ground water. Use of this product in areas with coarse soils and high water tables may result in ground water contamination."

Additional data were required of the registrant in order to register glufosinate-ammonium on soybeans. These data were needed to assess the likelihood and magnitude of glufosinate residues in the soybeans. The registrant had to provide the EPA with an in-depth metabolism study for the herbicide-tolerant crop. The nature of the residues and the magnitudes of the components of the residues were determined. The residue of concern for dietary exposure considerations is defined as those components (parent compound and/or metabolites/degradants) for which there is a significant toxicological concern. The residue of concern for enforcement purposes is defined as the parent and/or possibly one or more metabolites, depending on the relative magnitude of the residue components, the toxicological concerns for the components, and the capabilities of proposed enforcement analytical methods. The process is essentially identical for herbicide-tolerant and traditional crops. Additionally, a full complement of field trials in the principle growing regions was required with the herbicide-tolerant crop variety to ascertain the magnitude of the residue under actual growing conditions. Again, the metabolism/residue requirements for herbicide-tolerant and traditional crops are generally the same.

For glufosinate-ammonium, tolerances on several traditional commodities such as almonds, grapes, and tree nuts (40 CFR 180.473(a)(1)) have been established for residues of glufosinate-ammonium and its metabolite, 3-methylphosphinico-propionic acid. For the glufosinate-tolerant soybeans, there was also the possibility that new metabolites or other products that could result from the presence of the new enzyme function. The transgenic herbicide-tolerant soybeans contain a gene for an enzyme (phosphiothrion-acetyl-transferase) that enables the plant to metabolize the herbicidally active moiety of glufosinate-ammonium into N-acetyl glufosinate (2-acetamido-4-methylphosphinico-butanoic acid), which is not herbicidally active. This metabolite is found only in the transgenic plants, and this information was obtained from metabolism studies conducted on the transgenic crop. The metabolite N-acetyl glufosinate was added to the residue definition for crops for which herbicide-tolerant versions have been developed (40 CFR 180.473(a)(2)), and an enforcement analytical method was required for this metabolite.

The enforcement method for the genetically unaltered (traditional) crops determines glufosinate-ammonium and 3-methyophosphinico propionic acid by a GC/FPD method after extraction, anion-exchange chromatography, and derivitization with trimethylorthoacetate. The method inadvertently includes N-acetyl glufosinate in the measured residue by converting it and the parent to the same derivative. The inclusion is of no consequence, since the metabolite is not expected to occur in crops lacking the acetyl transferase enzyme. A modified method was developed to determine each of the compounds of interest in transgenic crops. Both field trial data and processing study data were required for the transgenic soybeans.

In the Federal Register of October 25, 1995 (60 FR 54689) (FRL-4982-4), EPA issued a notice pursuant to section 408(d) of FFDCA, 21 U.S.C. § 346a (d), announcing the filing of a pesticide tolerance petition by AgrEvo USA Co. The petition requested that 40 CFR 180.473 be amended by adding tolerances for residues of glufosinate-ammonium and its metabolites 2-acetamido-4-methylphosphinico-butanoic acid and 3-methylphosphinico-propionic acid, in or on the following raw agricultural commodities (RACs): corn, field, grain at 0.2 part per million (ppm); corn, field, forage at 4.0 ppm; corn, field, silage at 3.5 ppm; corn, field, fodder at 5.5 ppm; soybean seed at 2.0 ppm; and soybean hulls at 6.0 ppm. In the Federal Register of July 31, l996 (61 FR 39964)(FRL-5384-7), EPA issued a notice of an amendment to the petition. The tolerances requested were changed to residues of glufosinate-ammonium and its metabolites, 2-acetamido-4-methylphosphinico-butanoic acid and 3-methylphosphico-propionic acid expressed as glufosinate free acid equivalents, in or on the following RACs: corn, field, grain, at 0.2 ppm; corn, field, forage, at 4.0 ppm; corn, field, fodder, at 6.0 ppm; soybeans, at 2.0 ppm; aspirated grain fractions, at 25.0 ppm; eggs, at 0.05 ppm; poultry, meat at 0.05 ppm; poultry, fat at 0.05 ppm; and poultry, meat by-products (mbyp) at 0.10 ppm. The revised petition also requested that a maximum residue level be established for the same residues in or on the processed commodity under section 701 of FFDCA: soybean hulls at 5.0 ppm.

In the Federal Register of November 18, l996 (61 FR 58684) (FRL-5572-7), EPA issued a third Notice of Filing to amend the petition to bring the petition in conformity with FQPA (Pub. L. 104-170). The notice contained a summary of the petition prepared by the petitioner and this summary contained conclusions and arguments to support its conclusion that the petition complied with section 408 of the FFDCA, as amended by FQPA. In this instance the petitioner proposed to amend 40 CFR 180.473 by establishing tolerances for residues of glufosinate ammonium in or on the following RACs: corn, field, grain, at 0.2 ppm; corn, field, forage, at 4.0 ppm; corn, field, fodder, at 6.0 ppm; soybeans, at 2.0 ppm; soybean hulls, at 5.0 ppm; aspirated grain fractions, at 25.0 ppm; eggs, at 0.05 ppm; poultry, meat at 0.05 ppm; poultry, fat at 0.05 ppm; and poultry, mbyp at 0.10 ppm. The residues of glufosinate-ammonium were defined as butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt and its metabolites: 2-acetamido-4-methylphosphinico-butanoic acid and 3-methylphosphinico-propionic acid expressed as glufosinate free acid equivalents.

There were no comments or requests for referral to an advisory committee received in response to the notices of filing. The Notice of Filings were incorrectly stated for eggs and the poultry commodities because the residue chemistry data showed only the parent chemical and one metabolite, 3-methylphosphinico-propionic acid. The subject regulation was therefore amended accordingly. The data submitted in the petition and other relevant material have been evaluated and time-limited tolerances established for residues of the herbicides glufosinate ammonium (butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt) and its metabolites: 2-acetamino-4-methylphosphinico-butanoic acid and 3-methylphosphinico-propionic acid, in or on various raw agricultural commodities (RACs), derived from transgenic field corn and transgenic soybeans. The final rule announcing the new soybean tolerances for glufosinate-ammonium was published in the Federal Register on February 5, 1997 (Volume 62, Number 24, pp. 5333 - 5338).

An important aspect of EPA's risk assessment methodology is the use of monitoring as a condition of use to provide information for further assessment and refinement as the crop and herbicide are used. This is described further in section 6, below.

FDA

As for all plant foods, FDA is the federal agency responsible for overseeing the safety and appropriate labeling of glufosinate tolerant soybeans, apart from the safety issues presented by pesticide chemicals and metabolites. FDA has a voluntary consultation process through which companies resolve any safety or other regulatory issues prior to marketing foods from bioengineered plants. FDA considers, based on agency scientists' evaluation of submitted information, whether any unresolved issues exist regarding a food derived from a new plant variety that would necessitate legal action by the agency if the product were introduced into commerce. Examples of unresolved issues may include, but are not limited to, significantly increased levels of plant toxicants or anti-nutrients, reduction of important

nutrients, new allergens, or the presence in the food of an unapproved food additive. FDA has just published a proposed rule, that, if finalized, will require companies to notify FDA at least 120 days in advance of marketing a bioengineered plant food and provide FDA with data and information to demonstrate that the food is as safe as its conventional counterparts.

Interagency Coordination

At the time of the soybean case study, interagency coordination between APHIS and EPA was based on individual contacts between agency scientists conducting reviews. Both agencies felt the need to improve their coordination, especially on the review of herbicide tolerant crops. Since January 2000, the USDA and EPA have been identifying procedures that will improve coordination between the two agencies in their reviews of herbicide-tolerant crops and their respective herbicides. Currently, the APHIS reviews of the GEO and EPA reviews of the herbicide are done without any formalized joint reviews or sharing of information.

The improved coordination being discussed is likely to include an ad hoc interagency work group that will establish a protocol for exchanging completed scientific reviews between the agencies, whereby potential gaps and differences could be identified more readily and more expertise could be systematically brought to bear in these analyses. This would also speed the reviews in some instances by providing insight and perspective to agencies trying to answer very similar questions.

Specific coordination measures that are likely to be implemented include the following. APHIS will provide EPA a copy of APHIS petitions for non-regulated status for herbicide-tolerant crops. After APHIS drafts its Environmental Assessment (EA), APHIS will consult with EPA, especially as to any discussions of available herbicides for a given crop and their practical utility, i.e., efficacy on key weed pests. To this end, EPA will supply APHIS with current lists of herbicides registered for use on the crop in question, and any readily available information as to their efficacy. APHIS would also supply the work group with copies of extensions to existing petitions. This would keep the work group informed of any new transformation events in a crop that encode the same herbicide-tolerant phenotype from the same company.

APHIS will ask each petitioner of herbicide-tolerant crops to submit a voluntary stewardship plan for the management of pesticide resistance and potentially weedy volunteer crops in their herbicide-tolerant crops. Since APHIS receives petitions from registrants of herbicide-tolerant crops far in advance of EPA's receiving an application for registration of the herbicide on that crop, APHIS will consult with EPA as to the viability of the stewardship plans while preparing the APHIS EA. Having the two agencies concur on a stewardship plan early on in the registration process will ensure that the concerns of both agencies are addressed, and that these concerns are discussed in the EA along with the details of the plan and its implementation. The opportunity for the public to comment on both the petition and EA ensures transparency in the joint review process.

APHIS will, on an annual basis, keep EPA and the work group informed of what is in the registration pipeline by supplying a list of the herbicide-tolerant plants that are field tested each year. This advance notification system could alert the EPA to potential high-risk uses that might be of concern from an environmental or human health perspective.

4. Information and Data

USDA

APHIS requires different types of data depending on the particular regulatory process at hand. The particulars are described below for notifications and permits for importation, interstate movement, or field testing, and for the petition for determination of non-regulated status.

