

CASE STUDY No. II

Bt-MAIZE

Overview

This case study examines maize (corn) that was genetically engineered to produce a protein that is toxic to certain insects. A gene from the bacterium *Bacillus thuringiensis* (Bt), which produces the toxin, was modified and added to the corn. Promoters (genetic material that initiates transcription of the gene) and terminators (genetic material which stops transcription of the gene) were also added, from a virus and another bacterium, respectively, which are known plant pathogens. The Bt-maize considered in this case study is referred to as MON810.

MON810 was subject to regulation primarily by Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), 7 U.S.C. §§ 136-136y, Federal Food, Drug and Cosmetic Act, (FFDCA), 21 U.S.C. §§371-379d, and by the Animal and Plant Health Inspection Service (APHIS) under the Federal Plant Pest Act (FPPA), 7 U.S.C. §§ 150aa-150jj, and the Plant Quarantine Act (PQA), 7 U.S.C. §§ 151-164a, 166-167, as amended. EPA issued an Experimental Use Permit for field testing MON810; and it later registered MON810 for commercial sale and use subject to a time limit and specified conditions (which subsequently have been strengthened) and exempted the pesticidal portion from the requirement of having a residue limit (tolerance) in food. APHIS authorized field testing of MON810 and subsequently granted it non-regulated status, i.e., APHIS determined that MON810 is not subject to APHIS' regulatory oversight based on current knowledge. APHIS conducted an Environmental Assessment under National Environmental Policy Act (NEPA), 42 U.S.C. §§ 4321-4370e, on the basis of which it issued a finding of no significant impact on the environment (FONSI) and also concluded that there were no issues under the Endangered Species Act (ESA), 16 U.S.C. §§ 1531-1544.

1. Description of Proposed Organism and Its

Maize or corn, *Zea mays* ssp. *mays*, is a member of the Poaceae (grass family) and is grown for forage, silage, but most notably for its grain, which is borne in ears (or cobs). *Zea mays* is a wind-pollinated, monoecious, annual species with imperfect flowers. This means that spatially separate tassels (male flowers) and silks (female flowers) are found on the same plant, a feature that limits inbreeding. A large variety of types are known to exist (e.g., dent, field, flint, flour, pop, sweet) and have been selected for specific seed characteristics through standard breeding techniques (Hitchcock, 1971). Maize cultivars and landraces are known to be diploid ($2n = 20$), that is they contain a set of 10 paired chromosomes which contain the DNA or genetic material, and are interfertile to a large degree.

However, some evidence for genetic incompatibility exists within the species (*e.g.*, popcorn x dent crosses; Mexican maize landraces x Chalco teosinte). *Zea mays* has been domesticated for its current use by selection of key agronomic characters, such as non-shattering rachis (ear), grain yield and resistance to pests (Kiesselbach, 1949). The origin of corn is thought to be in Mexico or Central America, based largely on archaeological evidence of early cob-like maize in indigenous cultures approximately 7200 years ago. These issues are considered when examining the potential for outcrossing / pollination between a crop and its wild relatives.

The Monsanto Company has developed a genetically modified line of field corn (also referred to as “maize”) that produces a protein throughout the corn plant that is toxic to certain insect species. The resulting plant line is identified commercially as “MON810,” and the crop is one of several transgenic corn varieties generally referred to as Bt-corn or Bt-maize. The substance produced through the genetic alteration is identified as δ -endotoxin or the Cry1Ab protein.

MON 810 was developed by co-transforming corn with two vectors, one carrying a synthetic *cry1Ab* gene and the second bearing the two herbicide resistance genes (*EPSPS* and *gox*). The genetic alteration that produced MON810 incorporated a truncated form of the syntheticform *cry1Ab* gene from the bacterium, *Bacillus thuringiensis* subsp. *kurstaki*, into a Hi-II type corn line (see details below). *B. thuringiensis* subsp. *kurstaki* is a common soil bacterium that has been isolated worldwide. The modified *cry1Ab* gene maintained its ability to produce the δ -endotoxin in plant tissues at levels that are toxic to certain lepidopteran insects. The *cry1Ab* gene is expressed from an enhanced 35S promoter (E35S) derived from cauliflower mosaic virus, a known plant pest, and is joined to the nopaline synthase 3' transcription terminator, NOS 3', derived from *Agrobacterium tumefaciens*, a plant pathogenic bacterium. These genetic elements (promoter, gene, termination sequence) are all necessary for proper expression of the introduced trait (*i.e.*, Bt protein) in the maize plant.

The corn line that was the recipient of the added genes is a derivative of the A188 and B73 inbred lines of corn. These are publicly available inbred lines developed by the University of Minnesota and Iowa State University, respectively. Designated "Hi-II", the recipient material is approximately 50:50 of the two lines (Armstrong et al, 1991). The material was developed to have a higher regeneration potential (from the combination of genes from A188 and B73) along with acceptable commercial performance in hybrids (from B73).

These genes were introduced into corn line MON810 via microprojectile bombardment transformation, wherein microscopic beads of gold are coated with DNA and physically forced into maize cells such that the DNA can integrate with the maize DNA / chromosomes in the nucleus. The two plasmid vectors were introduced by microprojectile bombardment into cultured plant cells. This is a well-characterized procedure that has been used for over a decade for introducing various genes into plant genomes. Southern blot analysis and Mendelian genetics data demonstrate that the introduced

gene is stably integrated into the corn genome and stably inherited. Glyphosate-tolerant transformed cells were selected, then cultured in tissue culture medium for regeneration of whole plants.

No marker genes (*i.e.*, *npt II*, *C4 EPSPS*) are expressed in the subsequent generations of corn plants / progeny from this transformation. Through traditional breeding practices, the marker genes (*i.e.*, antibiotic and herbicide resistance) have been segregated out of the final commercial hybrid (*i.e.*, MON810) and are no longer present in the genome or DNA of the plant as demonstrated by molecular analyses.

Southern analysis indicated one integrated DNA segment which included a truncated copy of the *cry1A(b)* gene, inserted without rearrangement. The corn line has been crossed into several diverse corn genotypes for 4 generations and the protection against ECB has been maintained. MON810 was derived from the third generation of backcrossing and therefore the single insert appears to be stably inherited.

MON810 Bt-maize was developed for control of various insect pest species that cause serious pest problems in corn: corn earworm (CEW), European corn borer (ECB) and Southwestern corn borer (SWCB). Monsanto's petition to APHIS only claimed that MON810 was developed for control of ECB. These insect species feed on corn causing plant damage that ultimately results in decreased quality and quantity of yields. During field testing of plants of corn line MON810, ECB infestations were significantly reduced as compared to non-transgenic control plants.

Monsanto markets MON810 Bt-maize throughout the corn growing areas of the United States that include the primary Corn Belt of the Midwest, Great Plains and significant acreage in the southern states, including areas that also raise cotton. The grain harvested from MON810 is used in animal feed and processed into numerous food products for human consumption and non-food uses. The harvested product is used both in domestic foods and feed products and is exported to numerous foreign countries. Most corn is stored and marketed as a bulk commodity, and this practice means that, absent special handling procedures, transgenic corn would normally be mixed with conventionally bred types of corn.

2. Relevant Regulatory Agencies, Regulatory Authority and Legal Measures

Two federal agencies, EPA's Office of Pesticide Programs (OPP) and USDA's Animal and Plant Health Inspection Service (APHIS), share the primary responsibility for regulating Bt-maize, and other "plant-pesticides." Whenever claims are made for reducing damage caused by pests, the product becomes a "pesticide" subject to EPA oversight. EPA registers and regulates pesticides, including the plant-pesticides, genetically engineered plants and products (GEOPs) that contain a gene for controlling a pest, such as an insect, or plant disease organism (FIFRA 2(u)). EPA's review includes an

assessment of the potential impacts on human health, as well as impacts on non-target wildlife and the broader environment. It is well established that, because FIFRA is the functional equivalent of NEPA, EPA is not required to prepare an Environmental Assessment (“EA”), an Environmental Impact Statement (“EIS”), or a Programmatic EIS (“PEIS”) when registering pesticides pursuant to the procedures established in FIFRA. Environmental Defense Fund v. EPA, 489 F.2d 1247, 1256-57 (D.C. Cir. 1973).

If there are residues of a plant-pesticide in or on food or feed, EPA would also be involved in establishing maximum limits (tolerances) for the amount of residues of such pesticide in food. USDA / APHIS analyzes genetically engineered organisms for potential impact on agriculture, as well as for impacts on the broader environment. APHIS regulates organisms; products that are not viable are not covered under APHIS regulations 7 CFR part 340. APHIS regulates the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products under authority granted by the Plant Protection Act (PPA), 7 U.S.C. §§ 7701-7772.

In addition, the U.S. Department of the Interior (DOI) and the Food and Drug Administration (FDA) have consultative and regulatory roles. DOI has a consultation role under the Endangered Species Act and Migratory Bird Treaty Act. FDA evaluates information provided by developers during a consultation process to ensure that human and animal food safety issues or other regulatory issues (*e.g.*, labeling) are resolved prior to commercial distribution.

Statutory authority

EPA

EPA administers two statutes that contain authority to regulate Bt-maize and other plant-pesticides: FIFRA and the FFDCA. The Food Quality Protection Act (FQPA), that amended both FIFRA and FFDCA, was enacted shortly after MON810 was conditionally registered. In addition, the ESA and the federal Migratory Bird Treaty Act (MBTA), 16 U.S.C. §§ 703-712, apply to EPA. EPA fulfills its obligations in these respects in consultation with the DOI. EPA’s Field and External Affairs Division serves as a contact point for informal or formal consultations with DOI where listed species are considered as possibly affected. Consultations were not considered necessary in the case of MON810.

1. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

FIFRA defines a “pesticide” as “any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest . . .” (FIFRA 2(u)). Unless it is exempted or falls within certain minor exceptions, under FIFRA, a pesticide may be sold or distributed in commerce only if EPA has issued either an experimental use permit or a registration for the product (7 U.S.C. §

136). Plants themselves are exempted from FIFRA oversight (40 CFR 152.20). In general, EPA may approve the sale and distribution of a pesticide only if the Agency determines that use of the product will not cause "unreasonable adverse effects on the environment." FIFRA defines "unreasonable adverse effects on the environment" to mean (1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of a pesticide, or (2) in the case of a pesticide that requires approval under FFDCA, a human dietary risk from residues from a use which causes a pesticide residue in food that is not "safe." (FIFRA 2(bb)) The latter portion of the standard is a "risk-only" standard, while the first part of the FIFRA standard involves balancing risk and benefits. The proponent of the pesticide use bears the burden of showing that the pesticide meets the applicable statutory standards. The statute authorizes EPA to establish requirements for information that an applicant must satisfy in order for the Agency to consider its request to sell or distribute the pesticide.

Under FIFRA, EPA may establish requirements concerning the composition, packaging, and labeling of a pesticide. In particular, the labeling of a pesticide may specify the manner in which the pesticide is allowed to be used. FIFRA prohibits the use of a pesticide in a manner inconsistent with its labeling. Once a product is registered, FIFRA requires the registrant to report to EPA any information concerning the unreasonable adverse effects of the pesticide on the environment (FIFRA 6(a)(2)). FIFRA also authorizes EPA to issue "data call-in notices," which require the registrants of a pesticide to develop and submit any additional information the Agency needs to evaluate the pesticide to determine whether the registration may remain in effect. For MON810 Bt-maize, a time-limited registration was enacted to provide for a reassessment of the conditions of registration after five years.

Because the development of plant-pesticides represented a novel approach to pest control and a new technology, a provision for re-evaluation of the active ingredients was included at the time of registration. This provides for the reassessment of the status of certain Bt-crops (*i.e.*, corn and cotton) and to determine if further data will be required to ensure that an adequate risk assessment can be performed. Any outstanding data requirements would have to be fulfilled in order to support the renewal of the existing registrations. The currently registered Bt-corn crops all have registrations expiring in September, 2001.

2. Federal Food, Drug, and Cosmetic Act (FFDCA) 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.” In 1996, the Food Quality Protection Act amended the FFDCA to define “safe” to mean that “the Administrator has determined 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.”¹ 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.

Implementing regulations

EPA has defined a “plant-pesticide” as “a pesticidal substance produced in a living plant and the genetic material necessary for the production of the substance, where the substance is intended for use in the living plant.”² In other words, EPA regulates the pesticidal substance - the pesticidal substance produced in a living plant and the nucleic acid sequence (DNA) or genetic material necessary for directing synthesis of such a substance - but not the plant itself.

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. The Agency will typically allow small scale field tests (less than 10 acres of land or 1 acre of water, per pest being examined) following notification of the EPA that a GEOP is being evaluated in a field situation and some methods of confinement are being instituted. If a larger field test is planned, then an EUP is required. 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 of GEOPs for more than 5000 acres.

In addition, if the experimental design involves the production of food for distribution in interstate 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” requirement would then be included in the EUP.

Granting 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.