Movement, importation, and field testing (introduction). Permits are required for importation, interstate movement and field testing for articles which do not qualify for notification; these include microorganisms, arthropods, pharmaceutical-producing plants, and insect viruses. In the permit, the applicant lists:

- the regulated article or product,
- donor organism,
- recipient organism,
- vector or vector agent,
- date of the importation, movement or release,
- quantity of the regulated article, and
- the port of importation or site of release.

In addition, detailed information is required as applicable on:

- the anticipated or actual expression of the altered genetic material in the regulated article and how it differs from a non-modified parent organism,
- the molecular biology of the system,
- the country or locality where the donor, recipient, and vector were collected and produced,
- the experimental design at the release site,
- the facilities at the destination,
- the measures to ensure confinement, and
- the final disposition.

This data is required so that a decision can be made to conclude that the transgenic plant is adequately characterized, that no transgenic plant material will persist in the environment, and that any unintentional or unanticipated effects, if any, can be restricted to the confined field site and be managed in such a way that there are no environmental risks after the confined field release is terminated. All field test approvals require that a field data report be filed after the experiment is complete. In the case of importation and movement, the information allows for a decision which can conclude that the transgenic plants are adequately characterized and not considered to pose a plant pest risk, and/or can be considered to be contained in the receiving facility ensuring no dissemination into the environment, and thereby, posing no plant pest risk.

Under notification, much of the same information is required as for permits, but the format is more rigid and is streamlined such that the information is more easily catalogued and assessed by APHIS and thus allowing for a more rapid review process. The applicant must state that his article meets the eligibility requirements and that any actions taken will meet certain performance standards mandated in the regulations and described in the notification user's guides. It should be understood that the primary emphasis for field releases under both notification and permit is confinement and that the constraints imposed should effectively eliminate the potential for significant impact to the environment.

Petitions for determination of non-regulated status. The most comprehensive data packages received by APHIS for scientific review are the Petitions for Determination of Non-regulated Status. The petition process allows for removal of a transgenic plant from regulatory obligation. De-regulation may be a practical requirement for commercialization of common agronomic crops that are to be grown on a large scale, but may not be for certain specialized applications, for example, commercialization of pharmaceutical-producing plants. In order to make the determination on a petition, APHIS uses specified information and data supplied by the applicant to make risk assessments relative to the hazards listed previously.

The assessments rely on answers to a number of specific questions that are included as Appendix C. Information requirements may vary with plant species, the specific types of modifications, and end use. The information criteria listed in Appendix C are currently being developed mainly for crop plants with the exception of trees and aquatic plants. They represent a compilation of a range of issues that have been considered in past decisions depending on the specific case. Reviews are still conducted on a case-by-case basis that allows for reviewing additional or fewer criteria. These assessments are conducted by APHIS scientists.

EPA

FIFRA and section 408 of the FFDCA give EPA the authority to require studies necessary to determine whether a pesticide and its residues meet the statutory standards contained in each of the statutes. EPA regulations (40 CFR Part 158) detail the standard data requirements for pesticides.

Appendix A contains a list of reports that were submitted to the EPA in support of the registration of glufosinate-ammonium on glufosinate-tolerant soybeans, and for the establishment of a tolerance. Applicants may request waivers for required studies if they believe such studies unnecessary for a risk assessment. Guidelines determine the protocols that should be used for most of the required toxicity tests. Any significant variations from the protocol proposed by an applicant normally require independent validation of the novel test method. Additionally, primary literature (peer-reviewed) is a key source of new developments that may influence the type of data requested from registrants and whether EPA will accept waivers for certain studies. After reviewing any waiver requests, agency scientists determine, on a case-by-case basis, what studies will be required for a specific herbicide registration on a herbicide-tolerant crop.

Generally, EPA-required data for product characterization and toxicity tests are generated directly by the applicant or through the use of a commercial laboratory that specializes in performing chemistry / toxicity studies. Fate data (i.e., where the chemical eventually is found in the environment), field expression data, and product characterization studies are also generally performed by the applicant. Non-target studies are usually done by an outside contract lab that has experience in toxicology and the application of EPA guideline requirements.

All submitted studies are reviewed by agency scientists. Outside scientific experts may be contacted for the purpose of verifying scientific background information as needed. On particularly critical scientific issues, EPA may consult with its FIFRA Science Advisory Panel (SAP), a Federal Advisory Committee Act-chartered group of independent experts in scientific issues related to pesticides. The SAP's advice may concern broad issues, e.g., modifying existing guidelines or creating new ones, or may concern a specific pending regulatory action. No SAP reviews have been required as of the date of this writing for issues related to herbicide-tolerant crops.

Appropriate scientific and regulatory expertise exists within APHIS, EPA and FDA to review all submissions for scientific accuracy and interpretation.[2] EPA evaluates data for scientific soundness based on experience with the types of studies and the anticipated results. Agency scientists have the right to question any data that appear to be erroneous, falsified or otherwise questionable in nature. This may take the form of a request for clarification or another study with modifications.

Penalties for falsification of data can range from a monetary fine to imprisonment and combinations thereof. An extensive auditing program exists within EPA's Office of Enforcement and Compliance Assurance to ensure that laboratories are capable of carrying out the prescribed studies and that their equipment is in satisfactory working order. These audits can be carried out on a random

[2]Current resources are adequate to evaluate GEOs submitted for review. EPA, however, expects the number and complexity of submissions to increase, and it is clear that future budget appropriations may need to be increased to ensure continued adequate staffing.

basis or targeted to a specific laboratory if there is reason to believe that data have been falsified or in any manner misrepresented.

There are some areas in need of additional baseline research. At the top of this list would be field studies to assess the potential for the development of weed resistance. Weed resistance to herbicides could result from high selection pressure from the excessive use of a single herbicide mode of action. The agency is concerned that the development of weed resistance could result in farmers using higher application rates, additional applications of herbicides, or having to use additional, and potentially less environmentally benign herbicides to control their weeds.

Additionally, the agency would like to learn more about the environmental effects of gene transfer to other plants, the benefits of herbicide usage on herbicide-tolerant crops to growers and consumers, and would like to obtain improved usage data reporting for herbicide-tolerant crops and pesticides used in herbicide-tolerant crops.

5. Mitigation and Management Considerations: Approvals and Conditions on Research, Production Distribution, Marketing, Use and Disposal.

USDA

Interstate movement, importation, and field testing (introduction). APHIS regulations require that measures must be taken to minimize dissemination of the engineered organism into the environment during movement and while in the receiving facility (laboratory, growth chamber, or greenhouse) as specified in 7 CFR 340. The risk mitigation measures include: (1) adequate identification, packaging and segregation measures to prevent or minimize mixing, spillage and dissemination of viable transgenic plant material, including the flow of fertile transgenic pollen to sexually compatible plants during transit and in the receiving facility; (2) when applicable, methods to minimize the flow of fertile transgenic pollen to other sexually compatible plants within the contained facility or to such plants on the outside; (3) devitalization/disposal of transgenic plant material by suitable means, when no longer in use or authorized. Means of devitalization/disposal could include, but are not limited to, dry heat, steam heat, crushing, deep burial and/or chemical treatment.

For field tests, measures must be taken to confine the transgenic plants to the field site during the defined period of the release and to prevent the transgenic plants or their progeny from persisting in the environment in subsequent growing seasons either within or outside of the site of the confined release. Both the reproductive isolation measures and post harvest land use restrictions should be based on the reproductive biology and seed dormancy characteristics of the species**,** surrounding land use, proximity of sexually compatible plants and presence of pollinators**.** Additional mitigation measures may be necessary based on the nature of the introduced trait(s).

During the growing season, measures must be taken to achieve reproductive isolation from plants of the same species and other sexually compatible species that are not part of the confined release, whether they are cultivated, weedy or wild species. Depending on the plant species, this can be achieved by the use of one or a combination of the following: isolation distance, pollen or pollination-proof caging, netting or bagging of plants prior to flowering, guard rows/ border rows of plants to attract pollinators or trap transgenic pollen, flower removal prior to pollination, use of male sterile lines, use of plant growth regulators to block reproductive development, different flowering time, and/or termination of the confined field release prior to flowering. Generally, isolation distances that are used to ensure purity of certified seed **(**such as breeder seed or foundation classes of certified seed) may be adapted successfully to prevent or minimize outcrossing of transgenic pollen to sexually compatible plants that could produce viable progeny capable of persisting outside the confined field release site. When isolation distances are used, these zones are also monitored for the presence of the same species, related species and for proximity of fields of the same species.

Post-harvest land use restrictions may be necessary for a certain number of years following harvest of the transgenic plant material to allow monitoring, removal and destruction of volunteers. Generally, for soybeans, this would involve monitoring for volunteers either immediately after harvest in warm climates where conditions favorable for germination can be maintained, or in the next growing season in colder climates. Generally, the post-harvest periods used to ensure purity of certified seed may be adapted successfully. For certain plant species, and for certain specific cases, post-harvest land use restrictions may also be necessary for the perimeter of the confined field site itself to monitor for volunteers resulting from potential dissemination of seed, e.g., during mechanical harvesting operations.

Other risk mitigation activities for field tests include: (1) adequate identification, packaging and segregation measures to prevent seed mixing, spillage and dispersal into the environment during transit; (2) adequate cleaning of seeding and transplanting machinery at the confined field site prior to removal to another location to prevent dissemination of viable transgenic plant material into the environment; (3) devitalization/destruction of surplus seed or seedlings, and any viable transgenic plant material remaining after transplantation or after harvesting at the confined field site by suitable means which could include, but are not limited to, dry heat, steam heat, crushing, deep burial, discing into the soil, burning, treatment with appropriately labeled herbicides and/or chemicals (harvested transgenic seed and/or plant material from the confined field site may only be retained in an approved facility if requested at the time of the submission and authorized by the regulatory authority, and should be clearly identified, securely transported, and stored separately from other seed/or plant material to avoid mixing); (4) a contingency plan for destruction of viable transgenic plant material in case of accidental release. The plan should include site marking and monitoring to ensure destruction of viable material and immediate notification of regulatory authorities.