¹EPA initially registered and established tolerances for MON810 before the FQPA amendments. But, because the MON810 registration expires in 2001, EPA will be reevaluating both the registration and the tolerance exemption under the new statutory standard created by FQPA, as the Agency decides whether to renew its approvals.

The data required to support a request for an EUP are detailed in 40 CFR Part 158 and as discussed under Section 3, EPA's Hazard Identification, Risk Assessment and Regulatory Review of Bt-Maize. Site visits to the experimental plots can and have been performed, resulting in plot destruction in one instance for failure to follow the conditions established in the EUP (this example did not involve Bt-maize).

Registration. Like an EUP, a person must apply for registration of a pesticide. 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 registration for individual products. For example, EPA may issue a "seed increase registration" which allows a registrant to plant a GEOP for the purpose of producing seed for propagation and future sale. The genetically altered seeds, however, could not be sold until a new "full-scale" registration was approved. If the identical *cry* gene (as in MON810) encoding the insecticidal protein was transformed into another crop species, a new registration or an amendment to the existing registration would likely be required for sale and distribution of this plant-pesticide.

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 toxicity data related to human health comparable to that to support a registration. Environmental and non-target effects are not considered within a food tolerance petition. 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. Following review of the petition and any comments from the public, 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. Once the pesticidal substance is assessed for its toxicity to man, a determination is made to provide a tolerance limit for pesticide residues or to exempt the pesticidal substance from the requirement. Any exemption from the requirement of a tolerance is tied to the levels of pesticidal substance (*e.g.*, Cry protein) expressed and accumulated within the plant as compared to the levels of test substance utilized in the toxicity studies. That is, if the levels of active ingredient or pesticidal substance accumulate above the level tested in the toxicity studies, then the tolerance exemption would not be supported by the data and such studies would have to be repeated with increased levels to maintain the food tolerance (*i.e.*, distribution of the crop into the food supply).

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 MON 810, 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 (1) 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 corn, 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 corn, 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). The announcement marks the start of a 60-day public comment period on the petition, after which any

comments are considered in the final determination and Environmental Assessment (EA). The EA is conducted pursuant to the National Environmental Policy Act (NEPA). Since mid-1999, in addition to the 60-day comment period on the petition itself, notice of the availability of an 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 subject 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 demonstrate 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. Likewise, issues and risks that 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 Environmental Protection Agency and the Food and Drug Administration. For example, the consultation process between FDA and Monsanto for MON 810 maize was not completed until 1997, and so the product was not used as 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 non-target effects, and worker exposure, and with the National Institutes of Health and FDA for potential negative impacts on animals and humans.

The Bt-corn line MON 810, 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 seven field test releases were conducted after APHIS approval from 1992 through 1996 when Monsanto filed a petition for non-regulated status on January 17, 1996. Following a review of the petition, a deficiency letter was sent to Monsanto 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”). A determination of non-regulated status under 7 CFR 340 was granted for MON 810 on March 15, 1996. APHIS decision documents are available at <http://www.aphis.usda.gov/biotech>.

In the case of MON810, Monsanto submitted a petition in January 1996 asking APHIS to extend to MON 810 the same determination of non-regulated status that APHIS had granted August 22, 1995, to a very similar maize line, MON80100 (APHIS Petition 95-093-01P). In fact, MON 810 had been one of several lines included in petition 95-093-01P, but Monsanto withdrew MON 810 from consideration until it could be more thoroughly characterized. By 1996, the new petition provided the more thorough characterization and documentation of MON810 and another line, MON809, to support the conclusion that these lines posed no plant pest risk and should no longer be considered regulated articles. After careful review of all available data, APHIS published an announcement in the Federal Register on March 15, 1996, stating that “the APHIS determination of non-regulated status of August 22, 1995, applies as well to Monsanto’s two new transformed corn lines, MON 809 and Mon 810.” Both of the decision documents are available from APHIS (USDA, 1996).

In 1997, APHIS amended its regulations to provide a more formal procedure for developers to seek an extension of a previous determination of non-regulated status (7 CFR 340 Part 340.6). APHIS has also provided a complementary Users’ Guide for Extensions at the agency web site (<http://www.aphis.usda.gov/biotech/gaddbeg.htm>). Under the current system, in place since 1999, APHIS publishes in the Federal Register a notice that it has received a petition for an extension for a determination of non-regulated status. APHIS conducts its analysis and prepares an Environmental Assessment that is then made available to the public for comment during a 30-day period. The agency then considers any comments received from the public before making its final decision and announcing the decision in the Federal Register.

FDA

FDA considers, based on Agency scientist’s’ evaluation of available information, whether any unresolved issues exist regarding a food derived from a new plant variety that would result in 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.

DOI

The Department of Interior (DOI) administers the provisions of the federal MBTA and the ESA, with the Fish and Wildlife Service (FWS) being the sole administering Agency for the MBTA and shared authority for the ESA between DOI/FWS and the National Marine Fisheries Service (NMFS)

at the Department of Commerce (DOC). Under the ESA, any 'take' of a listed species is prohibited unless otherwise exempt or authorized. As appropriate, EPA will contact DOI /FWS or DOC to initiate a consultation through its Field and External Affairs Division when an EPA action may affect a listed species. These consultations involve only the resource agency (FWS or NMFS) and the action Agency or Agencies.

The MBTA prohibits 'take' of migratory birds. However, what qualifies as 'take' varies between the two statutes. Under the ESA, for example, 'take' includes harassment of any species listed as threatened or endangered. Any action that rises to the level of 'take' under the respective statute could be subject to a governmental action or a suit brought by a private citizen. The provisions of ESA and MBTA apply to both genetically engineered species and non-engineered species equally. In the case of MON 810, a determination that no biological impact or affect to listed species would result from registration of this plant-pesticide was made following the Agency's risk assessment. Therefore, no formal consultation was required between EPA and DOI.

Interagency Coordination

Under the Coordinated Framework for the Regulation of Biotechnology published in 1986, EPA and USDA have the major regulatory responsibilities for genetically modified plants with pesticidal properties. EPA's role is to protect human health (both dietary and worker exposure) and the environment. Related to environmental effects for products such as MON810, EPA conducts analyses on ecological effects to non-target species, environmental fate, threatened and endangered species, and insect resistance management. APHIS' authority overlaps considerably with that of other federal agencies, namely EPA, for a host of environmental concerns such as non-target effects. In addition, APHIS is to ensure that the product will not be a threat to agriculture. FDA's role is to protect the food supply and as such shares regulatory responsibility with EPA under the Federal Food, Drug, and Cosmetic Act. Under the FFDCA, EPA is authorized to establish, modify, or revoke tolerances for pesticide chemical residues on food. Thus, EPA is responsible for establishing maximum allowable residues of the Bt protein produced by the Bt maize that may be present in edible corn. In addition, the Department of the Interior is responsible for potential effects to fish and wildlife and their responsibility overlaps with EPA for ESA and MBTA. Of course, the provisions of NEPA must be followed by the DOI, while EPA / OPP's review of environmental impact is considered as functionally equivalent as that expected from NEPA oversight (although EPA maintains a record keeping and review of implementation function under NEPA guidelines).

Agencies consult with each other as warranted to properly review a new genetically engineered organism. There are currently no regularly scheduled meetings to review submissions that might be made to each Agency, but rather they are dealt with on a case-by-case basis. In many instances the

registrants of plant-pesticides have previously been through a review process with the USDA-APHIS biotechnology group, and although the focus of the risk assessment differs between agencies, there is significant overlap in some areas. Dialogue is initiated in those areas of mutual interest, especially in the consideration of non-target impacts, so that both groups benefit from the combined expertise. More recently, an effort is underway to hold scheduled conference calls between the EPA and APHIS when submissions regarding the same regulated article or plant-pesticide are made to both agencies. The timing and frequency of these conferences will be determined by the rate of the review process and the novel aspects of the plant-pesticide at hand. Currently, there is an Herbicide Tolerance Working Group with both EPA and APHIS members and a BT working group is about to begin cooperation. Issues of confidential business information and proper clearance under the statutory guidelines, however, may prohibit free exchange of data or review materials in some instances.

Additionally, scientists from EPA and APHIS regularly attend any relevant SAP (Science Advisory Panel) meetings and scientific workshops, which provide for exchange of ideas and information in areas of mutual interest. The NC-205 (North Central States Committee) meetings are another example of joint efforts wherein the Agencies meet informally to discuss regulatory matters.

USDA/APHIS, FDA, DOI and EPA

1. Endangered Species Act (ESA) and Migratory Bird Treaty Act (MBTA)

The Endangered Species Act (ESA), jointly administered by the Secretaries of the Interior and Commerce, could also affect the use and dispersal of plant-pesticides. The Endangered Species Act requires importers of plants to file declarations, and limits importation to designated ports. 16 U.S.C. §§ 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.*, at § 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.*, at § 1536(b). Any take of an endangered or threatened fish or wildlife species unless otherwise authorized is unlawful under the statute. *Id.*, at sec.1538. § 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 GEOP.

A Biological Opinion from the Department of Interior Fish and Wildlife Service was issued on December 18, 1986, concerning possible effects of foliar spray of *B. t. subsp. kurstaki* on threatened and endangered species. Based on difference in exposure routes between foliar spray and expression in plants, APHIS believes that the Biological Opinion is inapplicable, and that re-initiation of consultation is not necessary. The majority of endangered lepidopterans have very restrictive habitat ranges; and their larvae typically feed on specific host plants, none of which include corn or its sexually compatible relatives. An examination of county distribution of endangered lepidopterans shows that, for the most part, they do not occur in agricultural settings where corn is grown.

Additionally, the federal MBTA also requires that any federal action that might impact migratory avian species be minimized or excluded so as not to harm populations.

Voluntary Standards

For most crop plants there are breeders' organizations and seed certifying agencies, some of which are state, regional or national in scope. Compliance with the guidelines proposed by these organizations is necessary if a breeder wants to sell seed as being officially certified. Their oversight, however, is voluntary in the sense that it is not necessary to obtain their certification in order to produce or sell seed. Companies buying crops for processing into foodstuffs may mandate in their contracts that only certified or foundation seed of a particular variety be planted for their use; however, these are not covered by any statute or regulation. Additionally, very little certified maize seed is grown in the U.S. for processing. Most of what is grown as 'certified' is meant for export. In no instance is the scrutiny accorded GE (genetically engineered) crops approached by non-GE voluntary oversight in terms of safety assessment (*i.e.*, toxicity or environmental impact). All crop varieties, regardless of the genetic techniques used to produce them, receive a great deal of review and analysis to meet the demands of producers and consumers.

When the commercial line of a plant species is developed through classical breeding without subsequent transformation (*i.e.*, is non-GE), state seed foundations and crop councils prescribe distances between breeders' lots or fields to ensure a degree of purity from pollen spread / cross hybridization. Guidelines for seed certification are defined by the Association of Official Seed Certifying Agencies (AOSCA) and they are followed by member organizations or agencies. There are also parameters to ensure that the amount of weed seed inadvertently carried with the maize seed is minimal. The percent germination of seed lots is also tested. The details of distances between seeds and the definitions of plant types, etc., may vary state to state. AOSCA defines the number of generations of backcrossing required in generation of a hybrid and what constitutes an inbred line.

Commercial companies follow these guidelines to ensure that their seed is competitive in the

marketplace and of general high quality. In some instances this may include outside testing by a state seed foundation or other university associated program to establish seed quality and genetic purity / varietal identity. Presence of disease organisms is also examined in some crops or certain regional situations where a known pathogen is problematic.

A. EPA.3. Hazard Identification, Risk Assessment, and Regulatory Review of Bt-Maize

EPA

FIFRA requires EPA to consider all relevant factors in reviewing and approving a plant-pesticide for registration. This includes a risk / benefit analysis and any safety assessment to preclude unreasonable adverse effects.

EPA regulatory authority only covers the actions occurring within the borders of the United States and its possessions and territories. If a potential problem existed at the border with another country due to proximity of the GEOP, the Agency could exclude distribution of that plant-pesticide in a defined area (as has been done with other plant-pesticides for reasons of outcrossing to wild relatives). FIFRA § 2(bb) mandates that EPA ensure a reasonable certainty of no harm to man and the environment. This includes the potential for environmental impact to non-target species, such as those wild or feral relatives of crops. If a plant-pesticide were known to result in adverse effects in another country, it is possible that this matter could be considered under a risk / benefit analysis performed in accordance with FIFRA.

EPA's focus in considering these issues is on the statutory determination of unreasonable adverse effects the Agency must make with respect to pesticides, rather than on the engineered plant itself. In particular, these plant-related issues may potentially impact use patterns of pesticides, which are of relevance to the Agency.

1. EPA's hazard identification and risk assessment of Bt-maize.

Product characterization requirements for MON810 included details of the gene source (what organism), DNA and protein sequence data, details of the plasmid (DNA) used in plant transformation (annotated map), method of transformation, the pesticidal substance encoded by the gene, expression levels under field conditions and where in the plant the Cry1Ab endotoxin accumulates, glycosylation of the pesticidal protein (presence / absence and similarity of sugar residues on proteins produced in microbial and plant forms), serological relatedness of plant and microbial forms of Cry1Ab (Western blot and Enzyme Linked ImmunoSorbent Assay), and bioassayed against larvae of the corn ear worm or the European corn borer.

The Agency also required the applicant to explain the use pattern (*i.e.*, will the crop be used for human consumption, animal feed only, ornamental uses, etc.). MON810 is used as a traditional field corn *i.e.*, the whole grain is not directly used by humans for food, although processed products from the grain are used both for human food products, and for non-food items, *e.g.*, wallboard and paper components, adhesives, pharmaceuticals. The majority of harvested MON 810 maize will be used as an animal feed. in human dietary, *e.g.*, starches, fructose, alcohol and non-food items, *e.g.*, wallboard and paper components, adhesives, pharmaceuticals, ethanol fuel additive. The majority of harvested MON810 maize will be used as an animal feed.

Once the product (*i.e.*, the plant and associated pesticidal substance) and its proposed uses are adequately identified by the registration applicant, EPA evaluates the potential hazards of plant-pesticides in two broad areas: human health and environmental effects. Risks to humans and animals via the dietary route (*e.g.*, as food and feed) and to non-target organisms through exposure via water, wind, soil and direct consumption of the GEOP are all considered within the risk assessment. If other routes of potential exposure exist, such as dermal absorption, these risks are also addressed.

Human health. The human health assessment includes the evaluation of the pesticidal substance in an oral toxicity assay, typically performed on rats or mice. This is a maximum hazard dose assay in which the laboratory test animals are dosed with purified pesticidal substance (all proteins to date) at the rate of 4000 to 5000 mg/kg body weight. Animals are then observed for any clinical manifestations, decreases in body weight gain, and mortality. After a 14 day observation period, animals are sacrificed and a gross necropsy performed to ascertain if there were any major changes in organ size or evidence of pathology.

Because these human health studies require large amounts of pure protein, it is sometimes necessary to use protein produced in a microbial system, such as *E. coli* or *B. thuringiensis*, as the test substance. In such cases, the applicant is required to demonstrate that the protein is identical to the substance produced in the plant. Assays to verify this may include the sequence of the genes used in plant and microbial systems as well as the protein sequence and any glycosylation sites that may be present on the processed protein. This was done with MON810 Bt-maize, and the microbially (in *Escherichia coli*) produced protein was found to be equivalent to the plant product and was, therefore, allowed as a test substance (EPA / BPPD, 1995). Moreover, no toxicity was noted. In these toxicity studies, the dose level of endotoxin administered to the test animals far exceeded the possible human consumption levels via the diet or exposure in the environment through soil and water. Having this leeway one can expect that the level of human exposure to Cry1Ab from MON810 will fall far below any potential effect level.

EPA also requires a study on the digestion of the protein in a simulated gastric assay, to

determine the stability of the protein after ingestion. Proteins and digested fragments are analyzed on a polyacrylamide gel to separate them and characterize molecular mass. Proteins that are resistant to digestion are considered as potentially more allergenic or toxic as they may remain intact for longer periods in the stomach and intestines where they could be absorbed. This does not imply that any protein which remains intact after passage through the stomach is an allergen or a toxin, it only means that those proteins which do exhibit these properties generally remain intact, or largely so, following gastric passage. Cry1Ab endotoxin was found to degrade in the gastric assay.

Amino acid sequence data from the pesticidal substance are also subjected to analysis by comparison of similarities to known allergens using a database of protein sequences and a program that will highlight any sequence homology of 8 amino acids or longer. This is considered the minimum length of amino acids that may constitute an allergen. At the time MON810 was registered this database and search capability did not exist. Given the rapid degradation of the Cry1Ab protein in the gastric environment, the opportunity to be absorbed and act as an allergen is not afforded.

Environmental assessment. Non-target organism studies include the toxicity characterization for:

- fish (catfish or trout),
- aquatic invertebrates (Daphnia),
- earthworms,
- Collembola (springtails),
- beneficial insects (green lacewing, ladybird beetle, honey bee, parasitic wasp),
- birds (Bobwhite quail or Mallard duck) and
- any other species considered as being exposed or at risk from the pesticidal substance.

The species chosen for testing are representative of the main groups of organisms likely to be affected by a pesticide in a typical agricultural and environmental scenario. In addition, these species are readily available for testing and allow for valid comparisons between studies, even when performed by different testing laboratories. Other organisms are chosen as needed to conduct any further toxicity or pathogenicity testing based upon the evidence of any specific risk to that group of organisms or if the proposed use pattern of the pesticide indicates that a species or group of organisms not represented in the standard toxicity tests may be exposed during use of the pesticide. A maximum hazard dose is used to detect toxicity based upon the recommendations of a scientific advisory panel during formulation of the testing guidelines. Endangered or threatened species are given special consideration, and EPA may require testing with related, abundant species to assess possible non-target effects. Scientists within EPA review the proposed application sites of the plant-pesticide and the potential for exposure to any endangered species to produce a risk assessment. Consultations with the Fish and Wildlife Service of DOI are also carried out wherein questions arise. With MON810 there were no concerns for harm to endangered species based on the areas planted with Bt-corn and the containment of the pesticidal substance within the plant.

Studies of non-target species are typically designed as single dose, maximum hazard toxicity assessments, and animals are observed for varying time frames depending on the species (14 to 30 days typically). Toxicity studies with the organisms listed above are carried out on dried whole-plant tissues (*e.g.*, grain) as opposed to purified endotoxin, although there are some exceptions to this. Grain and Cry1Ab endotoxin had no observed effect in the studies outlined above. With proteins as potential toxicants, short-term toxicity assessments are considered as satisfactory in assessing the potential for long term effects through consumption since they are generally degraded rapidly within the digestive system. Similarly, proteins are not usually considered as mutagens or teratogens the way that some other organic molecules might be. Tests to measure these possible effects are in the guidelines (40 CFR) for biochemicals and traditional chemical pesticides.

No adverse effects were observed on larval honey bees at a maximum hazard dose of 20 ppm *B.t.k.* HD-1 protein. An LC₅₀ was not possible to calculate since this was a single dose test. Therefore, the no observable effect level (NOEL) is greater than 20 ppm. There were no statistically significant differences among the various treatment and control groups due to the sizable mortality that occurred in all treatments of adult honeybees. *B.t.k.* HD-1 protein at 20 ppm resulted in a mean mortality of 16.2%. Because mortality was observed at the single dose tested, a NOEL could not be determined from this study, but it was less than 20 ppm. It was determined that 20 ppm is significantly higher than exposure conditions in the environment.

No adverse effects were observed at a maximum hazard dose of 20 ppm *B.t.k.* HD-1 protein to *Brachymeria intermedia*, an insect parasitic wasp. Since this is a single dose study, an LC₅₀ cannot be calculated. The NOEL is greater than 20ppm. With green lacewing bioassays, there were no adverse effects observed at a maximum hazard dose of 16.7 ppm *B.t.k.* HD-1 protein after 7 days. The NOEL is, therefore, greater than 16.7 ppm. Similarly, there were no adverse effects observed in lady beetle bioassays at a maximum hazard dose of 20 ppm *B.t.k.* HD-1 protein. The NOEL is greater than 20 ppm.

Oral toxicity (feeding) studies with Northern Bobwhite Quail indicated no treatment related mortality or differences in food consumption, body weight or behavior occurred in birds fed 50,000 or 100,000 ppm transgenic corn meal derived from Monsanto's MON801 corn line (which contains Cry1Ab protein) relative to birds fed corn meal made from parental corn lines which did not express Bt toxin. Although this study utilized Monsanto's MON801 Bt corn for testing, the test material was considered sufficiently similar to the MON810 corn grain to bridge the data because of the similarity in Cry1Ab levels.

The 14-Day LC₅₀ value for earthworms exposed to Cry1Ab insecticidal protein derived from *E. coli* in an artificial soil substrate was determined to be greater than 200 mg/kg (ppm), which

was the single concentration tested. There were no statistically significant effects at the single dose tested. Therefore, the NOEL is greater than 200 ppm. Although this study was graded supplemental, Bt toxins expressed in the corn plant are not expected to generate a toxic effect in the earthworm; therefore, no additional follow-up of this study was required.

Impacts on non-target soil organisms are of interest because of the residual *B.t.k.* protein that exists in the corn plant at physiological maturity and the potential for incorporation into the soil. In the study submitted by Monsanto on the toxicological effect on two species of Collembola (*Folsomia candida* and *Xenylla grisea*), *B.t.k.* leaf tissue containing Cry1Ab insecticidal protein had an LD₅₀ over a 28 day exposure period that was > 50 % of the diet formulation by weight. The NOEL for mortality was 50 % of the diet. The estimated concentration of Cry1Ab in the lyophilized tissue was 50.6 µg/g dry weight.

The study "Evaluation of the European Corn Borer Resistant Corn Line MON801 as a Feed Ingredient for Catfish" was reviewed to determine potential impacts on channel catfish from Monsanto's MON810 corn lines. Feed per fish, feed conversion ratios, final weight, percentage weight gain and survival were not significantly different between fish fed the control MON 800 diet when compared to those fed the diet containing transgenic corn from the test line MON801. Body composition data exhibited no significant differences in percentage moisture, fat, or ash, with a higher protein content in the test fish on a dry weight basis. This difference in protein content disappears when one expresses the results on a wet weight basis. Data in this study are consistent with historical controls for catfish grown at the Delta Research and Extension Center. Although this study utilized Monsanto's MON801 Bt corn for testing, the test material was considered sufficiently similar to the MON810 corn grain to bridge the results for the data requirement since the levels of Cry1Ab in the MON801 grain tested were similar to MON810 levels.

After a 48-hour exposure of aquatic invertebrates to corn pollen containing the Bt Cry1Ab toxin (100 mg/L), no mortality was seen to *Daphnia magna*, a sensitive aquatic invertebrate. The data suggest that at the expected environmental concentration, no effects are expected on aquatic invertebrates.

The Agency also examines the environmental fate of the endotoxin. At the time of registration of MON810, the fate of Cry1Ab toxin in the soil from plant residues was considered to be essentially the same as other proteins added to the soil ecosystem, namely that they would be degraded by physical, chemical and biological processes associated with soil. With the measured lack of toxicity to soil invertebrates and other non-target organisms (as noted above), the presence of the δ -endotoxin in the soil profile is not considered to adversely impact the soil microflora and microfauna. *B.t.k.* Cry1Ab protein bioactivity, added to the soil as a component of Bt-maize tissue, decreased with an estimated 50 % degradation in 1.6 days and an estimated DT₉₀ of 15 days. The bioactivity of purified Cry1Ab

protein in soil decreased 50 % within 8.3 days and an estimated DT_{90} of 32.5 days (Sims and Holden, 1996). Subsequent laboratory studies simulating field conditions measured the rate of degradation of the endotoxin in soil and in the plant tissue, without soil present. In the Workshop on Ecological Monitoring of Genetically Modified Crops (Stotzky, 2000), it was reported that no differences were observed in terms of soil microbes (bacteria, fungi, protozoa, nematodes) and enzymes when comparisons were made between soil from Bt crops versus conventional crops. The same degree of Cry protein persistence was observed in soils treated with microbial Bt applications as with Bt from modified crops.

EPA also analyzes the potential for the development of insect resistance. The development of resistance to Cry1Ab endotoxin within the insect pest populations (CEW, ECB, SWCB) is another way adverse effects could occur. Larval resistance could potentially occur by selection of genotypes that are resistant to the toxin, whether these genotypes already exist in the population or develop through evolutionary forces, such as genetic drift, mutation or gene flow. For an insect species that feeds on maize, natural selection of insects on Bt cultivars of maize would tend to increase the frequency of resistant insects within the population. As these genotypes increased within the insect populations, greater crop damage would occur and lead to an increasing need for alternative control measures (*e.g.*, other Bt endotoxins, biological and chemical controls). The cross resistance of ECB to different forms of Bt endotoxins is not well understood (Denolf *et al.*, 1993; U.S. EPA, 1998; Bolin *et al.*, 1999).

In general, the more widely Bt plant-pesticides are used, the greater the possibility for the development of insect resistance to the endotoxin. Thus, for MON810, EPA considered the existing and potential distribution of the MON810, as well as other Bt plant-pesticides. The Agency's analysis suggested that, without restrictions on MON810, there was some potential for the development of resistance in the pest species, but with conditions on the use of MON810, the risk could be minimized to a biologically acceptable level. That is, the impact of the MON810 plants when deployed as indicated in the registration documents, would not significantly hasten the potential development of resistance in the target pest species to Cry1Ab.