Even in the granting of a notification, APHIS still retains the option of requiring additional information from an applicant about the conduct of the trial if there is concern that in the particular

instance a performance standards may be difficult to meet or if new information or data becomes available. No such requirement was necessary in the case of the glufosinate tolerant soybeans.

Petitions for Determination of Non-Regulated Status. Once an article has been granted non-regulated status APHIS has no authority to impose conditions on research, production, distribution, marketing, use, or disposal other than phytosanitary restrictions that may be applicable. However, if new information indicates that a de-regulated article is causing harm as a plant pest, APHIS can revoke non-regulated status and again regulate under its authority as previously described.

EPA

Using the information gathered for a risk assessment, EPA decides whether to approve a pesticide chemical and/or use, as proposed, under FIFRA or whether additional protective measures are necessary to eliminate unreasonable risks and unsafe dietary exposures. For example, the Agency may prohibit a pesticide from being used on certain crops because of environmental risks that would be associated with that cropping system. Buffer zones around the cropped land might be required to protect vulnerable surface or ground water sources. Application rates and the number of applications could be altered to ensure protection of the environment from risks from the use of the pesticide.

If, after considering all appropriate risk reduction measures, the pesticide still does not meet FIFRA's safety standard, the Agency will not allow either 1) any uses of the proposed chemical, or 2) specific high-risk uses of the chemical.

6. Monitoring and Consideration of New Information

USDA

Interstate movement, importation, and field testing (**introduction**). APHIS personnel and appropriate state officials may inspect a site or facility where regulated articles are proposed to be released into the environment or contained after their interstate movement or importation. Failure to allow the inspection of the premises prior to the issuance of a permit or notification shall be grounds for the denial of the permit (7 CFR 340.4 (d) 7**).** APHIS has qualified inspectors in every State and Territory to perform inspections and take remedial action if necessary.

APHIS regulations (7 CFR 340.4(f) (10) require applicants to notify the agency within the time periods and manner specified below, in the event of the following occurrences: (1) orally notified immediately upon discovery and notify in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article; (2) in writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application, or suffers any unusual occurrence (excessive

mortality or morbidity, or unanticipated effect on non-target organisms). APHIS was not notified of any such occurrences with the glufosinate tolerant soybeans.

A final data report is required regardless of whether a field test is authorized under notification or permit. The regulations require that these reports include: methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, and the environment (specific instructions to applicants can be found on **http://www.aphis.usda.gov/biotech/notgen.html** under section B). APHIS coordinates the approval processes with the states, and federal regulations require that access to facilities, field test sites and pertinent records be allowed by officials from APHIS and the states. APHIS site inspections help to ensure the compliance with the mandated performance standards. Violations can result in fines or termination of the field test.

Petitions for Determination of Non-Regulated Status. Once an article has been granted non-regulated status, APHIS has no authority to require monitoring, perform site inspections, or require data reporting. If it were found later to pose a plant pest risk, however, it could return to regulated status and the authorities to conduct these activities would then be available.

EPA

As discussed above, EPA has considerable authority to regulate the post-registration use of a pesticide. EPA has the legal authority, technical capacity, and resources to prescribe monitoring requirements for the use of the herbicide on herbicide-tolerant crops should risk concerns warrant it. This authority includes: 1) issuance of data call-in notices to obtain additional information from registrants needed to evaluate the safety of a pesticide, and 2) assuring compliance with conditions imposed on the pesticide's registration. The EPA has required, as conditions of herbicide registration on herbicide-tolerant crops, that the registrant report annually on 1) changes in herbicide usage on the crop, and 2) on whether any adverse changes in agronomic practices accompany the use of the herbicide on the herbicide-tolerant crop. This additional reporting resulted, in part, from public concerns that herbicide-tolerant crops would foster farmers' reliance on herbicides, and that these registrations might adversely affect the use of no-till or other conservation tillage practices.

Data reported to EPA show that herbicide-tolerant crops often require lower application rates or fewer herbicide applications. In many cases, herbicide-tolerant crops also allow farmers to use more benign herbicides instead of more harmful ones, and allow farmers to use them prescriptively as post-emergent herbicides instead of making prophylactic applications before the crops and weeds emerge. The herbicides registered thus far for use with herbicide-tolerant crops have also proven to be highly compatible with conservation tillage practices. The Agency is currently assessing the quality of the usage information provided by the registrants, and is considering providing guidance on more robust collecting and reporting. If herbicide usage on herbicide-tolerant crops results in an increase in environmental risks, risk mitigation can be required.

EPA has authority to obtain additional data about a pesticide post-registration. Section 6(a)(2) of FIFRA (which contains an adverse effects reporting requirement) requires registrants to inform the agency of "additional factual information regarding unreasonable adverse effects on the environment of the pesticide" (see 40 CFR Part 159 for specific reporting requirements). Section 3(c)(2)(B) of FIFRA allows EPA to require submission of additional data where necessary. In addition, to reflect the current science, FIFRA proides for a periodic review of all pesticides under section 3(g) and/or 4.

7. Enforcement and Compliance

USDA

Interstate movement, importation, and field testing (introduction). Failure of applicants to submit complete and accurate information for all introductions may result in a fine of not more than $10,000 or imprisonment for not more than 5 years or both (18 U.S.C. § 1001 APHIS has qualified personnel in every State that can inspect field sites for compliance to the performance standards for all field testing. In addition, headquarters staff has inspected (in 2000), and will in the future inspect, field sites that raise new confinement issues, as decided on a case-by-case basis. Failure to comply with performance standards under notification or permit conditions can result in compliance infractions and the applicant can be ordered to take remedial action (7 U.S.C. § §7714(b)(1)) if necessary to prevent the spread of plant pests (7 CFR 340.4 d 7). From 1995 through 2000, APHIS recorded a total of 63 compliance infractions. After an infraction has been identified, APHIS decides on the appropriate course of action. In some cases, such as minor infractions where the applicant identifies the infraction, notifies APHIS immediately, and takes prompt and appropriate remedial action, an formal written APHIS response may not be necessary. In other cases, written warnings are issued. For the most serious of infractions, an investigation is conducted by APHIS Investigations and Enforcement Services Staff that usually results in applicants being fined. The applicant can also be assessed a criminal or civil penalty for failing to comply with the regulations (7 U.S.C. § 7734). If necessary, to protect the environment or public health, the transgenic organisms can be subjected to the application of remedial measures (including disposal) if determined by the Administrator (7 CFR 340.4 d 7). If the owner fails to take such action, the Department can take the action and recover the cost of the action from the owner (7 U.S.C. §7714(b)(2)). These remedial actions include removing the plants by burning, spraying herbicide, hoeing or discing. No infractions were identified in the case of the glufosinate-tolerant soybean.

Petitions for determination of non-regulated status. Every applicant must sign the following statement when submitting a petition for non-regulated status:

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition".

APHIS knows of no peer reviewed or anecdotal evidence that suggests that any plant that has been deregulated is a plant pest or has behaved in a manner significantly different with respect to its plant pest characteristics than a similar cultivar developed by traditional plant breeding. As explained above, APHIS has no authority to require monitoring *per se* after granting non-regulated status, however, if data becomes available that an organism granted non-regulated status does pose a plant pest risk, a deregulated organism could again be deemed a "regulated article" and could be subjected to the application of remedial measures (including disposal) if determined by the Administrator to be necessary to prevent the spread of plant pest (7 CFR 340.4 d 7).

EPA

FIFRA generally provides the authority to enforce all provisions regarding regulation of pesticides. EPA can take regulatory action to impose penalties or to restrict or prohibit the sale and distribution of any registered product, if necessary, to prevent unreasonable adverse effects on the environment, or necessary to prevent threatened violations of the FIFRA. This could include, for example, seizure of pesticide product or the assessment of civil and/or criminal penalties. FIFRA Sections 6, 8 and 9 provide statutory authority for the Agency to inspect the producing establishment, inspect books and records, and, although rarely needed, to cancel or suspend registration.

The EPA's Toxics and Pesticides Enforcement Division (TPED) within the Office of Enforcement and Compliance Assurance (OECA) is responsible for case development, policy and enforcement issues for the FIFRA. Although FIFRA Section 26 provides states with the primary enforcement responsibility for pesticide use violations, TPED enforces FIFRA violations other than use violations and proposes penalties for such violations. Congress, in FIFRA, describes the various unlawful acts that may be committed in connection with the sale and distribution of pesticide products. For example, it is unlawful for persons to sell or distribute an unregistered, misbranded, or adulterated pesticide, as well as a pesticide whose claims made for it substantially differ from claims made for it in connection with its registration under Section 3. Moreover, it is unlawful to use any pesticide in a manner inconsistent with its labeling. EPA's enforcement response may include the issuance of a civil administrative complaint, a stop sale use and removal order, or the imposition of criminal sanctions. When proposing a penalty the Agency must consider the violator's size of business, the effect the penalty will have on the violator's ability to continue in business, and the gravity of the violation.

EPA receives its legal authority to enforce FIFRA through the following sections of the Act:

FIFRA Section 6(a)(2) Adverse Effects Reporting

TPED sends referrals to the appropriate regional office for review and potential case development. TPED may develop and issue the case if the matter is nationally significant. There have been no adverse effects reported regarding glufosinate.

FIFRA Section 7 Registration of Pesticide Producer Establishments

The Section 7 database is managed by the Office of Compliance within OECA. The regional offices take the majority of the enforcement actions for Section 7 violations.

FIFRA Section 17 Import/Export Notification

TPED is presently involved in efforts to strengthen the enforcement program and provide guidance to the regional offices.

Worker Protection Standard

TPED sends referrals to the appropriate regional office for review and potential case development. TPED may develop and issue the case if the matter is nationally significant.

8. Public Involvement and Transparency

USDA

APHIS has involved and informed the public on a broad range of agency biotechnology activities through an array of mechanisms. The public has been involved in establishing the criteria for the regulatory and environmental assessment framework and subsequent amendments as the agency gained experience and adapted to the developments in the technology. The public has been informed through written regulations (the first government biotechnology regulations), guidance documents, and through both formal notice in the Federal Register and informal information systems such as home pages on the Internet. Stakeholders to the agency, such as the Agriculture Biotechnology Advisory Committee have played a significant role in providing a public source of advice to the agency.

When the APHIS biotechnology regulations (7 CFR 340) were first established in 1987, there were a number of public meetings involving a broad spectrum of interested individuals and groups to discuss the types of data necessary to make informed decisions for safe field testing of genetically engineered organisms. Those discussions included the scope, breadth, and specific environmental concerns that should be considered in environmental analysis under NEPA.

APHIS continues to hold public meetings as needed to inform and involved the public. Meetings have included topics such as program efficiency, timeliness of review, clarity of regulations and guidance documents, applicant satisfaction, paperwork reduction, and identification of scientific or environmental considerations for future reviews by APHIS. All APHIS-sponsored meetings, such as our regular customer service meetings, are announced on the internet and in the Federal Register and

are open to the public. No public meetings were held specifically for review of glufosinate tolerant soybeans. From time to time, APHIS also holds more focused public meetings on specific issues of scientific interest, such as the meeting in 1999 on the ecological effects of pest resistance genes in managed ecosystems. Comments at these meetings are considered in evaluating the need for regulation changes, changes in review procedures or criteria, and for the scope of consideration of environmental issues in NEPA documents.

The APHIS biotechnology home page, http:\\www.aphis.usda.gov\biotech, was one of the first government home pages to be established. It has been one of the primary sources of information globally on biotechnology regulation and a source of information on actual developments in the technology. The Internet has been used by APHIS as a mechanism to compliment and augment other more traditional information and transparency processes such as Federal Register notices, NEPA documents, and public meetings. The home page contains copies of the regulations; guidance documents; lists of notifications, permits, and determinations of non-regulated status; recent environmental assessments; and numerous links to other sources of information on biotechnology.

Interstate movement, importation, and field testing (introduction). Every permit and notification for the introduction of a genetically engineered organism is announced on the APHIS Internet home page (http://www.aphis.usda.gov/batik/status.html) the day after it had been received. The information listed includes: the name of organism, the State where the introduction will take place, and whether the proposed action has been authorized. Every application is sent to the State regulatory official where the introduction will take place and the State must concur with APHIS before any action can take place. The public can also comment on the permits and notifications either by contacting APHIS directly or by contacting the State official if the field test is in their state. Contacts for State Departments of Agriculture can be found on the APHIS website at (**http://www.aphis.usda.gov/biotech/lt_sta.html**). Additional information on each application is available by searching the APHIS on-line database (http://www.nbiap.vt.edu/cfdocs/fieldtests1.cfm), a service provided by Virginia Tech's Information Systems for Biotechnology (ISB) web server.

If an environmental assessment is prepared for a specific field test performed under permit (not done for notifications) in accordance with APHIS' NEPA implementation regulations (7 CFR 372), a Federal Register notice will announce a 30-day public comment period on the EA. Copies of the EAs and Finding of No Significant Impact (FONSI) will be distributed via mail or electronically.

Petitions for determination of non-regulated status. Every petition submission is announced on the APHIS Internet home page (http://www.aphis.usda.gov/batik/status.html) the day after it has been received. After petitions have been reviewed by APHIS scientists and have been deemed complete, USDA announces the receipt of the petition in the Federal Register and the public has 60 days to submit comments. All petitions are available for reading at the Reading Room at the South Building of the USDA Headquarters in Washington, DC and when requested, APHIS provides the public with free copies of all petitions. Subsequently, when a draft environmental assessment is

completed, APHIS announces in the Federal Register that the EA is available (electronically or a hard copy) and the public has 30 days to submit comments. APHIS considers all public comments in its decision-making. APHIS announces in the Federal Register when it has reached a FONSI for the EA that the engineered organisms do not meet the definition of regulated articles. The FONSI, analysis of public comments (if any), the EA, and the determination of non-regulated status are all available electronically at the APHIS home page or in hard copy. Copies of APHIS decision documents are available at APHIS web site "http://www.aphis.usda.gov/biotech/pubs.html".

As the biotechnology regulations have matured over the years, so have procedures implementing NEPA for decisions subject to those regulations. Initially, environmental assessments were completed before the decision on the issuance of every permit for release to the environment (field test) and notice of availability was published in the Federal Register for each one. After a few years, notice of availability for environmental assessments was published first monthly and then quarterly, as the number of requests for copies of individual environmental assessments decreased and as web-based information became the preferred mode for receiving that information.

In 1995 APHIS established NEPA implementing regulations in 7 CFR 372 that established criteria for the level of documentation for agency action including biotechnology decisions. The implementing regulations set the following environmental assessment triggers for biotechnology:

> "(b)(4) Approvals and issuance of permits for proposals involving genetically engineered or nonindigenous species, except for actions that are categorically exclude, as provided in paragraph (c) of this section (7 CFR 372.5)."

The relevant categorical exclusion reads as follows:

> "(c) (ii) Permitting, or acknowledgment of notifications for, confined field releases of genetically engineered organisms and products. . ."

except for

> "(d) (4) When a confined field release of a genetically engineered organism or product involves new species or organisms, or novel modifications that raise new issues."

As a matter of policy, APHIS also completes an environmental assessment before making a decision of non-regulated status in response to an applicants petition. Since 1999, notice of availability of draft environmental assessments for determinations for non-regulated status are published in the Federal Register for 30 days comment. Comments are considered before completion of findings of impact.

A fairly large volume of environmental assessments and technical decision documents are made available to the public. These are made available in paper copy or electronically at the preference of the recipient.

APHIS will complete an EIS when an EA does not support a finding of no significant impact. To date, environmental assessments to support biotechnology decisions have resulted in findings of no significant impact. EIS documents would also be available for public comment.

Notifications do not have environmental assessment prepared in accordance with APHIS' NEPA implementation regulations (7 CFR 372). The rationale is that these are not exposed to the environment due to the performance standards that ensure confinement Due to the changes in the regulations regarding notification in 1993 and 1997, species currently under notification may have had EAs prepared in the past, when the same species were required to apply for a permit that may have required an EA.

EPA

EPA publishes Federal Register notices announcing the receipt of applications for an Experimental Use Permit (EUP) where the EUP is of regional or national significance, and for registration of a new active ingredient or a new use pattern; and EPA invites public comment on the proposed action. In addition, Federal Register notices announcing approval of EUPs and registration of pesticides containing new active ingredients are also published. 40 CFR 172.11(c); 40 CFR 152.102. EPA also publishes Federal Register Notices announcing the notice of receipt of a request for a food tolerance or exemption, and provides opportunity for filing public comment. Within 60 days after a final rule granting a tolerance or exemption is issued, any person may file objections to the petition. The Federal Register Notice for the final rule announcing the food tolerances for glufosinate ammonium on soybeans can be found on the EPA web site at http://www.epa.gov/fedrgstr/EPA-PEST/1997/February/Day-05/p2838.htm. Although not required by statute, EPA also may hold meetings with groups and individuals interested in particular pending regulatory actions, either at its own initiative or at the request of others.

The EPA makes many individual decisions in its regulation of pesticides, including herbicides, e.g., in its registration, reregistration, and special review programs. The Office of Pesticide Programs (OPP) uses a variety of tools to guide these decisions and inform its many stakeholders. Various advisory committees have been established under the Federal Advisory Committee Act (FACA), including the Pesticide Program Dialogue Committee (PPDC). This Committee provides a forum for a diverse group of stakeholders to provide feedback to the Pesticide Program on various pesticide regulatory, policy and program implementation issues. Membership to the Committee includes environmental and public interest groups, pesticide manufacturers and trade associations, user and commodity groups, public health and academic institutions, Federal and State agencies, and the general

public. OPP develops regulations, policy documents, guidelines and analyses covering scientific, legal, and international matters. Proposed regulations are published for notice and comment in the Federal Register (FR) and are publicized on the Agency's web site as well as oftentimes in the press. OPP makes policy, guidance and other documents available through a variety of mechanisms as well, such as the Government Printing Office (GPO), direct mailings, and increasingly, through electronic dissemination. When final, regulations are incorporated in the Code of Federal Regulations (CFR) that is available to the public.

The agency also employs a Scientific Advisory Panel (SAP) that provides scientific advice on pesticides and pesticide related issues as to their impact on health and the environment. The role of the SAP has been expanded to that of a peer review body for current scientific issues that may influence the direction of OPP's regulatory decisions. Open meetings of the SAP are held on an average of six times per year. The agenda items for the meetings are chosen by OPP Division Directors at the beginning of the fiscal year, although emergency meetings may be called if the need arises, such as in the case of a proposed pesticide cancellation. Specific actions that OPP is required to present to the SAP include proposed and final regulations (which also require review by the Secretary of Agriculture), operating guidelines utilized by EPA personnel, and notices of intent to cancel or change a pesticide registration or classification undertaken under the procedures of FIFRA 6(b). The agency does not typically go to the SAP for routine registration decisions on new chemical uses.

Finally, EPA maintains a public docket that contains a large number of documents available for inspection and copying, including scientific reviews on safety issues and Reregistration Eligibility Decisions (REDs) on individual pesticides. Because glusofinate was registered relatively recently, it has not yet been subject to registration review.

No public comments were received in response to the Federal Register notices regarding the applications for an EUP or registration for the use of glufosinate on soybeans (which was also the notification for the tolerance). When the registrant petitioned the agency in request of tolerances for glufosinate-ammonium on soybeans, a docket number was cited in the Federal Register Notice, (PP-5F4578/R-2277) but no public comments were received. The Freedom of Information Act (FOIA) also provides for the request of documents submitted to support a pesticide registration as long as they do not contain confidential business information.