EPA also evaluates the potential for outcrossing or hybridization of Bt-maize pollen with wild relatives of maize that are sexually compatible. In other words, is there a possibility of transfer of the pesticidal substance to other plant species that might result in an entirely new exposure scenario? The potential for transfer of the pesticidal gene to wild relatives is assessed based upon the basic biology of the crop plant and the distribution of related species. Since the only relatives of maize that exist in the United States or its territories and have the potential to cross hybridize are in special plantings, herbaria and research plots, this is not considered a problem (U.S. EPA/BPPD, 2000A). Of course, Bt-maize can cross with other maize hybrids (*i.e.*, sweet corn, popcorn, field corn), but these are harvested and

do not persist in the environment where chance escapes or volunteer plants occur.³ Plants that may develop from scattered seed are not aggressive or competitive and are dealt with by cultivation or herbicides used for weed management.

The potential for outcrossing to traditional cultivars of maize from MON810 or other registered plant-pesticides is not currently reviewed within the guidelines (40 CFR). Since the mammalian toxicity and environmental evaluations have indicated that the plant-pesticidal substance (*i.e.*, δ -endotoxin) is not a threat to man or the environment, there is not a risk associated with MON810 pollen fertilizing traditional maize. Traditional culture methods and breeding (*i.e.*, seed production) have resulted in cross-pollination between open pollinated varieties, hybrids and inbred lines for centuries with no known ill effects. This has similarly transferred genes for disease and insect resistance between varieties in the past.

EPA does not attempt to evaluate the possible future changes in social and ecological conditions (*e.g.*, climate) nor the marketability of the grain produced. Predictability of climatic change is difficult at best. The GEOP is evaluated under different conditions (*e.g.*, temperatures, drought stress) to examine expression of the pesticidal gene as the local environment changes. The limit dose testing performed for the assessment of toxicity is at a level that exceeds the amounts of pesticidal substance present in the plant under any foreseeable conditions. The longevity of a cultivar and associated resistance genes is considered finite at the time of deployment and by the time any significant climatic or social changes alter the ability to grow certain crops or tastes for specific foods, it is likely this GEOP (MON810) will no longer be of utility (*i.e.*, will be replaced by other plant-pesticides). The EPA regulatory process does not end with registration, but continues and has the ability to modify the registration at a later date as warranted.

³With other genetically modified crops that EPA has reviewed for registration where the potential for outcrossing exists (*i.e.*, sexually compatible relatives are present in the U.S. or its territories), these plant-pesticides have been precluded from distribution in those areas where wild relatives occur. For example, Bt-cotton is restricted from planting in Hawaii by EPA due to the presence of a wild relative of upland cotton (Stewart, 1991; U.S. EPA/BPPD, 2000B). If it became known that a sexually compatible relative of maize had established itself within U.S. borders, then it would be necessary to consider whether regulatory measures to prevent outcrossing are appropriate or necessary in those areas as a means of preventing outcrossing.

The focus of EPA's analysis of plant-pesticides is prescribed by FIFRA and FFDCA based upon the presence of a pesticidal substance and the gene(s) necessary to produce it being present in the plant.

Finally, EPA takes into account any other relevant information. In the case of MON810, EPA considered the long history of the use of Cry1Ab in microbial sprays. This experience showed that the endotoxin has virtually no toxicity to mammalian and other non-target species, based upon studies reviewed by EPA and a review of the relevant literature.

2. EPA's Consideration of the Risks and Benefits of Bt-maize.

As noted above, FIFRA's standard for registration decisions involves an assessment of risks and benefits of using a pesticide. One of the primary benefits of a plant-pesticide is the replacement of pesticides that may pose greater risks, *e.g.*, groundwater contamination, toxicity to non-target organisms, or dietary risks to infants and children. To date, however, decisions to register plant-pesticides have relied primarily on their lack of toxicity to all organisms tested, except target pests. Nonetheless, EPA has also considered possible benefits that might result from use of MON810. Planting of MON810 likely will reduce the use of other insecticides and thereby will avoid the types of risks those insecticides might have had, if applied to the same acreage as MON810.

Targeting the δ -endotoxin to the point of feeding of pest insects should minimize the impact of pesticides on non-target organisms and minimize ground water contamination, as may occur with use of some conventional chemical pesticides. Since many of the previously deployed insecticides were broad-spectrum in their activities, the potential for impacts on the beneficial insect populations was significant. Populations of beneficial insects should increase over time as more GEOs are planted and fewer toxic (*i.e.*, broad spectrum) pesticides are used. Even though Bt expressing maize is an effective control method for the target pests, many species of the beneficial insect community associated with a maize field do not prey upon or parasitize the target insects. Those that do, typically rely on several hosts or prey insects to sustain their populations throughout the season. Since some insecticides have effects on non-insect organisms (*e.g.*, earthworms, nematodes), the reduction or elimination of these pesticides will help to nurture these populations as long as cultural practices of soil management are adequate.

Additionally, the hazard to farm workers, pesticide applicators and the public in general is reduced when a plant-expressed pesticide takes the place of a more toxic chemical spray alternative. Residues on food are also less a concern with MON810, since the δ -endotoxin is known to be non-toxic to humans and other mammals. Fuel costs for transporting, packaging in containers, disposal and application are also not expended when using GEOPs as compared to conventional chemical pesticides or microbial sprays. Spray drift is often problematic with chemical applications, but this is not an issue

with plant-pesticides / GEOPs.

3. History of EPA's regulatory review of Bt-maize.

Based on the hazard identification, risk assessment, and risk-benefit analysis described above, EPA determined that the applications for MON810 met the statutory standards for issuing an experimental use permit, registration, and an exemption from the requirement of a tolerance (40 CFR 180.1173; 61 FR 40343, Aug 2, 1996). Because MON810 and other similar Bt-maize products involve a new technology about which there is some uncertainty, EPA issued time limited registrations. At the time of registration, the details of insect resistance management plans were under development and a time line for reassessment was determined. Currently, all Bt-maize registrations will expire in 2001; MON810 registration expires on September 30, 2001. EPA is presently re-evaluating all registered Bt-GEOPs and will determine if continued registration is warranted and if all food tolerance exemptions will be continued without change.

As noted above, Bt-maize is generally non-toxic to all species, except certain lepidopteran insects. These characteristics led EPA to focus primarily on two types of potential risks: 1) the development of insect resistance; and 2) the risk to non-target insects. These risk scenarios require proper monitoring of insect resistance, implementation of resistance management plans, and examination of potential non-target influence on insects inhabiting the area of Bt-maize planting to prevent or mitigate adverse effects. That is, if an adverse event should be observed, the potential hazard could be mitigated by halting further seed sales, altering the distribution of a specific pesticidal substance (region of growth of crop) or other remedial measures. Failure by the registrant to monitor or analyze the data gathered from such assessments would be a potential avenue for proliferation of these risks by allowing resistance to develop unchecked. Growers are required by contract to implement a refuge plan for insect resistance management and their compliance is monitored by the registrant. The registrants of Bt-maize plant-pesticides, the Biotechnology Industry Organization and the National Corn Growers Association have formed a consortium (Agricultural Biotechnology Stewardship Technical Committee) to closely coordinate the sampling and bioassay of insect populations for resistance to Bt δ -endotoxins in cooperation with university and USDA/ARS scientists.

Insect resistance. To minimize the potential for the development of insect resistance, EPA imposed several different conditions on the registration of MON810: 1) limitations on the amount of MON810 that could be planted in certain regions of the country; 2) a requirement that each grower plant an appropriately sized refuge of non-Bt maize (based on recommendations from the USDA NC-205 [http://biotech-info.net/NC_205.html] research committee on ecology and management of European corn borer and other stalk-boring Lepidoptera working group on insect resistance management and sanctioned by EPA); and 3) a requirement that the registrant perform post-registration monitoring of field insect populations for all Bt-crops in order to detect the development of resistance as

early as possible.

Because EPA had previously approved a Bt cotton plant-pesticide, at the time of the initial registration of MON810, the Agency's greatest concerns about insect resistance centered on the southern part of the United States. Bt-cotton was targeted against some of the same insect pest species as MON810, and was already widely planted in the South. To constrain the overall use of Bt-based products in this region of the country, the Agency limited use/planting of MON810 in the South to 100,000 acres. Since the cotton acreage in the south was significantly greater than the maize acreage and the pests which crossover move from maize to cotton (and have different developmental ontogenies within the two crops), maize acreage was initially restricted. This restriction did not apply to other parts of the country and later was relaxed to allow for more planting in the South after February 1999, based on EPA's conclusion that insect resistance could be managed by adopting a requirement for non-Bt refuges.

In February 1998, EPA requested that the FIFRA Scientific Advisory Panel (SAP) subpanel on Bt plant-pesticide resistance management review existing IRM strategies for Bt crops (SAP, 1998). Following the recommendations of this SAP subpanel, EPA began to mandate specific structured refuge options for new Bt corn registrations (those products registered prior to that time were still expected to implement voluntary refuge options). The specific structured refuge requirements were based on the technical recommendations of the February 1998 FIFRA SAP subpanel and USDA NC-205 research committee on ecology and management of European corn borer and other stalk-boring Lepidoptera. The NC-205 regional research committee, consisting of USDA/ARS and university scientists published IRM recommendations in 1997 and 1998. In 1998, NC-205 recommended at least a 20-30% untreated (not treated for the target pests) refuge or 40% treated (not treated with microbial Bt products, but other insecticides acceptable) refuge planted within close proximity. That is, the refuge should be planted within the same half section (section = 640 acres) wherein the Bt maize is planted.

Following registration of MON810 Bt-maize, a proposed draft refuge management plan was mandated by the Agency for submission by 8/98. The final structured refuge management plan was required to be in place by 1/99. These initial insect resistance management plans were proposed voluntarily by the registrant and agreed upon by the Agency in the early stages of Bt-maize registrations (*i.e.*, 1995-1997). Insect resistance management plans were mandated after 1/31/00 and required establishment of a refuge⁴ Currently, growers using MON810 must plant a 'refuge' of a non-Bt maize

⁴ EPA has established a condition on the registration of MON810 that requires Monsanto to enter into a contract with every grower who uses MON810. The contract must require the grower to plant an appropriate refuge. The condition on the registration also obligates Monsanto to take actions to assure that growers fulfill this aspect of the contract. A pattern of contract violations by growers would lead EPA to reconsider whether, without further restrictions on the registration, the use of

within 1/4 mile of their Bt-maize fields. The refuge must be equal to at least 20 % of the acreage planted to Bt-maize, and the plants in the refuge may be treated only with non-Bt insecticides. In areas of the South where cotton is grown in significant acreage, EPA requires growers to plant a refuge equal to 50% of the Bt-maize acreage, and it must be placed within 2 mile of the Bt-maize field. This is largely due to issues of cross resistance when insects feeding on maize are known to cross over to feed on cotton and experience selection for resistance to a similar Cry endotoxin protein in Bt-cotton.

Insect resistance management plans for MON810 also require Monsanto to monitor field insect populations for development of resistance and reporting to the Agency any adverse events. Further, the registration of MON810 requires Monsanto to instruct growers to report any sudden increase in insect damage to the Bt-maize crop, and the company is mandated both to examine such reports thoroughly and to pass such reports on to EPA⁵

Finally, to discourage overuse that might contribute to the emergence of insect resistance, the seed bags must also contain a statement indicating that this seed is for use in controlling specific pests, such as the ECB.

Impacts on non-target insects. The potential for toxicity to non-target organisms is another area where adverse effects could occur. In particular, one area EPA considered was potential impact on non-pest lepidopteran species (*e.g.*, butterflies) to determine if they might be adversely impacted. No threat to listed (threatened or endangered) species was found for MON810 following an analysis by EPA risk assessors. EPA considered the potential toxicity of Bt-maize to Monarch butterflies and related species. EPA concluded that Bt-maize poses an extremely low risk (Sims, 1995). This conclusion rested on an expectation that there would be relatively few milkweed plants (Monarch food source) near or in maize fields and on an expectation that the amounts of MON810 pollen which might land on adjacent milkweed plants would be below toxic levels. Since the demonstration by Losey *et al.* (1999) that Bt-maize pollen can be toxic to monarch larvae when present in significant amounts, a

MON810 may lead to the emergence of insect resistance.

⁵ If resistance to Cry1Ab were noted in a particular region, the Agency would need to decide whether further regulatory action would be appropriate. EPA would be able to choose among a range of measures to address the potential risk, including: requiring changes in refuge size and spray/treatment options; restricting sales of MON810 in an area; limiting distribution of Cry1Ab in other crops; or cancellation of the plant-pesticide registration.

wealth of field and lab data has been collected (Hansen and Obrycki, 2000; Herms *et al.*, 1997; Pilcher *et al.*, 1997; Pimentel and Raven, 2000; Wraight *et al.*, 2000). To date, these further studies have confirmed EPA's earlier finding that the risk to monarch butterflies from Bt-maize pollen is extremely low. In addition, Monarch larvae have been shown to avoid pollen in amounts that would be required to deliver a detrimental dose of Cry1Ab (Losey *et al.*, 1999). Finally, the aerodynamics of corn pollen are such that deposition of pollen grains in concentrations sufficient to harm other lepidopterans beyond 1 m from the field edge is highly unlikely as determined from field studies.