The Agency and OPP have also increasingly undertaken a variety of other communication and outreach efforts to ensure that the public has the information it needs to make responsible decisions about pesticides and to promote public health and environmental protection goals. To achieve this goal, OPP issues announcements and publications, provides information by telephone and electronic networks, responds to written and verbal inquiries, maintains a public docket, holds public meetings, and presents speeches and Congressional testimony.

REFERENCES

AgrEvo USA. 1996. Petition for Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (submitted to the United States Department of Agriculture, Petition Number 96-068-01p) and available from USDA-APHIS, Unit 147, 4700 River Road, Riverdale, MD 20737

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USDA (United States Department of Agriculture) 1996. Environmental Assessment and Determination of Non-Regulated Status - Petition Number 96-068-01p (glufosinate tolerant soybean). Available electronically at "http://www.aphis.usda.gov/biotech/pubs.html" or write to USDA-APHIS, Unit 147, 4700 River Road, Riverdale, MD 20737.

APPENDIX A

BIBLIOGRAPHY OF SUBMITTED STUDIES

Product: Liberty™ Herbicide

EPA Pesticide Petition Number: 5F4578

EPA File Symbol: 45639-ROO

Purpose of Submission: Data in Support of Application for Registration of Liberty™ Herbicide and Petition for Tolerance of Glufosinate-Ammonium on Corn and Soybean

Date of Submission: August 15, 1995

Volume Number	Study Title	Submitter Document Number	EPA Guidelines Reference Number	EPA Data Requirement	EPA MRID Number (Assigned by EPA on 9/6/95)
Reasonable Grounds in Support of the Petition and Safety Evaluation					
1 of 36	Use of Glufosinate-Ammonium on Glufosinate-Ammonium Resistant Corn and Soybean: Reasonable Grounds in Support of the Petition and Safety Evaluation	N/A	N/A	N/A	43778401
Product Chemistry Data Requirements					
2 of 36	Discussion of the Product Identity, Disclosure of Ingredients, Beginning Materials and Manufacturing Process, Formation of Impurities, and Certification of Ingredient Limits for Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54483	61-1, 61-2, 61-3 and 62-2	Chemical Identity, Begin. Mat. & Mfg. Proc; Disc. of Impurities, Cert. of Limits	43766901
3 of 36	The Validation of Analytical Method Used to Determine Glufosinate-Ammonium in Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54469	62-3	Analytical Method	43766902

4 of 36	The Determination of the Color of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54467	63-2	Color	43766903
5 of 36	The Determination of the Physical State of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54475	63-3	Physical State	43766904
6 of 36	The Determination of the Density of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54465	63-7	Density	43766905
7 of 36	The Determination of the pH of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54474	63-12	pH	43766906
8 of 36	The Determination of Oxidizing/Reducing Action of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54473	63-14	Oxidizing/Reducing Action	43766907
9 of 36	The Determination of the Flammability of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54471	63-15	Flammability	43766908
10 of 36	The Determination of the Explodability of Liberty ™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54464	63-16	Explodability	43766909
11 of 36	The Determination of the Viscosity of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54470	63-18	Viscosity	43766910
12 of 36	The Determination of the Miscibility of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18A5)	A54472	63-19	Miscibility	43766911
13 of 36	The Determination of the Corrosion Characteristics of Liberty™ Herbicide (Formulation Code: Hoe 039866 0H SL 18 A5)	A54466	63-20	Corrosion Characteristics	43766912
Toxicology Data Requirements *(EPA DP Bar Code D219070 Assigned 9/8/95)*					
14 of 36	Hoe 039866-^{14}C: Metabolism in Male and Female Rats Following Single Oral Administration of Test Substance at a Dose Level of 2 mg/kg Body Weight	A49981	85-1	General Metabolism	43766913

15 of 36	Hoe 039866-^{14}C, Glufosinate-Ammonium: Metabolism in Male and Female Rats Following Single Oral Administration of Test Substance at a Dose Level of 500 mg/kg Body Weight	A54334	85-1	General Metabolism	43766914
16 of 36	Hoe 039866-^{14}C: Sampling of Blood, Excrements, Organs and Tissues for Metabolism Studies in Male and Female Rats Following Single Oral Administration of Approximately 500 mg/kg Body Weight	A54450	85-1	General Metabolism	43778402
Environmental Fate Date Requirements *(EPA DP Bar Code D219073 Assigned 9/8/95)*					
17 of 36	Terrestrial Field Dissipation of Ignite® Herbicide Applied to Transgenic Corn	A54505	164-1	Terrestrial Field Dissipation	43766915
18 of 36	Terrestrial Field Dissipation of Ignite® Herbicide Applied to Transgenic Soybean	A54506	164-1	Terrestrial Field Dissipation	43766916
Residue Chemistry Data Requirements *(EPA DP Bar Code D219069 Assigned 9/8/95)*					
19 of 36	Uptake Of ^{14}C- Glufosinate-Ammonium Residues in Soil by Rotational Crops Under Confined Conditions	A54272	165-1	Confined Rotational Crop	43766917
20 of 36	Metabolism of [^{14}C]-Glufosinate in a Lactating Goat	A54158	171-4(b)	Nature of Residue - Livestock	43766918
21 of 36	Metabolism of [^{14}C]-Glufosinate in Laying Hens	A54159	171-4(b)	Nature of Residue - Livestock	43766919
22 of 36	Testing of Hoe-099730 Through FDA Multiresidue Protocols A Through G	A54502	171-4©	Residue Analytical Method - Plant	43766920
23 of 36	Magnitude of Glufosinate-Ammonium Residues in the Tissues and Milk of Dairy Cows Dosed with Glufosinate-Ammonium and Hoe-099730 At 1,3 and 10 Times the Estimated Maximum Daily Intake, for 28 Consecutive Days, USA, 1994	A54503	171-4(j)	Magnitude of the Residue - Meat, Milk and Eggs	43766921

24 of 36	Magnitude of Glufosinate-Ammonium Residues in the Tissues And Eggs of Chickens Dosed With Glufosinate-Ammonium and Hoe-099730 At 1,3 And 10 Times the Estimated Maximum Daily Intake, for 28 Consecutive Days, USA, 1994	A54485	171-4(j)	Magnitude of the Residue - Meat, Milk and Eggs	43766922
25 of 36	Magnitude of the Residue Of Glufosinate-Ammonium in or on Transgenic Field Corn Following Two Applications of Ignite® Herbicide	A54160	171-4(k)	Magnitude of the Residue - Crop Field Trials	43766923
26 of 36	Magnitude of the Residue of Glufosinate-Ammonium in or on Transgenic Soybeans Following Two Applications of Ignite® Herbicide	A54156	171-4(k)	Magnitude of the Residue - Crop Field Trials	43766924
27 of 36	Magnitude of Glufosinate-Ammonium Residues in or on Soybean Hay and Seed Resulting from Application of Ignite® Once at Third Node or Twice At Three Growth Stage Combinations, USA, 1994	A54108	171-4(k)	Magnitude of the Residue - Crop Field Trials	43766925
28 of 36	Magnitude of the Residue of Glufosinate-Ammonium in or on Transgenic Field Corn Processed Commodities Following Two Applications of Ignite® Herbicide	A54284	171-4(l)	Magnitude of the Residue - Processed Food	43766926
29 of 36	Magnitude of the Residue of Glufosinate-Ammonium in or on Transgenic Soybean Processed Commodities Following Two Applications of Ignite® Herbicide	A54283	171-4(l)	Magnitude of the Residue - Processed Food	43766927
PAT Protein Safety Studies					
30 of 36	L-Phosphinothricin N-Acetyltransferase Biochemical Characterization	A50188	N/A	N/A	43766928
31 of 36	L -Phosphinothricin N-Acetyltransferase Inactivation by Pig and Cattle Gastric Juice	A51230	N/A	N/A	43766929

32 of 36	Fate Of Introduced DNA in Gut: Degradation Of Phosphinothricin Acetyl Transferase Gene from Transgenic Rape HCN 92 (*Brassica Napus*) in Stomach Fluids From Pig, Chicken and Cow	A51613	N/A	N/A	43766930
33 of 36	Expression of the Phosphinothricin Acetyltransferase in Glufosinate Resistant T14 and T25 corn	A53356	N/A	N/A	43766931
34 of 36	Comparison of the Phosphinothricin Acetyltransferase Enzyme Expressed in *Escherichia coli*, Corn (T14 and T25) and Canola (HCN-92)	A53391	N/A	N/A	43766932
35 of 36	Digestion of the Phosphinothricin Acetyltransferase Enzyme in Human Gastric Fluid (Simulated)	A53425	N/A	N/A	43778403
36 of 36	Comparison of the Synthetic PAT Gene and the PAT Protein with Other Known Nuclotide and Protein Sequences	A53504	N/A	N/A	43766933

APPENDIX B

BIBLIOGRAPHY OF SUBMITTED STUDIES

Products: Liberty™ Herbicide

Temporary Tolerance Petition No: 5G4466

EPA EUP Number: 45639-EUP-56

Purpose of Submission: Application for Experimental Use Permit For Liberty™ Herbicide and Petition For Temporary Tolerance of Glufosinate-Ammonium on Corn and Soybean