Following registration of MON810, academic researchers conducted a laboratory study in which larval Monarch butterflies (*i.e.* caterpillars) were fed Bt-maize pollen combined with milkweed, the Monarch's food source. The study showed that the endotoxin in this form and dose level was toxic to the Monarch caterpillar (Losey *et al.*, 1999). In order to evaluate more fully the potential risk to Monarch butterflies, EPA imposed the following requirements for data on the registrant:

- determination of the land mass involved with growth of milkweed plants and inhabited by Monarch butterflies;
- distribution of milkweed plants near maize fields;
- species of milkweed actually fed upon by Monarchs;
- effect of herbicides used in maize on milkweed;
- toxicity of Cry1Ab to Monarch larvae;
- lethality of pollen from MON810 plants to Monarch larvae;
- palatability of maize pollen to Monarch larvae; and
- various information on the natural history of Monarch butterflies.

At the time of this writing, EPA is in the process of reviewing this information to assess the potential risks and the need for possible mitigation measures. Milkweed issues are addressed because milkweeds are the host plants for monarch larvae, so their distribution relative to corn agriculture represents an important component for evaluating the potential for effects of *Bt* corn on monarchs. Milkweeds are considered a weed in corn agriculture, and are therefore subject to control measures by cultural practices (*e.g.*, tillage, cultivation, herbicides). In areas where weed control is practiced this may result in much higher milkweed densities in non-corn areas, such as pastures, roadsides, and fallow fields. The larvae of approximately 90% of the monarch butterflies passing through the corn belt will feed on the 7 most common milkweed species found in that area (Monarch Watch, 2000).

Roughly 50% of the monarchs in the US may pass through the corn belt each year (Wassenaar and Hobson, 1998). Recent estimates (MBRS, 1999; USDA, 2000) are that approximately 1.5 million square miles represent the summer monarch breeding area, with 10.5 % of this area comprised of corn fields. In the corn belt, 16.4 % of the potential summer monarch breeding range is estimated to comprise corn fields. More recent estimates are in the 10 % range (USDA, 2000).

Based on data from the 1997 U.S. Census of Agriculture, the total area under corn cultivation in states that have been identified as breeding areas for the monarch butterfly is approximately 105,174 square miles. This represents 18 percent of the 570,045 square miles of crop, pasture, and range land associated with monarch breeding sites. If hazard to monarchs is limited to milkweed at the field edge, the analysis indicates that a 1-m field edge margin typically represents a 1 % increment of the planted field area. The field edge habitat estimate may have minor significance in light of new information collected in the summer of 2000. The new studies show that milkweed grows-well between corn rows and that monarch larvae were seen on these plants during the peak breeding period (Marcotty, 2000). This would indicate that monarch larva exposure to *Bt* pollen would take place in *Bt* corn fields in geographical locations where there is an overlap of pollen shed and monarch breeding. Here one needs to factor in the preliminary data showing that there is no pollen shed and monarch breeding overlap in most of the corn belt, except in the northern range. And in assessing hazard in the northern corn belt, one needs to look at the findings that MON810 corn pollen at levels found in the fields showed no detrimental effect on monarch development.

It is the larval stages of monarchs, not the adults, that are potentially affected by *Bt* corn pollen because it is the larvae that may ingest *Bt* corn pollen. The Cry proteins incorporated into *Bt* corn need to be ingested to exert their toxicity; *Bt* corn products do not represent contact toxins. Pollen from these events are unlikely to be found in densities that may affect non-target lepidopterans, even on milkweeds within a corn field. Additionally, modeling work on the overlap of pollen shed timing with the presence of monarchs indicates that, for most of the corn belt, except for the northern range, the monarch larvae are not present during pollen shed. Biological activity of *Bt* corn pollen against sensitive lepidopteran larvae is significantly reduced within approximately one week, or less in wind and rain, after pollen shed.

For MON810 no effects on larval survival were observed at pollen concentrations up to 1,445 pollen grains/sq. cm of leaf surface, although slight-to-moderate effects on larval weight were seen. No effects on larval weight were observed at 1,100 grains/sq. cm. 90% of the pollen distribution on milkweed leaves in corn fields is below 500 grains/sq. cm. Other studies show mean pollen deposition in fields as low as 60 to 150 grains/sq. cm. Several *Bt* and non-*Bt* corn field studies showed no differences in monarch larval survival. In general the field data show that larval survival increases with closeness to corn fields. In sweet corn treated with conventional spray pesticides a 90-100% larval mortality was seen within one hour of pesticide application. MON810 has only trace levels (<90 ng/g dry wt.) of Cry1Ab protein in pollen. [The above data were collected during the 2000 growing season were presented at the USDA Monarch Data Review Workshop, November 16-17, 2000, Chicago, IL.]

To the extent EPA identifies any potential effects on non-target insects, especially beneficial or non-pest insects, the Agency will require mitigation efforts to minimize exposure to the endotoxin. In the

case where pollen expresses the endotoxin protein and pollen falls in sufficiently heavy amounts on the host plants fed upon by non-target insects, a potential for harm to non-target insects might exist. This hazard, where it exists, could be alleviated by a change in the promoter sequences that drive expression of the endotoxin in pollen. Also, the planting of sufficient border rows of non-Bt maize to halt drift and deposition of Bt-pollen onto host plants (*e.g.*, milkweed) outside of the maize field would mitigate exposure of non-target insects to the endotoxin in that area. As mentioned above, this is not a problem with MON810 due to the low level of expression of Cry1Ab in the pollen and its low toxicity to monarch larvae.

USDA/APHIS

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 in 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.

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 (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 (Organization for Economic Cooperation and Development (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. (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 corn. 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 containment 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 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 Monsanto, available information about the crop (corn), and the engineered genes, APHIS assessed the risks of introduction of MON 810. 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 35S 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 MON 810 corn 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 very nature of the product, use of the MON 810 could be accompanied by a shift in insecticide usage patterns, depending on the prevalence of the target insect species and other crop insect pests which are not sensitive to the Cry1Ab protein. As noted above, EPA has the authority under FIFRA to regulate the pesticidal use of this Cry protein and other pesticides used in the cultivation of crops.
- Potential to harm nontarget organisms - APHIS considered the mode of action of the delta-endotoxin Cry1Ab, the documented low toxicity of EPA-registered microbial formulations, and field observations of MON810 that revealed no negative effects on nontarget organisms and on endangered species.
- Potential of the crop plant to become a weed - Central to the conclusion that MON 810 maize is not likely to become a weed is the significant evidence that maize does not possess weedy tendencies, nor is it listed in standard texts or references as a weed. The introduced characteristic of resistance to some lepidopteran species is not expected to add any characteristics of weediness to MON 810. In the highly unlikely event that there was a need to control MON 810 as a weed, a wide range of options are available.
- Potential to affect "weediness" of sexually compatible plants - In general, wild relatives of corn do not grow in the United States, except in some isolated plantings. Cultivated corn and wild diploid and tetraploid members of *Zea* can be crossed to produce fertile offspring. Nonetheless, in the wild, introgressive hybridization does not occur because of differences in flowering time, geographic separation, block inheritance, developmental morphology, seed dissemination, and dormancy.
- Potential impacts on biodiversity - APHIS concluded that MON 810 maize does not pose a threat to biodiversity based on: 1) It will not become a weed and does not significantly hybridize with related species, 2) The high specificity of the Cry protein and its lack of toxicity to humans, other mammals, and threatened and endangered species will result in an insignificant threat to nontarget species. 3) APHIS can envision no threat to biodiversity for MON 810 that does not apply to traditionally bred maize.

APHIS applied the foregoing principles of hazard identification and risk assessment in the course of authorizations for field testing and the later determination as to the regulated status of MON 810 maize. MON 810 was field tested from 1992-1996 under seven separate APHIS authorizations which specified biological confinement of viable plant material. Prior to the revision of the regulations in 1993 to include a notification procedure for APHIS authorizations, field tests were conducted under APHIS permits.

In 1995, Monsanto originally included line MON 810 among other BT-maize lines in a petition

submitted to APHIS for a determination of non-regulated status (APHIS# 95-093-01p). Monsanto later amended their petition to drop MON 810 from the petition, because MON 810 was not sufficiently characterized to meet APHIS standards. The other BT-maize line (MON80100) of this petition went on to be granted non-regulated status from APHIS in 1995. In 1996, Monsanto submitted a petition (APHIS # 96-017-01p) requesting an extension of nonregulated status granted in Petition 95-093-01p to lines MON809 and MON810, which are very similar to the antecedent organism (MON80100) granted non-regulated status under petition 95-93-01p.

In 1997, APHIS amended its regulations to provide a formal mechanism for addressing extensions of previous determinations. APHIS conducted its assessment of MON810 in the same manner as the antecedent organism, MON801. APHIS reached a determination of non-regulated status for MON810, and the agency adopted the previous environmental assessment through reference to 95-093-01p, because the organisms were so similar (the EA for 95-093-01 is available at (<http://www.aphis.usda.gov/biotech/pubs.html>)). APHIS concluded that extending the determination of non-regulated status to MON 810 would likewise pose no significant impact on the environment.

Announcement of the APHIS review of MON 810 under NEPA and APHIS' conclusion of FONSI was published in the Federal Register and made available for public reading and comments (see section on "Transparency and Public Involvement" for additional discussion)(the FONSI and determination documents can also be found at <http://www.aphis.usda.gov/biotech/pubs.html>).

Coordination of USDA, EPA and Other Federal Agencies

As indicated above, some overlap exists in the scope of the risk assessments conducted by APHIS and EPA with respect to determining the potential of a GEOP to impact the agricultural environment including issues of outcrossing, weediness and plant pathogenicity. Many of the registrants that submit a data package to EPA for consideration of an EUP or Section 3 registration have already submitted a data package to APHIS for their risk assessment. While the specific evaluations or tests requested from the company differ with respect to what they are trying to accomplish, enough relevance and common ground exists such that both Agencies may benefit from sharing of risk assessments and related information.

When areas of regulatory overlap indicate a need for EPA or APHIS to consult with the other Agency, this is most often accomplished by a simple telephone call or e-mail communication. More recently, an effort is underway to hold scheduled conference calls between the two groups when submissions regarding the same regulated article or plant-pesticide are made to both agencies. The timing and frequency of these conferences will be determined by the rate of the review process and the novel aspects of the plant-pesticide at hand.

The improved coordination being discussed is likely to include periodic meetings between the agencies. Specific coordination measures that are likely to be implemented include the following. APHIS will provide EPA a copy of its draft Environmental Assessment (EA) prior to its publication to discuss impact of plant-incorporated pesticide on nontarget organisms, threatened and endangered species, and issue associated with gene flow. EPA will provide list of the currently registered pesticides used to control the target pest.

APHIS and EPA have 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.

4. Information and Data (What and How is Data and Information Collected and Generated)

EPA

FIFRA and FFDCA give EPA the authority to require whatever studies are necessary to complete a risk assessment of a pesticide. EPA regulations (40 CFR Part 158) detail the standard data requirements for plant-pesticides. Applicants may request waivers for required studies if they deem such studies unnecessary for a risk assessment. Guidelines (885 series) determine the protocols that may 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 if 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 GEOP.

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, field expression data and product characterization studies are also generally performed by the applicant. Toxicity and non-target studies are usually done by an outside contract lab that has experience in toxicology and the application of EPA guideline requirements. If the guideline requirements are not met (*i.e.*, the study was not performed using accepted procedures), then an additional study may be required. In the case of MON810, an additional study on the aquatic invertebrate, *Daphnia magna*, was required to assess the toxicity of Cry1Ab to this organism because the original maize line submitted for review (MON801) showed a decrease in Cry1Ab expression due to inbreeding effects. The new line proposed, MON810, expressed Cry1Ab in pollen, potentially

increasing the exposure of organisms like *Daphnia* to the endotoxin.. MON810 and MON801 were each transformed with the same plasmid construct (PV-ZMCT01). The MON810 progeny express a slightly truncated version of Cry1Ab compared to MON801, but the active site is still retained. The MON810 progeny do not express in detectable levels the marker gene products found in MON801 progeny. On 5/29/96, BPPD registered *Bacillus thuringiensis* delta-endotoxin as produced by the *cry1Ab* gene and the genetic material necessary for its production (PV-ZMCT01) in corn. Although this new active ingredient is not limited to a particular corn line, the registration was originally limited to corn line MON801.

On 7/16/96, BPPD amended this registration to allow plantings of corn line MON810. However; additional studies of quail, catfish, and *Daphnia* were required for the full commercial registration of MON810. These studies were listed as data gaps because although some of the data in the nontarget organism database supporting the registration were generated using *E. coli* produced Bt protein, the test substance for the quail and catfish studies already reviewed was MON801 seed.

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 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.

Appropriate scientific and regulatory expertise exists within APHIS, EPA and FDA to review all submissions for scientific accuracy and interpretation. EPA evaluates data for scientific soundness based on experience with the types of studies and the anticipated results. EPA scientists (4 reviewing product chemistry and health effects data, 7 reviewing environmental effects and insect resistance management data) 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 basis or targeted to a specific laboratory if there is reason to believe that data have been falsified or in any manner misrepresented.