Date of Submission: January 18, 1995

1 of 12	Use Of Glufosinate-Ammonium on Glufosinate-Ammonium Resistant Crops:Toxicology Overview and Risk Assessment	N/A	N/A	N/A	43515601
2 of 12	^{14}C-Glufosinate-Ammonium: Nature of the Residue in Field Corn	Pan-Ag Study Number 93260; Sponsor Project Number 93-0025	171-4(a)	Nature of the Residue - Plants	43515602
3 of 12	Metabolism of [^{14}C]-Glufosinate Ammonium in Soybeans,Treated Under Normal Field Conditions	500BK Report A53607	171-4(a)	Nature of the Residue - Plants	43515603
4 of 12	Method Validation - Determination of Residue Levels of Glufosinate-Ammonium and Metabolites in Various Field Corn and Soybean Matrices	Xenos Number XEN 93-19A Sponsor Project Number 93-027	171-4(c)	Residue Analytical Method - Plant	43515604
5 of 12	Independent Laboratory Confirmation of the Analytical Method AE-24 (Draft) - Revision 4A for Glufosinate-Ammonium Residues in or on Crops, USA, 1994	BK-94R-05	171-4(c)	Residue Analytical Method - Plant & PRN # 88-5	43524601

6 of 12	Determination of Possible Analytical Interference From Other Pesticides During the Analysis of Crops for Residues of Glufosinate-Ammonium	Pan-Ag Study Number 94428 Sponsor Project Number BK-94R-07	171-4(c)	Residue Analytical Method - Plant	43515605
7 of 12	**Part 1 of 3:** Magnitude of the Residue of Ignite Herbicide in Transgenetic Field Corn Following Application of Ignite Herbicide	Pan-Ag Study Number 93224 Sponsor Project No. HRAVC 93-0006	171-4(k)	Magnitude of the Residue - Cropfield Trials	43515606
8 of 12	**Part 2 of 3:** Magnitude of the Residue of Ignite Herbicide in Transgenetic Field Corn Following Application of Ignite Herbicide	Pan-Ag Study Number 93224 Sponsor Project No. HRAVC 93-0006	171-4(k)	Magnitude of the Residue - Cropfield Trials	
9 of 12	**Part 3 of 3:** Magnitude of the Residue of Ignite Herbicide in Transgenetic Field Corn Following Application of Ignite Herbicide	Pan-Ag Study Number 93224 Sponsor Project No. HRAVC 93-0006	171-4(k)	Magnitude of the Residue - Cropfield Trials	
10 of 12	Magnitude of the Residue in Transgenetic Soybeans Following Application of Ignite Herbicide	Pan-Ag Study Number 93225 Sponsor Project No. HRAVC 93-0007	171-4(k)	Magnitude of the Residue - Cropfield Trials	43515607
11 of 12	Magnitude of the Residue of Ignite Herbicide in Transgenetic Field Corn RAC and Corresponding Processed Commodities Following Application of Ignite Herbicide	Pan-Ag Study Number 93230 Sponsor Project No. HRAVC 93-0006	171-4(l)	Magnitude of the Residue - Processed Commodities	43515608

12 of 12	Magnitude of the Residue in Transgenetic Soybean Processed Commodities Following Application of Ignite Herbicide	Pan-Ag Study Number 93231 Sponsor Project No. HRAVC 93-0007	171-4(l)	Magnitude of the Residue - Processed Commodities	43515609

APPENDIX C

1. Phenotypic expression

Phenotypic expression of the transgenic plant relative to its nearest nontransgenic counterpart and/or to a range of cultivated types**.** Observed changes may warrant further in-depth studies. Applicants may provide valid scientific rationale to demonstrate that certain information requirements are unnecessary or impossible to provide.

1.1. How does the transgenic plant compare to its non-transgenic counterpart with respect to the following reproductive and survival biological characteristics?

a. Growth habit - changes in basic morphology
b. Life-span - annual, biennial, perennial
c. Vegetative biomass / vigor
d. Overwintering capacity
e. Flowering period / Days to first flowering
f. Days to maturity
g. Seed production - number of seeds produced per plant and a description of the various environmental conditions, to evaluate number of seeds produced in favorable and in variable environments.
h. Continuous seed production -Length of time (days) of seed production
i. Seed dormancy
j. Seedling emergence -proportion of seeds planted that emerge as seedlings under field conditions and a description of the various environmental conditions, to evaluate emergence in more variable environments, especially those outside the managed ecosystems
k. Seedling survival to reproduction
l. Outcross frequency within species (e.g. 0-1, 2-20, 21-100%)
m. Cross pollination vectors -change in pollinator species
n. Pollen viability - proportion viable and length of survival
o. Fertility or infertility - male or female
p. Self-compatibility or -incompatibility
q. Asexual reproduction, i.e. vegetative reproduction
r. Dispersal ability, i.e., seed shattering, digestibility, or palatability to birds or mammals

1.2. How does the transgenic plant compare to its counterpart with respect to the following stress adaptations (specifically note which stresses were observed)?

a. Biotic stress factors: includes pathogens, competitors, symbionts, and herbivores
b. Abiotic stress factors: includes atmosphere (i.e., ozone, NOx), soil nutrients,

temperature, and moisture
c. Pesticides

1.3. Does the transgenic plant differ in nutritional composition from its nontransgenic counterparts (e.g., protein, lipids, etc.)?

1.4. Does the transgenic plant differ from its counterparts in levels of known naturally expressed toxicants?

2. Potential nontarget effects

2.1. Is the introduced gene product a novel part of the diet of humans, animals, or insects?

2.2. Does the introduced DNA directly or indirectly lead to the expression of a toxin or other product that is known to affect metabolism, growth, development, or reproduction of animals, plants, or microbes?

2.3. Is there a potential effect (toxic or nontoxic) to organisms that may be associated with the crop, including insect, avian, aquatic, or mammalian species, and organisms that are beneficial (pollinators, predators, parasites, biological control organisms, soil microbes), from both endogenous [naturally expressed] or non-endogenous [transgenic] compounds? APHIS considers routes of exposure to all plant parts that express the gene, i.e., direct feeding or other exposure to the plant or plant part, dispersed plant parts, or organisms that have fed on the plant.

2.3.1. In what parts of the plant is the gene product expressed and at what levels?

2.3.2. Has typical pollinator and other insect activity (i.e. feeding) been observed on the transgenic plant?

2.4. Is there potential for adverse human health effects, e.g., exposure to toxins, irritants, and allergens? APHIS considers estimated level and most likely route of human exposure to the gene products, breakdown products and by-product.

2.5. Does the transgenic plant differ from the nontransgenic plant in residual effects on soil microflora and microfauna?

2.6. Will the introduced trait directly or indirectly result in altered physiological or behavioral characteristics of animals (e.g., pheromones, hormones, or attractants; altered seed morphology; altered growth habit)?

3. Growing the Transgenic Plant - Interactions of the transgenic plant in the environment (Agricultural ecosystems)

3.1 Description of the growing area

3.1.1. Is the transgenic plant intended to be grown in all of the U.S.? If in a specific region of the country, please provide.

3.1.2. What is the projected total area being grown?

3.1.3. Will the transgenic plant be grown outside of the normal geographic areas for the species?

3.1.3.1 If yes, identify and describe the new geographical area(s) in which the transgenic plant can be grown.

3.1.4. Will the transgenic plant be grown outside of the usual managed ecosystems for the species?

3.1.4.1 If yes, identify and describe the new ecosystems in which the transgenic plant can be grown.

3.1.4.2 Will the introduced trait allow the plant to be grown or survive in a new habitat where it could impact nontarget organisms including populations of plants with which it can interbreed?

3.2. Description of cultural practices

3.2.1. Will the cultural practices (land preparation, fertilizer usage, weed and pest control, harvest, post-harvest protocols, etc.) involved in growing the transgenic plant vary from those traditionally used?

3.2.1.1 If yes, describe the change in cultural practices. Provide information showing the effect of these changes on sustainability, pesticide use, frequency of tillage, soil erosion and consequential changes in energy and soil conservation.

3.2.2. Will volunteer plants of the transgenic plant necessitate altered cultural practices for succeeding crops?

3.2.2.1 If yes, describe alternative practices to control volunteers?

3.2.3. Are any specific deployment strategies recommended for this transgenic plant?

3.2.3.1 Insect Resistance Management - Has an insect resistance management (IRM) strategy been

submitted to EPA or is this product under an existing IRM with EPA?

3.2.3.2 Herbicide Resistance Management - Describe any strategies that will be needed to delay the development of resistant weeds.

3.3. If it is anticipated that the transgenic plant will be grown only under contract/controlled conditions (e.g. Pharmaceuticals, biologics), describe:

- any control and mitigation procedures;
- post-harvest procedures, including procedures for disposal of remaining plant matter.

4. Introgression - Potential Environmental Effects Resulting from Introgression

4.1. Will the crop be grown in proximity to species with which it can interbreed?

4.2. Does the introduced trait increase the likelihood of introgression between the crop and species with which it can interbreed?

4.3. Where there is potential for gene flow from the transgenic plant into related species, detail the consequences of novel gene introgression into those species and resulting expression. Interactions identified for the transgenic plant should be considered, as appropriate, for these species.

4.3.2. Is the compatible wild relative considered a weed and/or is it invasive?

4.3.3. Does the introduced trait increase reproductive fitness or confer a selective advantage on the wild relative?

4.3.3.1 Is the potential for the trait to increase reproductive fitness or confer a selective advantage different than the potential for this to occur from a similar trait, if there is one, in a traditionally bred line of the same crop?

4.3.3.2 Is the introduced trait similar to a trait found currently in natural populations of the compatible wild relatives?

4.3.4. Does the introduced trait have a significant impact on the establishment and spread of populations of wild relatives?

SIDEBAR No. III.A

PHARMACEUTICAL-PRODUCING PLANT

Overview

This sidebar examines the proposed use of genetically engineered plants and plant viruses to produce protein biologics for use in human or animal therapy, referred to in the sidebar as "human biologics" and "veterinary biologics," respectively. Human biologics are regulated by the Food and Drug Administration (FDA), while veterinary biologics are regulated by the Center for Veterinary Biologics (CVB) of the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA). The plants that are engineered to produce the biologic, or infected with a virus engineered to produce the biologic, are regulated by Plant Protection and Quarantine Staff (PPQ) of APHIS. If they produce a human biologic, they are also regulated in part by FDA as part of its oversight of production of the biologic. FDA is responsible for ensuring that the plant is grown and maintained in a manner that will enable consistent production of a safe, pure, and potent biologic. If plants are engineered to produce a veterinary biologic, the plants are likewise also regulated in part by APHIS CVB as part of its oversight of production of the veterinary biologic.