USDA-APHIS

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 insure containment, 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 containment 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, which 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.

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

EPA

This case study has already discussed many different types of conditions that may be imposed on an experimental use permit or registration of a plant-pesticide. It has also described the conditions that were imposed with reference to MON810. In addition, for non-commercial field release (*i.e.*, > 10 acres, but plants not to enter the food supply; fields monitored for volunteer plants), containment of the test site can be mandated to preclude movement of the GEOP into the wild. As discussed in the review of APHIS' program below, this can be achieved in a variety of ways, with both physical and biological barriers.

USDA-APHIS

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 maize, this would involved 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 MON 810 maize.

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.

6. Monitoring and Consideration of new Information

EPA

As discussed above, EPA has considerable ongoing authority to regulate the post-registration use of a plant-pesticide. This authority includes: 1) issuance of data call-in notices to obtain additional information from registrants needed to evaluate the safety of a pesticide (see section 3. A. 1., above) and 2) assuring compliance with conditions imposed on the plant-pesticide's registration.

As a condition on the registration of MON810, EPA required Monsanto to develop and implement plans for monitoring insect resistance management. A key element of the monitoring plans for MON810 is the observation of ECB, CEW and SWCB for evidence of the buildup of resistance. The registrant must educate growers through a formal program to recognize and report any lack of efficacy in MON810 and unusual damage to plants from the target insects. Any adverse incident reports received by the registrant must be filed with Agency. The registrant must also conduct grower surveys to assess the degree of compliance with refuge implementation and any related monitoring issues. Because the Agency does not have the capacity to undertake the implementation of the monitoring plan, the registrant must supply the necessary infrastructure to ensure that the monitoring for resistance, grower education and refuge requirements are enforced.

EPA, however, performed and will continue to take an active oversight role in both the development and implementation of the monitoring plans, as well as in assuring that there is compliance with other requirements. The monitoring plans were developed by the registrant using a process that included input from Agency scientists, university researchers and guidance from the SAB / SAP. Once developed, the Agency required the registrant to submit detailed regional monitoring plans for review and approval.

The Agency has also requested data on compliance from registrants and has the legal authority to further investigate any issues associated with conditions of registration. Typically, EPA reviews the data submitted to assess the degree of monitoring performed and the potential need for further alterations to address specific concerns. The Agency has the authority to inspect and evaluate monitoring and other conditions of registration if it deems this necessary. Some degree of compliance is assured through a comparison of USDA-NASS data (<http://www.usda.gov/nass/pubs/histdata.htm>) with the information submitted by the registrant.

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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 MON 810 maize.

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 founded 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.

7. Enforcement and Compliance

EPA

FIFRA and FFDCA generally provide the authority to enforce all provisions regarding regulation of pesticides and presence of pesticide residues on food products. As noted above, EPA relies on an assessment by the registrant to determine compliance. Grower surveys have been conducted by some university scientists as well, to estimate the degree of compliance with insect resistance management requirements. As part of the Agricultural Biotechnology Stewardship Technical Committee and the NC-205 research committee, scientists sample insect populations for development of resistance. The registrant is required to report any adverse events, such as the development of insect resistance or the sudden increase in pest related crop damage, to the EPA under the FIFRA § 6(a)2 reporting requirement provision.

EPA can take regulatory action to impose penalties (fines) or to restrict or prohibit the sale and distribution of any registered product (*i.e.*, cancellation of the product), if it 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 (*i.e.*, seeds) 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.

USDA-APHIS

APHIS has qualified personnel in every State that can inspect field sites for compliance to the performance standards for all field testing. In addition, to APHIS field inspectors, officials of the State Department of Agriculture can inspect sites. Also, members of the headquarters staff will continue on a case-by-case basis to inspect sites that potentially raise unique containment issues with respect to engineered organisms.

USDA-APHIS reviews the experimental design protocols for field tests to ensure that performance standards are met. Failure to comply with performance standards under notification or permit conditions can result in the owner being 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). 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)). The owner can also be assessed a criminal or civil penalty for failing to comply with the regulations (7 U.S.C. § 7734). For example, some remedial actions might involve removing the plants by burning , spraying herbicide, hoeing or discing. No failure to comply was detected with MON 810 maize field tests.

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).

Failure to comply with performance standards under notifications or permit conditions can result in compliance infractions. From 1995 through 2000, APHIS recorded a total of 63 such 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, a 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. 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 to be necessary to prevent the spread of plant pests (7 CFR 340.4 d 7). These remedial actions include removing the plants by burning, spraying herbicide, hoeing or discing. No infractions were identified in the case of MON 810.

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).

8. Public Involvement and Transparency

EPA

EPA publishes Federal Register Notices announcing the receipt of applications for an Experimental Use Permit (EUP) and for registration, and invites public comment on the proposed action. For MON810, no comments were received from the public. In addition, Federal Register notices announcing approval of EUPs and registration of pesticides containing new active ingredients are also published. EPA also publishes Federal Register Notices announcing the notice of receipt of a request for a food tolerance or exemption and provides opportunity for public comment on the petition. Although not required by statute, EPA also holds meetings with groups and individuals interested in particular pending regulatory actions. During the course of registration of MON810, for example, several groups (*e.g.*, corn growers associations, biotechnology industry groups, environmental groups) visited EPA to discuss issues and concerns relative to this GEOP.

Additionally, the Agency has held workshops on GEOPs within which concerned citizens, non-governmental scientists and other researchers were provided the opportunity to discuss and add to the process. Open Scientific Advisory Panel (SAP) meetings have been held on various topics including insect resistance management, toxicity, allergenicity, non-target organism effects and other aspects associated with plant-pesticides (GEOPs). During these Panel meetings, the public is invited to make public statements and engage the panel in discussion of specific topics. The degree of public input varies with the topic of the meeting. Typically each presenter is allowed to make an oral presentation and subsequent interchange with the Panel occurs if the Panel raises questions. Written statements are also received and included in the docket. EPA considers all comments in making its regulatory decisions.

All studies submitted to the Agency that are not considered as confidential business information (CBI) and all submissions to the SAP are made available through the public docket. The docket number and contact information is published in the Federal Register Notice announcing the registration or tolerance associated with a pesticide. For an SAP meeting, a docket is similarly established for receipt of comments. Information on upcoming and recently held SAP meetings can be found on the EPA website listed below. Portions of the product chemistry section associated with the genetic sequence of introduced genes and the details of the transformation methodology are often restricted as CBI. This can vary based upon what is requested by the registrant and what EPA deems appropriate. Results of toxicity studies are not classified as CBI. EPA works with all stakeholders as part of an open and transparent regulatory process.

The Agency website (<http://www.epa.gov/oppbppd1/biopesticides/>) and published materials (*e.g.*, booklets, proceedings of workshops, pamphlets) help disseminate information related to GEOPs. Both APHIS and EPA websites provide a list and links to regulations and provides an explanation of the process. Regulatory decisions and the outcome of EPA's toxicology reviews are posted for public review. The website also provides for contact directly with Agency scientists and regulators to address issues of concern. Additionally, scientists may publish articles in trade and peer-reviewed journals, monographs and books that outline Agency position on topics related to regulation of GEOPs.

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 plant-pesticides. The Freedom of Information Act (FOIA) also provides for the request of any document submitted to support a pesticide registration as long as it does not contain confidential business information.

USDA-APHIS

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. Two advisory committees have had a significant role in providing a public source of advice from stakeholders to the Agency, the Agriculture Biotechnology Advisory Committee, and the Agricultural Biotechnology Advisory Committee.

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 involve 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 MON 810. 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 complement 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 has 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.

APHIS prepares EAs for field tests in accordance with its NEPA implementing regulations (7 CFR 372) and accepts written comments received following announcement of the EA in the Federal Register. APHIS distributes copies of EAs via mail or electronically.

Petitions for determination of nonregulated status. Every petition submission is announced on the APHIS Internet home page (<http://www.aphis.usda.gov/petday.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 Finding of No Significant Impact (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 nonregulated status are all available electronically at the APHIS home page or in hard copy. Copies of APHIS decision documents are available at the 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 excluded, 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 applicant’s petition. Since 1999, notice of availability of draft environmental assessments for determinations for non-regulated status are published in the Federal Register and provide for a 30-day comment period. 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 (see bentgrass sidebar for example of performance standards). 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.

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Appendix A - BIBLIOGRAPHY OF SUBMITTED STUDIES - EPA

See <http://www.epa.gov/oppbppd1/biopesticides/news/news-bt-crops-sap-oct.htm>
 EPA's Biopesticide Registration Action Document for Bt crops is available on the web site and details the studies submitted in support of MON810 Bt-maize.

Study Title	MRID #
Molecular Characterization of Insect Protected Corn Line MON 80100: Lab Project Number: MSL 13924. Unpublished study prepared by Monsanto Co. 100 p.	43533201
Compositional Comparison of <i>Bacillus thuringiensis</i> subsp. <i>Kurstaki</i> HD-1 Protein Produced in European Corn Borer Resistant Corn and the Commercial Microbial Product, DIPEL: Lab Project Number: 94-01-39-12: MSL 13876. Unpublished study prepared by Monsanto Co. 35 p.	43533203
Molecular Characterization of Insect Protected Corn Line MON 810: Lab Project Number: MSL 14204. Unpublished study prepared by Monsanto Co. 61 p.	43665501
Assessment of the Equivalence of the <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> HD-1 Protein Produced in <i>Escherichia coli</i> and European Corn Borer Resistant Corn: Lab Project Number: 94-01-39-09: MSL 13879. Unpublished study prepared by Monsanto Co. 94 p.	43533204
Acute Oral Toxicity Study of <i>Btk</i> HD-1 Tryptic Core Protein in Albino Mice: Lab Project Numbers: 92069: 11985:ML92069. Unpublished study prepared by Monsanto Co. 264 p.	43468001
Assessment of the In vitro Digestive Fate of <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> HD-1 Protein: Lab Project Number: 93-01-39-04. Unpublished study prepared by Monsanto Co. 44 p.	43439201
Stability of the CryIA(b) Insecticidal Protein of <i>Bacillus thuringiensis</i> var. <i>kurstaki</i> (B.t.k. HD-1) in Sucrose and Honey Solutions Under Non-refrigerated Temperature Conditions: Lab Project Numbers: IRC-91-ANA-11: MSL 13375:13375. Unpublished study prepared by Monsanto Co. 32 p.	43468002

Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Larvae: Lab Project Number: IRC-91-ANA-13. Unpublished study prepared by Monsanto Co. 51 p.	43439202
Molecular Characterization of Insect Protected Corn Line MON 810: Lab Project Number: MSL 14204. Unpublished study prepared by Monsanto Co. 61 p.	43665501
Evaluation of Insect Protected Corn Lines in 1994 U. S. Field Test Locations: Lab Project Number: 94-01-39-01: 14065: 14179. Unpublished study prepared by Monsanto Co. 147 p.	43665502
Activated Btk HD-1 Protein: A Dietary Toxicity Study With Green Lacewing Larvae: Lab Project Numbers: WL-92-155: 139-3388. Unpublished study prepared by Monsanto Co.; and Wildlife Int'l, Ltd. 21 p.	43468003
Activated Btk HD-1 Protein: A Dietary Toxicity Study With Ladybird Beetles: Lab Project Numbers: WL-92-156: 139-321. Unpublished study prepared by Monsanto Co.; and Wildlife Int'l, Ltd. 23 p.	43468005
Activated Btk HD-1 Protein: A Dietary Toxicity Study With Parasitic Hymenoptera (<i>Brachymeria intermedia</i>): Lab Project Numbers: WL-92-157: 139-320. Unpublished study prepared by Monsanto Co.; and Wildlife Int'l, Ltd. 24 p.	43468004
Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Adults: Lab Project Number: IRC-91-ANA-12. Unpublished study prepared by Monsanto Co. 70 p.	43439203
A Dietary Toxicity Study with MON80187 Meal in the Northern Bobwhite: Lab Project Number: WL 94-150: 139-387. Unpublished study prepared by Wildlife International Ltd. 32 p.	43533205
Corn Pollen Containing the CryIA(b) Protein: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (<i>Daphnia magna</i>): Final Report: Lab Project Number: WL-96-322: 139A-201: 95-152E2. Unpublished study prepared by Wildlife International Ltd. 23 p.	44271502

<p>Evaluation of the European Corn Borer Resistant Corn Line MON801 as a Feed Ingredient for Catfish: Lab Project Number: 94-01-39-16: 14066:95-459-720. Unpublished study prepared by Mississippi State University Delta Research and Extension Center. 39 p.</p>	<p>43887901</p>
<p>Effect of the <i>Bacillus thuringiensis</i> Insecticidal Proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on <i>Folsomia candida</i> and <i>Xenylla grisea</i> (Insecta: Collembola): Lab Project Number: 93-081E1. Unpublished study prepared by Monsanto Co. 22 p.</p>	<p>43941601</p>
<p>Chronic Exposure of <i>Folsomia candida</i> to Corn Tissue Expressing CryIA(B) Protein: Lab Project Number: 7140-97-0030-AC-001: XX-97-064: 95-152E2. Unpublished study prepared by Ricerca, Inc. 91 p.</p>	<p>44271501</p>
<p>CryIA(b) Insecticidal Protein: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Final Report: Lab Project Number: 139-417: WL-95-281. Unpublished study prepared by Wildlife International Ltd. 25 p.</p>	<p>43887902</p>

Appendix B - Pesticide Fact Sheet - EPA

See <http://www.epa.gov/oppbpd1/biopesticides/factsheets/fs006430t.htm> for a fact sheet detailing the product chemistry, health effects and environmental risk assessments, and regulatory history of MON810 maize.

APPENDIX C - Plant Phenotypic and Environmental Interactions- USDA-APHIS

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, NO_x), 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. II.A

BIOCONTROL USING A VIRUS (AcMNPV)

Overview

This sidebar examines a baculovirus that affects gypsy moths and which has been genetically modified to express scorpion toxin. The transgenic virus kills tobacco budworm and corn earworm, which are plant pests, more quickly than the unmodified virus. This GEO is still in small-scale field test stage.

1. Proposed Organism and Use

Granuloviruses (GVs) and nucleopolyhedroviruses (NPVs) belong to a family of insect viruses called baculoviruses. Baculoviruses infect insects, such as moths and beetles, and certain closely related species. Most of the research on GVs and NPVs has involved viral species that infect insect larvae that harm plants; all of the baculovirus species approved as of February 2000 for use in pesticide products act against moth larvae. Baculoviruses are relatively specific regarding their target insects. For example, the gypsy moth NPV seems capable of infecting only gypsy moth larvae and other Lymantriids.

GVs and NPVs have a more complicated structure than most viruses. Most known viruses exist as individual viral particles, with each particle consisting of viral nucleic acid surrounded by a protein shell. By contrast, GVs and NPVs are complex viruses, protected by a protein overcoat. For NPVs, there are usually one or more enveloped virus particles or virions embedded in a proteinaceous matrix, called polyhedrin. GVs, by contrast, have one enveloped virus particle or virion embedded in a protein matrix called granulin. For both kinds of insect viruses, the protein overcoat and everything within it is called an "occlusion body." It keeps the virus particles occluded, or separate, from the outside environment. Because the occlusion bodies are the actual structural units that infect larvae, EPA has registered the occlusion bodies of individual viruses as the pesticide active ingredient.

These insect viruses become active only after susceptible larvae ingest the occlusion bodies. In the larval gut, the protein overcoat quickly disintegrates, and the viral particles proceed to infect digestive cells. Within a few days, the larvae become unable to digest food, and they weaken and die.

Tests show that the GV and NPVs that EPA has registered as pesticide active ingredients

specifically infect only certain species of moth larvae. The viruses do not harm other organisms, including plants, beneficial insects, other wildlife, or the environment. These viruses occur naturally in their insect hosts.

The nuclear polyhedrosis virus (AcMNPV) of *Autographa californica* has been genetically modified to express the toxin of the scorpion, *Leiurus quinquestriatus hebraeus*. This multiple embedded wild type nuclear polyhedrosis virus (AcMNPV) has the ability to infect *Trichoplusia ni*, *Heliothis virescens*, *Helicoverpa zea*, and, to a lesser extent, *Spodoptera exigua* and *S. frugiperda*. The addition of the insect-specific scorpion toxin to the viral genome provides for a rapid mortality among infected insects, but does not alter the host range of the virus significantly (AcMNPV/LqhIT2), if at all.

In total, six submissions have been received for field testing of genetically modified baculoviruses from May 1995 through August of 1998. Four of these utilized the AcMNPV with additions of insect-specific toxin genes: three from two different scorpions and one from a mite. Two others are based upon modified *Helicoverpa zea* single-embedded nuclear polyhedrosis virus (HzSNPV) each using an insect-specific scorpion toxin from one of two scorpion species. Since the issues are very similar between the various baculovirus constructs, only the AcMNPV/LqhIT2 biopesticide will be discussed herein.

The AcMNPV/LqhIT2 would be sprayed onto leaf surfaces and thereby consumed by leaf feeding insects, such as the tobacco budworm / corn earworm (*Heliothis virescens* / *Helicoverpa zea*). The virus does not replicate to the same degree as wild type AcMNPV and, therefore, is somewhat limited in its spread. Since the mortality observed for wild type and modified AcMNPV/LqhIT2 was similar in the *H. zea* system, it is concluded that the genetic modification (i.e., addition of the scorpion toxin gene) does not alter the basic pathogenicity or host range of the AcMNPV.

The modified virus (GEO) will be applied to tobacco, cotton, cabbage, broccoli, and a few other vegetables for control of foliar feeding insects (e.g., corn earworm, cabbage looper). AcMNPV occlusion bodies are produced by mass infection of susceptible insects in the laboratory and harvest of the cadavers after a prescribed time. Insect cadavers contain large numbers of infective occlusion bodies, which contain individual viral particles in a membrane bound matrix. Homogenization and formulation of these particles is needed to prepare a workable biopesticide. When applied to vegetable and other crops, the particles reside on the external plant surfaces and are consumed by feeding insects. Once ingested, the particles will find their way into the cells of susceptible individuals. There they take over the host cell machinery and produce more infective virions within the occlusion body matrix. This may take several days and the inclusion of the insect-specific scorpion toxin to the viral genome speeds up the time to death for the infected insects. This, of course, results in less feeding damage to the crop as even infected insects will continue to feed.

Since the virus is not particularly stable on the plant surface, especially when exposed to sunlight and temperature extremes, and the transfer of virus from deceased host to a new living host is minimized compared to wild type virus, the AcMNPV/LqhIT2 will not persist very well in the environment. The host range of AcMNPV/LqhIT2 could conceivably include other insects than the target pest. Although the host range is known to be broader than some other NPV, the insects coming into contact with this biopesticide are likely to be pests as well, given the application site scenario. The AcMNPV/LqhIT2 is modified to provide a quicker death to the target insect, but does not show indications that the host range is enhanced or broadened. Field test and laboratory evaluations were conducted and reviewed, indicating that the host range is the same as wild type viruses already present in the environment. These baculoviruses are known from the literature to be specific to lepidopteran insects.

If the AcMNPV/LqhIT2 was able to proliferate and outcompete wild type NPV, it is conceivable that persistence of this biopesticide could alter the mortality of the subset of lepidopteran insects known to be susceptible. This is not likely to occur however, since the behavioral changes in infected larvae (with AcMNPV/LqhIT2) are known to be contrary to that contributing to a sustained epizootic. Larvae infected with AcMNPV/LqhIT2 form small, hard cadavers after being paralyzed by the insect-specific scorpion toxin and fall to the ground. In contrast, wild type NPV infected larvae liquefy and spread their viral load onto the leaf surface after death.

Given the specificity of AcMNPV/LqhIT2 and the target pests proposed, a general reduction in use of more broad-spectrum, chemical insecticides should ensue. This could encourage the proliferation of beneficial insect predators. Additionally, some of these pests do considerable damage even after being infected with wild type NPV or other entomopathogens, while the time to death following infection with the modified NPV reduces this window. The reduction in use of less specific (i.e., more toxic) control agents is a plus and the more rapid kill of the target pests results in less damage (i.e., better yield). For some crops (e.g., cabbage), the cosmetic appearance of the harvested product is key to marketability. By having the AcMNPV/LqhIT2 result in a faster kill, the overall marketable harvest may be drastically increased.

2. Relevant Regulatory Agencies, Regulatory Authority and Legal Measures

Two federal agencies, EPA's Office of Pesticide Programs and USDA's Animal and Plant Health Inspection Service (APHIS), share the primary responsibility for regulating microbial pesticides. Whenever claims are made for reducing damage caused by pests, the product is a "pesticide" subject to EPA oversight. EPA registers and regulates pesticides, including genetically engineered organisms (GEOs) intended to be used to control a pest, such as an insect, or plant disease organism. EPA's review includes an assessment of the potential impacts on human health, as well as impacts on non-target wildlife and the broader environment. If there are residues of a microbial pesticide in or on food or feed, EPA must establish either a tolerance or a tolerance exemption for the food or feed bearing

those residues to move in interstate commerce (i.e., be sold). USDA analyzes GEOs for potential impact on agriculture, as well as for impacts on the broader environment.

In addition, the U.S. Department of the Interior and the Food and Drug Administration (FDA) have consultative and regulatory roles. FDA acts to review any GEOs that may cause an alteration in the nutritional state of a food or otherwise contribute to a food safety issue.

The following discussion focuses on those aspects of the regulatory regime most relevant to this sidebar. More details may be found in the accompanying case study (Bt-Maize).

Statutory authority

EPA

EPA administers two statutes which contain authority to regulate AcMNPV/LqhIT2 and other microbial pesticides: the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), 7 U.S.C. 136-136y, and section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 371-379d. Section 408 of the FFDCA was amended by the Food Quality Protection Act (FQPA), Pub. Law 104-170 (1996), after AcMNPV/LqhIT2 was approved for a small-scale field test. In addition, the Endangered Species Act (ESA) and the federal Migratory Bird Treaty Act (MBTA) impose obligations on EPA, which the Agency discharges in consultation with the Department of the Interior.⁶ Conducting these small-scale field tests in agricultural areas is not expected to expose any endangered or threatened species to this biopesticide. No listed species are known to feed on the crops being evaluated. A narrow host range for this baculovirus also minimizes the possibility a listed species might be harmed by exposure to AcMNPV/LqhIT2. Based on these provisions, no formal consultation with the DOI was required.

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.” AcMNPV/LqhIT2 was approved for field testing on a “crop-destruct” basis, hence, no tolerance was required.

Implementing regulations

⁶ Courts have determined that EPA’s risk assessment process is functionally equivalent to the NEPA process and therefore EPA is not required to conduct an EA or EIS as aprt of its registration process.

At least 90 days prior to conducting any small scale test of a genetically modified microbial pesticide, other than those described at 40 C.F.R. 172.45(d), a Notification must be submitted to the EPA in which the details of the genetic modification, proposed application methods and sites, and any potential toxicity or non-target organism effects are delineated. 40 C.F.R. 172, subpart C. Measures must also be outlined in the Notification submission which indicate the methods of containment and monitoring used to ensure the GEO does not become established in the ecosystem. 40 C.F.R. 172.48. The data required to support a request for a Notification are detailed in 40 C.F.R. Part 172.48. If the proposed field test is to be greater than 10 acres of treated land per pest evaluated, or greater than 1 acre for aquatic uses, then an experimental use permit is necessary. 40 C.F.R. 172.3.

Both of the genetically modified NPV biopesticides that have been considered by the Agency were processed through a Notification procedure for small-scale field tests. Neither NPV has been approved for an EUP or registered.

EPA's regulation of the pre-registration sale or distribution of a pesticide occurs primarily through its experimental use permit process. 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 of GEOs for more than 5000 acres.

In addition, if the experimental design involves the production of food for distribution in interstate commerce, a tolerance or temporary exemption is necessary to allow the food to be moved in commerce. 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. See 40 C.F.R. 172.4(b)(2).

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. Site visits to the experimental plots can and have been performed, resulting in plot destruction in one instance for failure to follow the conditions established in the EUP.

USDA-APHIS

The USDA's Animal and Plant Health Inspection Service (APHIS) has the authority to regulate plant pests and other articles, including insect viruses, to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. Under authority granted by the Plant Protection Act, 7 U.S.C. 7701-7772, APHIS regulates the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. 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 §340.2 of the regulations and is also a plant pest; if it is unclassified; or, if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk (7 CFR Part 340).

The National Environmental Policy Act (NEPA), 42 U.S.C. 4321-4375, applies to the APHIS review process and mandates consideration by APHIS of a broad range of environmental issues. In fulfilling its NEPA responsibilities, APHIS prepares an Environmental Assessment (EA) in which APHIS determines whether it has adequate information to conclude its proposed regulatory action will have no significant impact on the environment. If APHIS can make a “finding of no significant impact” (FONSI), NEPA requires no further analysis. If APHIS cannot make a FONSI, APHIS must prepare a draft environmental impact statement (EIS) and make it available for interagency and public comment.

As with other genetically engineered products, particularly insect viruses, USDA examines whether application of AcMNPV/LqhIT2 poses a direct or indirect plant pest risk to agriculture or the environment under its regulatory authority. AcMNPV/LqhIT2 was determined not to pose a plant pest risk, and therefore not regulated by APHIS. Thus further regulatory activities, including the environmental review, were deferred to EPA.

3. Hazard Identification, Risk Assessment and Regulatory Review of Product

FIFRA allows EPA to consider all relevant factor in reviewing and approving a pesticide for registration. This includes a risk / benefit analysis of the potential environmental and occupational impacts, and an assessment of all potential human health impacts. EPA may only register a pesticide if it finds that, when used in accordance with widespread and commonly recognized practice, the pesticide will not generally cause unreasonable adverse effects on the environment. FIFRA sections 2(bb) & 3(c)(5).