The principal example used in the sidebar is that of a tobacco mosaic tobamovirus (TMV) engineered to cause tobacco plants to produce thrombopoetin, a hematologic growth factor that stimulates the production of platelets by bone marrow. The thrombopoetin would be extracted from the tobacco plants and purified for use in treating human cancer patients who have received chemotherapy. The sidebar also notes some issues posed by food crops engineered to produce pharmaceuticals or other non-food material, such as the need to ensure that such products do not inadvertently enter the food supply. Because no products from pharmaceutical-producing plant systems have completed the federal regulatory process, the sidebar cannot be as detailed or definitive as it otherwise might be.

1. Description of proposed organism/product and its use

TMV is a small RNA virus (that is, its genome is ribonucleic acid (RNA), rather than DNA). It causes a severe disease in tobacco world-wide. TMV occurs naturally only in solanaceous plants (Gibbs 1986). Tobamoviruses, (the class of viruses to which TMV belongs) reach high quantities in infected plants. They are easily transmitted by mechanical inoculation, but not by insects or other common agents. Most tobamoviruses are not transmitted via the embryo (true seed transmission), but the virus often contaminates the external mucilage, testa, and sometimes the endosperm of tobacco plants. Surface virus particles can infect tobacco seedlings during transplanting, but not if the seeds are undisturbed (Broadbent 1965). Tobamoviruses cause a wide variety of symptoms from mild yellowing to necrosis depending on virus strain and the host plant. Tobamoviruses are controlled by a number of

methods, including the use of resistant or tolerant cultivars, elimination of sources of inoculum such as weeds and infected debris, decontamination of infested equipment, and the use of mild strains of the virus to cross protect against virulent strains of the virus.

TMV RNA codes for at least four proteins, all of which are required for efficient viral multiplication. To engineer the virus to produce thrombopoetin, scientists modified a strain of TMV known as U1. They replaced most of the U1 coat protein (except for its promoter) with the coat protein from the most distantly related tobamovirus. The thrombopoetin gene was inserted next to the remaining U1 coat protein promoter. (see appendix A for details). One of the advantages of this construction is that the fusion gene is not stably maintained by the virus. Experiments have shown that after passing through four or five generations of tobacco plants, the replicating virus will no longer contain the thrombopoetin gene, thereby minimizing any long-term environmental risk that persistence of such engineered viruses might pose.

The manufacturing schema includes mechanically inoculating tobacco plants with the engineered TMV in ten-acre fields. After viral infection, thrombopoetin is produced in the infected plant cells. It generally is harvested from the intracellular spaces in the leaves about a month after inoculation, prior to flowering. Pharmaceutical-producing tobacco plants are routinely deflowered to allow vegetative growth to continue, and to put more of the plant's energy into leaf (and therefore pharmaceutical) production. The tobacco is to be grown and harvested by contract farmers and the processing of the plant material will ultimately be covered by an approved Biologics License Application. Any unharvested tobacco plants, and the solid material remaining after extraction of the harvested plants, will be plowed into the field on which the plants were grown. Other wastes will be sent to the local wastewater treatment facility after deactivation with chlorine bleach, in accordance with 9 CFR 114.15.

The process of infecting the tobacco plants and growing the infected plants is under the jurisdiction of FDA from the point of view of ensuring the safety, purity and potency of the biological product to be licensed, and under the jurisdiction of USDA from the point of view of controlling any plant pest risks posed by the virus and infected plants.

Pharmaceutical Producing Plants

While this sidebar addresses a TMV in a plant, using plants themselves to produce pharmaceuticals is increasingly of interest, and confined field trials of such plants are currently ongoing. Researchers and companies are interested in using crop plants to produce pharmaceuticals for a number of reasons. The need for very large quantities of biologics, projected to be 500 to 1000 kilograms per year for some human biologics, is growing rapidly. Production costs may be lower than with traditional fermentation technology, both because of reduced energy costs and reduced cost of raw materials. The energy-expensive process of cleaning and sterilization of large fermentors is not necessary and the need for large volumes of purified culture medium is eliminated. In addition, the use of crop plants removes the potential for contamination of the biologic with animal viruses that potentially can be pathogenic to humans. An inherent risk with biologics produced in animals or animal cells is that the animals or animal cells will become infected with a pathogenic virus that may then contaminate the product. This risk is avoided by producing the biologic in plants, because there are no known plant viruses

Because TMV is classified as a regulated article under 7 CFR 340.2, anyone wishing to import it, transport it across state lines, or release it into the environment must apply for and be issued a permit from APHIS prior to engaging in these activities. The permitting process provides federal regulatory oversight by APHIS over not only the release of the agent into the field, but also the disposal of potentially contaminated waste material. All of the controls outlined in the analogous APHIS section of the glufosinate-ammonium (GA) tolerant soybean case study apply to this product, too. It is noteworthy, however, that APHIS' regulations (7 CFR 340.3(b)4(iii)) clearly state that plants that encode products intended for pharmaceutical use do not qualify for simple notification under 7 CFR 340.3 and therefore are required to apply to APHIS for a permit for interstate transport and field testing.

Thus, before initiating field trials of the TMV in tobacco plants, a sponsor would need to go through the APHIS permitting process described in the accompanying glufosinate-ammonium tolerant soybean case study. Similarly, before initiating field trials of a food crop, such as corn, that is itself engineered to produce a pharmaceutical or other non-food material, a sponsor would need to go through the same APHIS permitting process.

To clarify what pharmaceutical means, APHIS has provided the following guidance. If commercialization of the pharmaceutical produced in plants will require approval from FDA's CBER (human biologic), CDER (human drug), CVM (animal drug), or USDA's CVB (animal biologic), then the engineered plant is intended for pharmaceutical intent. The term "commercialization" with plant-derived biologic means that the biologic is approved by FDA or CVB for its intended use. It does not mean that the plants or plant viruses could be grown in the U.S. without APHIS authorization. APHIS cannot currently envision plant-derived biologics that would be granted a determination of nonregulated status, nor could they qualify for release under notification procedures (7 CFR 340.3). APHIS believes that the plant-derived biologics will always be grown under APHIS permit and concurrently regulated by either FDA or CVB.

FDA

The production of bioengineered plant-derived biologics or drugs, intended for diagnostic, preventive, or therapeutic use in humans is regulated by the FDA under authority of the Public Health Service Act (PHS Act), 42 U.S.C. §§ 201 et seq. and the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. §§ 301 et seq.

Individuals or organizations wishing to perform clinical testing of a biologic product in humans must submit an Investigational New Drug application (IND) to the Center for Biologics Evaluation and Research (CBER) of the FDA for prior review. If, after completing clinical trials to demonstrate that the biologic is safe and effective, the sponsor wishes to market the biologic for human therapy, the sponsor must submit a Biologics License Application (BLA) to CBER for review and approval.

FDA oversight of the thrombopoetin, and of the engineered TMV and TMV-infected tobacco plants (and similarly, FDA oversight of a human biologic derived from a bioengineered plant), would begin at the time the sponsor submitted an Investigational New Drug (IND) application. This typically would occur near the time the sponsor was ready to begin clinical trials with the plant-derived biologic. Under the PHS Act and FFDCA, FDA regulatory authority encompasses environmental issues that pertain to human health.

In addition, under the National Environmental Policy Act of 1969 (NEPA), 42 U.S.C. §§ 4321 *et. seq.*, FDA would be responsible for identifying and evaluating any potential environmental impacts related to FDA's action on the IND or biologics license application. (The Center for Veterinary Biologics of APHIS has the same responsibility under NEPA when reviewing a license application for a veterinary biologic such as an animal vaccine.) The FDA will review the environmental assessment or the request for a categorical exclusion submitted in the IND and BLA in accordance with the Council on Environmental Quality (CEQ) regulations implementing NEPA (40 CFR Parts 1500-1508) and FDA's regulations (21 CFR Part 25) that implement CEQ's regulations.

Although INDs are ordinarily categorically excluded, given the nature of the environmental issues potentially posed by pharmaceutical-producing bioengineered expression systems, it is likely FDA would consider that such products represent an "extraordinary circumstance" and would therefore not receive a categorical exclusion. If, after FDA reviews the environmental assessment submitted to it, FDA determines that an environmental impact statement is necessary, this will be prepared in accordance with the CEQ and FDA requirements implementing NEPA. It is envisioned that should an environmental impact statement be needed, it would likely be jointly prepared by FDA and APHIS, and potentially by other interested or affected agencies as well.

Because such pharmaceutical-producing plants or plant-virus systems will always be grown under APHIS permit, and because permits enabling field trials will always be obtained prior to submission of an IND to FDA for human clinical trials of the plant-derived biologic, APHIS will have the responsibility to identify and evaluate the environmental effects potentially posed by such plants and plant-virus systems, and FDA's NEPA analysis will take into account APHIS' environmental reviews.

APHIS PPQ provides confidential copies of all importation, interstate movement, and field testing permits for plant-derived biologics to FDA or APHIS CVB. Also, since 1999 FDA and USDA have been cooperating on preparing a document entitled, "Guidance for Industry: Draft Guidance on Plant-Derived Biologics for Use in Human and Animals". As part of the information-gathering for preparing this guidance document, the Agencies held a joint scientific meeting and public hearing in April 2000 in Ames, Iowa (transcripts of the meeting and public hearing are available at: http://www.fda.gov/cber/minutes/plnt1040500.pdf and http://www.fda.gov/cber/minutes/plnt2040600.pdf.)