1. EPA’s Hazard Identification and Risk Assessment of AcMNPV/LqhIT2

Product characterization requirements for AcMNPV/LqhIT2 included details of the gene source (what organism), DNA and amino acid sequence data, details of the vector used in transformation (annotated map), method of transformation, the pesticidal substance encoded by the gene, expression levels under field conditions wherein the insect-specific toxin is expressed in susceptible insects, glycosylation of the pesticidal protein where appropriate (presence/absence and similarity between microbial and invertebrate forms), and bioassays against larvae of the corn ear worm or the cabbage looper.

The Agency also required the applicant to explain the use pattern (i.e., will the crop be used for human consumption, animal feed only, ornamental uses, etc.). AcMNPV/LqhIT2 is used for control of leaf feeding insects in various vegetable crops and tobacco.

Once the agency has understood the product and its proposed uses, EPA evaluates the potential hazards of microbial pesticides in two broad areas: human health and environmental effects. Risks to humans and animals via the dietary route, and to non-target organisms via water, wind, soil and direct consumption of the GEO are all considered within the risk assessment. If other routes of potential exposure exist, such as dermal absorption, these risks are also addressed.

Human health

No toxicity was noted in mammalian toxicity evaluations with the insect-specific scorpion toxin. In these toxicity studies, the dose level of toxin administered to the test animals far exceeded the possible human consumption levels via the diet or exposure in the environment through soil and water. Having this leeway one can expect that the level of human exposure to scorpion toxin from AcMNPV/LqhIT2 will fall far below any potential effect level.

Environmental assessment

Non-target organism studies include the toxicity characterization for: fish (catfish or trout), aquatic invertebrates (Daphnia), earthworms, Collembola (springtails), beneficial insects (green lacewing, ladybird beetle, honey bee, parasitic wasp), birds (Bobwhite quail or Mallard duck), and any other species considered as being exposed or at risk from the pesticidal substance.

Endangered or threatened species are given special consideration, and EPA may require testing with related, abundant species to assess possible non-target effects. Studies of non-target species are typically designed as single dose, maximum hazard toxicity assessments, and animals are observed for varying time frames depending on the species (14 to 30 days typically).

EPA does not attempt to evaluate the possible future changes in social and ecological conditions (e.g., climate). Predictability of climatic change is difficult at best. The limit dose testing performed for the assessment of toxicity is at a level that exceeds the amounts of pesticidal substance present on the plant surface under any foreseeable conditions. The EPA regulatory process does not end with registration, but continues and has the ability to modify the registration at a later date as warranted.

Finally, EPA takes into account any other relevant information. In the case of AcMNPV/LqhIT2, EPA considered the history of the use of baculoviruses as a microbial biopesticide and the wealth of literature regarding host range and mode of activity of these agents. This experience showed that the NPVs

have a limited host range and the insect-specific toxin has virtually no toxicity to mammalian and other non-target species. Any effect on other species vis-a-vis the insect-specific scorpion toxin would require infection of that species' cells as a prerequisite. Hence, the introduction of the toxin to the non-target organisms would be precluded.

2. EPA's Consideration of the Risks and Benefits of AcMNPV/LqhIT2

As noted above, FIFRA's standard for registration decisions involves an assessment of risks and benefits of using a pesticide. One of the primary benefits of a biopesticide is the replacement of pesticides that may pose greater risks, e.g., groundwater contamination, toxicity to non-target organisms, or dietary risks to infants and children. To date, however, decisions to approve NPVs have relied primarily on their lack of toxicity to all organisms tested, except target pests. Nonetheless, EPA has also considered possible benefits that might result from use of AcMNPV/LqhIT2. Application of AcMNPV/LqhIT2 will likely reduce the use of other insecticides and thereby will avoid the types of risks those insecticides might have had, if applied to the same acreage as AcMNPV/LqhIT2.

Targeting the insect-specific toxin to the point of feeding of pest insects should minimize the impact of pesticides on non-target organisms and minimize ground water contamination, as may occur with use of some chemical pesticides. Because many of the previously deployed insecticides were broad-spectrum in their activities, the potential for impacts on the beneficial insect populations was significant. Populations of beneficial insects should increase over time as more GEOs with host specificity are used and fewer broad-spectrum pesticides are applied. Since some insecticides have effects on non-insect organisms (e.g., earthworms, nematodes), the reduction or elimination of these pesticides will help to nurture these populations as long as cultural practices of soil management are adequate.

Additionally, the exposure of farm workers, pesticide applicators and the public in general is reduced when a biological pesticide takes the place of a chemical spray alternative. Residues on food are also less a concern with AcMNPV/LqhIT2, because the insect toxin is known to be non-toxic to humans and other mammals. Spray drift is often problematic with chemical applications, but this is not a significant issue with target specific NPVs.

3. History of EPA's Regulatory Review of AcMNPV/LqhIT2.

Based on the hazard identification, risk assessment, and risk-benefit analysis described above, EPA determined that the notifications submitted for AcMNPV/LqhIT2 met the statutory standards for issuing approval for a small-scale field test. Because AcMNPV/LqhIT2 and other similar modified NPV involve a new technology about which there is some uncertainty, EPA issued the permission with some restrictions: all crops treated must be destroyed; field tests must not be conducted in areas of

endangered species habitat; and each field test must include a non-recombinant NPV control inoculation to ensure a source of competing baculovirus and thereby reduce the persistence of AcMNPV/LqhIT2.

As noted above, AcMNPV/LqhIT2 is generally non-toxic to all species, except certain insects. These characteristics led EPA to focus primarily on two types of potential risks: (1) the persistence of AcMNPV/LqhIT2 in the environment, and (2) the risk to non-target insects. These risk scenarios require proper monitoring of proliferation of the AcMNPV/LqhIT2 based biopesticide, and examination of potential non-target influence on insects inhabiting the area of application to prevent or mitigate adverse effects. That is, if an adverse event should be observed, the potential hazard could be mitigated by halting further applications, altering the distribution of this biopesticide geographically or other remedial measures. Failure to monitor or analyze the data gathered from such assessments would be a potential avenue for proliferation of these risks.

4. Information and Data

FIFRA and FFDCA give EPA the authority to require whatever studies are necessary to complete a risk assessment of a pesticide. EPA regulations (40 CFR Part 158) detail the standard data requirements for plant-pesticides. Applicants may request waivers for required studies if they deem such studies unnecessary for a risk assessment. Guidelines (885 series) determine the protocols that may 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 if EPA will accept waivers for certain studies. After reviewing any waiver requests, agency scientists determine on a case-by-case basis whether studies will be waived, or additional studies will be required for a specific GEO.

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, field expression data and product characterization studies are also generally performed by the applicant. Toxicity and 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.

Appropriate scientific and regulatory expertise exists within APHIS, EPA and FDA to review all submissions for scientific accuracy and interpretation. 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 basis or targeted to a specific laboratory if there is reason to believe that data have been falsified or in any manner misrepresented.

5. Mitigation And Management Considerations: Approvals And Conditions On Research, Development, Production, Distribution, Marketing, Use And Disposal

This paper has already discussed many different types of conditions that may be imposed on an experimental use permit or registration of a microbial pesticide. In addition, for non-commercial field release, containment of the test site can be mandated to preclude movement of the GEO into the wild. This can be achieved in a variety of ways, with both physical and biological barriers.

6. Monitoring And Consideration Of New Information

As discussed above, EPA has considerable ongoing authority to regulate the post-registration use of a microbial pesticide. This authority includes: (1) issuance of data call-in notices to obtain additional information from registrants needed to evaluate the safety of a pesticide (see section 3. A. 1., above) and (2) assuring compliance with conditions imposed on the pesticide's approval for field testing.

As a condition on the approval for field testing of AcMNPV/LqhIT2, EPA required DuPont to develop and implement plans for monitoring persistence of AcMNPV/LqhIT2. A key element of the monitoring plans for AcMNPV/LqhIT2 is the observation of environmental persistence. The registrant must ensure the safe application to the area of the target insects while precluding any exposure to endangered species or other susceptible non-target organisms. Any adverse incident reports must be filed with Agency.

EPA, however, performed and will continue to take an active oversight role in both the development and implementation of the monitoring plans, as well as in assuring that there is compliance

with other requirements. The monitoring plans were developed by the registrant using a process that included input from Agency scientists.

7. Enforcement and Compliance

FIFRA and FFDCA generally provide the authority to enforce all provisions regarding regulation of pesticides and presence of pesticide residues on food products. As noted above, FDA is responsible for enforcing EPA's tolerance requirements. With respect to FIFRA compliance, as noted in the preceding paragraph, EPA relies on the independent assessment by researchers and the registrant to determine compliance.

EPA can take regulatory action to impose penalties or to restrict or prohibit the sale and distribution of any approved pesticidal product, including AcMNPV/LqhIT2, if it 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 (i.e., formulated NPV) or the assessment of civil and/or criminal penalties. FIFRA sections 13 & 14. FIFRA sections 8 and 9 provide statutory authority for the Agency to inspect the producing establishment, inspect books and records. In addition, if a pesticide does not comply with the provisions of FIFRA, or as a result of widespread misuse, EPA may cancel a pesticide registration. FIFRA section 6(b). EPA may also cancel or suspend a pesticide if necessary to prevent unreasonable adverse effects on the environment. FIFRA sections 6(b)-(c).

8. Public Involvement and Transparency

In addition to the general description in the accompanying case study (Bt-maize), open Scientific Advisory Panel (SAP) meetings have been held on various topics including insect resistance management, toxicity, non-target organism effects and other aspects associated with GEOs. During these panel meetings, the public is invited to make public statements and engage the panel in discussion of specific topics.

The Agency website (<http://www.epa.gov/oppbppd1/biopesticides/>) and published materials (e.g., booklets, proceedings of workshops, pamphlets) help disseminate information related to GEOs. Both APHIS and EPA websites provide a list and links to agency regulations and provides an explanation of the process. Regulatory decisions and the outcome of EPA's toxicology reviews are posted for public review. The website also provides for contact directly with Agency scientists and regulators to address issues of concern. Additionally, scientists may publish articles in trade and peer-reviewed journals, monographs and books which outline Agency position on topics related to regulation of GEOs.

Finally, EPA maintains a public docket, which contains a large number of documents available for inspection and copying, including scientific reviews on safety issues and Reregistration Eligibility Decisions (REDs) on individual plant-pesticides. The Freedom of Information Act (FOIA) also provides for the request of any document submitted to support a pesticide registration as long as it does not contain confidential business information. Comments were received following publication of Federal Register Notices describing genetically engineered baculoviruses. Comments were concerned with persistence of the virus in the environment and methods used for containment of field tests. The Agency responded by imposing stricter containment provisions on the field tests. Studies described above also indicate that the host range of the modified virus is not extended and the replication of biopesticide is decreased relative to wild type forms. Hence, the opportunity for persistence through a sustained infection cycle is lessened. Given the rapid death of AcMNPV/LqhIT2 hosts as compared to wild type AcMNPV and the production of fewer new virus particles per cadaver, greater exposure of other organisms to the modified NPV are not expected.

Brief Overview of Regulation of Genetically Modified Arthropods

Genetic engineering of arthropods that may be released into the environment might include: engineering for more effective biocontrol (which may invoke FIFRA), and engineering of disease vectors (such as mosquitoes) in disease control for human or animal health. The effectiveness of the above in meeting the desired goals when they are in the environment depends on understanding of the complex interactions between the arthropods, their hosts, other organisms and the environment. Future uses might include production of chemicals or pharmaceuticals in insects and genetic engineering of pet arthropods.

APHIS authorities over arthropods

APHIS has established regulations (7 CFR 340) under the Federal Plant Pest Act and the Plant Quarantine Act to provide oversight for genetically engineered (transgenic) arthropods that are plant pests or that can impact plant pests. These regulations cover plant pests, vectors of plant diseases, and biocontrol agents. Various APHIS procedures are in place to process permit applications for importation, interstate movement, and release into the environment.

APHIS has overall statutory authority (21 U.S.C. 111) and general regulatory authority (9 CFR 122) to take whatever measures deemed necessary to prevent the introduction and/or dissemination of contagious/infectious/communicable diseases of animals (21 U.S.C. 134), and to restrict the importation and movement of organisms and vectors of those diseases (51 FR 23341).

At the moment, however, APHIS statutes and regulations do not specifically address the issue of Agency oversight for genetically engineered vectors of animal diseases. APHIS presently is considering whether there is a need for specific regulations in this area, and may eventually develop new regulations by publishing an Advance Notice of Proposed Rulemaking and a subsequent Proposed Final Rule and a Final Rule.

Further information on permits issued and an environmental assessment for a field trial of a genetically engineered mite can be found on the APHIS website:
“<http://www.aphis.usda.gov/biotech/arthropod/>”.

EPA Authority

Under TSCA, EPA has jurisdiction with respect to the manufacture of new and existing chemicals for commercial purposes. Production of new chemicals by use of arthropods is required to be notified to EPA. The arthropods themselves may also be regulated by EPA if they qualify as chemical substances under TSCA.