3. Hazard identification and environmental evaluation

In order to evaluate whether there are any significant impacts to the environment posed by the recombinant TMV, APHIS requires that various laboratory and field studies be conducted, and the results submitted in the APHIS permit application. These studies include evaluations of the ability of the virus to spread from infected plants to other plants in the vicinity. Spread of the virus is tested in plants in direct contact and plants at a distance.

APHIS is aware of no evidence that plant viral genes can be transferred to the genomes of microorganisms, plants, or animals (Amabile-Cuevas and Chicurel 1993). However, genes can be transferred from TMV to other closely related tobamoviruses, e.g. tomato mosaic tobamoviruses (ToMV), via recombination. Fortunately, ToMV (the closest related plant virus) is unlikely to be present in tobacco fields (Gooding 1986). Additionally, those viruses likely to infect tobacco plants via insect vectors such as aphids (e.g., potyviruses) are unlikely to recombine with TMV (Falk and Bruening 1994). To further minimize the chances of viral gene transfer, and also because infection of the experimental plants with other plant viruses would jeopardize data collection, APHIS requires applicants to make every effort to exclude other plant viruses from infecting the experimental plants.

Any unanticipated effects on non-target organisms must be reported to APHIS within five working days. However, the potential for adverse effects of the TMV-based pharmaceutical production system on non-target beneficial or threatened and endangered species is believed to be minimal. Probably because of the production of nicotine, few organisms feed on tobacco plants. The only organisms that are routinely associated with tobacco plants are its pests, tobacco budworm, tobacco hornworm, and tobacco aphid. There are no birds or mammals that eat tobacco, so no effects on these species are expected. Similarly, earthworm populations decline in tobacco fields, probably because of nicotine production by tobacco plants. In addition, because these plants generally will be deflowered or harvested before flowering occurs, bees and any other potential pollinators generally will not visit these plants, and therefore, generally will not be affected.

Generally speaking, the potential risk to threatened or endangered species will be evaluated on a case-by-case basis, taking into account the pharmaceutical product, the host-plant and expression system, the location and size of the field, the time period of growth, harvest, and clean-up, and the potentially effected species. Such information is generally included in the environmental assessment component of the sponsor's permit application to USDA and will be reviewed by APHIS. APHIS does not anticipate a risk from the TMV- tobacco plant system to any threatened or endangered species, because it has not identified a direct or indirect effect of the field release of the engineered TMV on any wild plant or animal species.

For expression systems that utilize germ-line transformed plants for production of biologic

products, pollen and seed-production are of greater concern than in the TMV system. For these expression systems, the sponsor must comply with APHIS permit requirements to avoid pollination of nearby agricultural crops or wild relatives. This may include de-flowering, the use of sterile male plants, or other measures. In situations where the biologic-producing plant (or a plant engineered to produce other non-food-use products, like plastics or industrial enzymes) is from a species that is also used for food or feed (for example, corn or other cereal grains), APHIS and FDA are considering what mechanisms will be needed to ensure that the pharmaceutical-producing plant, and grain or other products of the plant, are kept completely segregated from food/feed-use varieties of the crop (both in the field and when harvested and distributed for processing), and that such segregation is effectively monitored This is a new area, and may require new legislation or new regulations.

4. Information and data

APHIS

APHIS requires data and information on the host plant, the genes that have been introduced into the plant, and the interaction of the engineered plant and the environment. This process is described in greater detail in the Herbicide-tolerant Soybean case study (No. III).

FDA

To satisfy the requirements of the FFDCA and PHS Act, sponsors will have to provide to FDA data and information about the plant or plant-virus system necessary to demonstrate that the biologic produced by the plant system will consistently be safe, pure and potent.

In addition, FDA generally requires an applicant to prepare an environmental assessment to enable the agency to determine whether the system will have a significant impact on the environment. (see 21 CFR 25.40 (b)). The FDA is ultimately responsible for the scope and content of environmental assessments and may supplement the information provided by the applicant in environmental documents when warranted. The reliability of sponsor-generated data is assessed during review of the licensing application and upon inspection of the manufacturing site. As noted in section 2, FDA intends to base its environmental assessment upon the environmental assessment made by APHIS as part of its evaluation of permit applications for such plant systems, and thus FDA would likely request a sponsor to submit a copy of the APHIS permit and the environmental documentation for an environmental assessment.

5. Mitigation and management

Under the APHIS permitting process, the distribution of the recombinant virus can be controlled by the sponsor in a number of ways. The following is a list of mechanisms that have been used in field

tests of TMV-infected tobacco plants. The permittee is responsible to ensure that these conditions are complied with:

1) No plants in the Solanaceae family are grown near the field site. The closest commercial tobacco production site would likely be used to grow TMV-resistant cultivars because they are readily available and effectively control this disease. A strip of fallow ground would be maintained around the field of tobacco that is to be infected with the TMV and the Solanaceous weeds that are hosts for TMV (horsenettle, black nightshade and ground cherry) would be controlled on site by either herbicide application or roguing.
2) A non-host species (e.g., corn) is grown in the arable land adjacent to this strip of fallow ground to act as a barrier to the spread of the virus to other fields.
3) Inoculation of the tobacco plants with the virus is performed by hand-held spray applicators to control the distribution of the virus.
4) Plants are de-flowered to eliminate seeds that would produce 'volunteer' plants that could act as hosts for the virus in the following year.
5) Harvest of the tobacco is performed with a crosscut mower and the plant material is collected in covered containers for transport to the purification facility.
6) All farm implements that come in contact with infected plants will be washed thoroughly with soap and then will be cleaned with 1% sodium hypochlorite solution (Gooding 1986).
7) Because TMV persists in soil only when infected tissue is present (Gooding 1986), the field site will be redisked at least twice after final harvest to facilitate natural decay of plant material. Additionally, any solid waste plant material resulting from the extraction and purification process is also plowed into the field. The following year a non-host species is grown in the field to allow additional time for remaining TMV to biodegrade. This crop of non-host species is not harvested for use as food or feed, but is again plowed into the field. In the next year, the field again may be used for any purpose.
8) Potentially infectious liquid waste from the purification process is inactivated in accordance with USDA regulation (9 CFR 114.15) and sent to the local waste water treatment facility.
9) Evidence submitted by the applicant and other published data (reviewed by Mushegian and Shepherd 1995) show that the engineered virus either reverts to wildtype virus or is not competitive in mixed infections with wildtype TMV.
10) The gene inserted into the virus has never been shown to be involved in plant pathogenicity and its expression within the plant would not broaden the host range of the TMV.
11) If the engineered virus did escape and infect another susceptible plant, the engineered virus would be at a competitive disadvantage to endemic tobamovirus of that host.
12) Although TMV is a problem in tobacco growing regions in US, it is not routinely a problem in tomato fields. ToMV out competes TMV in mixed infection in tomatoes. Therefore, TMV is eliminated during mixed infections.

These containment conditions have been used in previous tests of engineered TMV and have

proved adequate to contain the virus.

6. Monitoring

Under permits, APHIS will require monitoring programs to test for residual virus in surrounding fields on which recombinant TMV has been previously used. The results from monitoring activities can be reviewed by APHIS during site inspections and as part of the review of applications for permit renewal. If the sponsor were found to be out of compliance, their permit may be cancelled or not renewed and it could trigger an enforcement action. If monitoring led to the detection of adverse effects, APHIS would review them to determine what appropriate mitigating actions might be required.

7. Enforcement

The penalties imposed by the USDA as outlined in the Herbicide-tolerant Soybean case study (No. III) would apply to this sidebar as well. FDA has authority to take actions to enforce requirements instituted to ensure the safety, purity, or potency of a human biologic produced from a bioengineered plant. FDA also has authority to take actions against adulterated and misbranded foods, such as would likely be the case if a food product contained a pharmaceutical (e.g., if corn from a corn plant engineered to produce a pharmaceutical inadvertently entered the food supply).

8. Public involvement

APHIS permits for interstate transport and field studies can be viewed on the USDA/APHIS website as described in the soybean case study.

Public involvement in FDA actions will be in accordance with 21 CFR Part 25, subpart E. Prior to approval of a biologics license application for a plant-derived biologic, FDA would consider convening an advisory committee of outside experts to review the environmental concerns and provide additional guidance as necessary. Advisory committee meetings are generally open to the public, although on occasion they may have closed segments to deal with confidential commercial information. At this time, the existence of an IND is considered confidential and therefore information in an IND, including NEPA documentation, would not be releasable to the public. NEPA information and other non-confidential safety-related information would be releasable once a BLA had been licensed or denied.

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Appendix 1: Engineering of Tobacco mosaic virus. Dotted lines represent ribonuleotides.

1. Genome of TMV. The ?up? represents the promoter sequence for the coat protein from the TMV U1 strain.

5' end----RNA polymerase/helicase---movement protein----^{UP}-coat protein--3' end

2. Exchange of coat protein from the common strain for coat protein and its promoter from the orchid strain (labeled ?op? and highlighted in bold). Notice the promoter for U1 strain is still present.

5' end----RNA polymerase/helicase---movement protein----^{up}-$^{\mathbf{OP}}$-coat protein--3' end

3. The gene sequence of thromopoetin is inserted in the genome between the two promoters. The orchid strain was selected because the sequence of its coat protein promoter is the most divergent (different) from the tobacco strain while still being able to encapsidate TMV viral RNA. When the promoters have high degree of homology, the inserted gene is rapidly deleted via homologous recombination between the identical promoter sequences. With the sequence difference between orchid and tobacco coat protein promoters, the engineered virus loses the insert protein at a lower frequency than when they have identical sequences.

5' end---RNA polymerase/helicase---movement protein----^{up}*thromopeotin*-$^{\mathbf{OP}}$-coat protein--3' end

4. Eventually the thromopeotin gene is deleted. The resulting virus is very similar to the initial virus described above in number 1 expect the coat protein is now from the orchid strain not tobacco strain that has identical biological properties.

5' end---RNA polymerase/helicase---movement protein----^{up}-coat protein--3' end