

Pesticide Registration (PR) Notice 2007-2
**NOTICE TO MANUFACTURERS, FORMULATORS, PRODUCERS AND
REGISTRANTS OF PESTICIDE PRODUCTS**

ATTENTION: Persons Responsible for Registration of Pesticide Products

SUBJECT: Guidance on Small-Scale Field Testing and Low-level Presence in Food of Plant-Incorporated Protectants (PIPs)

This PR Notice provides clarification on the process by which EPA reviews and ensures the safety of residues of plant-incorporated protectants (PIPs) potentially present in low levels in food or feed, and the conditions under which a tolerance or exemption from the requirement of a tolerance would be required for field tests for biotechnology-derived food and feed crop plants containing plant-incorporated protectants.

There are no new rules, policies or interpretations in this PR Notice. This PR Notice summarizes, explains, and provides guidance to researchers and others using or testing plant-incorporated protectants regarding compliance with existing rules under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Some researchers and other users of PIPs may not be aware of their obligations regarding small scale field studies. Because those obligations are contained in several different rules, the Agency seeks to assist these individuals in understanding the existing requirements to facilitate compliance, and therefore prevent potential violations.

I. BACKGROUND

The use of bioengineered plants for food production, including plants engineered to express plant-incorporated protectants, has markedly increased over the past decade. A plant-incorporated protectant is a pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. It also includes any inert ingredient (such as selective markers used to ensure the active ingredient is inserted into the plant) contained in the plant, or produce thereof.

This PR Notice elaborates on policies the U. S. Environmental Protection Agency (EPA) described in the August 2, 2002, Federal Register Notice (67 FR 50578) on “Proposed Federal Actions to Update Field Test Requirements for Biotechnology Derived Plants and to Establish Early Food Safety Assessments for New Proteins Produced by Such Plants” issued under the auspices of the Office of Science and Technology Policy (OSTP). The OSTP notice was issued to outline what measures federal agencies would take to prevent low levels of biotechnology derived genes and gene products from being found in commercial food and feed until the appropriate safety standards have been met. The OSTP notice stated that EPA would rely on

existing processes and publish guidance for individuals and organizations conducting field testing of Plant-Incorporated Protectants.

As discussed further in Section II, regulations promulgated under the Federal Insecticide, Fungicide and Rodenticide Act typically allow small-scale field trials (<10 acres) without the issuance of an Experimental Use Permit (EUP), and do not require the issuance of a temporary tolerance for residues in food if the crop is destroyed or fed only to experimental animals (animals used for research purposes that will not enter the human food chain). Such small scale tests are thus not expected to result in residues in food that have not been evaluated for safety by EPA. This is important since food containing pesticide residues that have no tolerance or tolerance exemption is adulterated under the FFDCA and may not be moved in interstate commerce.

Potential users of PIPs or researchers conducting such small scale field trials should however be cognizant of the possibility of dispersal of PIPs and PIP residues. PIPs are produced and used in living organisms and living organisms have the potential to spread genetic material and subsequently produced pesticidal proteins through several routes among plant populations. Depending on the biology of the crop expressing a PIP, agronomic practices employed and the conduct of the studies, there is the potential for the PIP to inadvertently spread from test plants into other commercial, breeding or experimental crops and into the food supply. Transfer can occur through cross-pollination with surrounding crops, or the inadvertent mixing of seeds or other plant propagative or food/feed material after harvest. If PIPs are transferred, the consequence may be that low-levels of biotechnology-derived genes and gene products that have not been evaluated for dietary safety and do not have needed residue tolerances enter the commodity stream and the food supply.

Depending on the specific circumstances of a field trial (e.g. biology of the crop, location of the trial, etc.), EPA urges potential registrants and researchers to consult early with the EPA to ensure that appropriate physical and/or biological controls are in place to restrict the flow of genetic material, including seeds, from field tests to minimize the potential for such adulterants to enter the food supply. During this consultation, EPA would discuss the planned confinement measures, and provide recommendations as to whether those measures would adequately ensure that residues of the tested PIP do not enter the food supply. In general, EPA would consider field trials less than 10 acres to have sufficient physical or biological controls if they are conducted under APHIS authorization and are in compliance with APHIS requirements. As part of the consultation, EPA could also recommend measures that could be taken to destroy affected crops, or measures to prevent the commingling of seeds or other plant material if the crop is to be held and used only for future research purposes. In some cases, EPA may recommend that the developer seek a temporary tolerance. EPA might also conclude that an EUP is required in order for the test to proceed. If there is a reasonable expectation that residues of a PIP being tested could enter the food supply (through any route, e.g., pollen flow, mixing of seed), albeit at low levels, all crops affected by such tests must either be destroyed, be kept from the food/feed supply while additional studies using the crop are conducted, or a tolerance determination must be made at any size of field test (i.e., including any field test at or less than 10 acres of land). It is the responsibility of the company and/or researcher to ensure that all studies comply with this regulatory requirement.

A. Scope

The purpose of this PR Notice is to provide guidance to those individuals conducting field trials with plant-incorporated protectants that have either not received a registration or an EUP and do not have a tolerance/exemption or temporary tolerance/exemption. As described in Part 174.3 of Title 40 of the Code of Federal Regulations (CFR), a plant-incorporated protectant is a pesticidal substance that is intended to be produced and used in a living plant, or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. It also includes any inert ingredient contained in the plant, or produce thereof. Part 174.3 defines living plant as “a plant, plant organ, or plant part that is alive, viable, or dormant. Examples of plant parts include, but are not limited to, seeds, fruits, leaves, stems, flowers, and pollen.” PIPs are pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act, because they meet the section 2(u) definition of pesticide which reads in part: “(1) Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest”

B. FFDCA Section 408 Requirements

Residues of PIPs in food are pesticide chemical residues under the Federal Food, Drug, and Cosmetics Act. Food includes articles used for food or drink by humans or other animals (animal feed). Under FFDCA section 408, EPA regulates pesticide residues by establishing tolerances limiting the amounts of residues that may be present in or on food, or by establishing exemptions from the requirement of a tolerance for such residues. A tolerance is a legal limit or maximum amount of residues of the pesticidal substance allowed in food and feed. A tolerance (or exemption from a tolerance) is required for all pesticide residues on food or feed whether imported into the United States or domestically produced. An exemption from the requirement of tolerance can be issued if it can be shown that the aggregate exposure of any amount of the pesticide meets the legal standard of a reasonable certainty of no harm (FFDCA sec. 408(e)(2)(ii)). Typically a tolerance exemption may be granted for a PIP when testing indicates no toxicity from the pesticide and no indication of allergenicity from the pesticidal (or inert) proteins. A food containing pesticide residues may not be moved in interstate commerce without an appropriate tolerance or an exemption from the requirement of a tolerance. This applies also to food generated during field testing of a PIP and includes any food/feed crops not part of the testing that contain any detectable level of a PIP originating from such tests, with the exception that tolerance issues need not be addressed for plant materials containing the PIP provided that such materials are being moved only for the purposes of additional experimentation and do not enter the food/feed supply.

C. Small Scale Field Testing of Pesticides

Section 5 of FIFRA, and 40 CFR part 172 provide for issuance by the Agency of EUPs for the testing of new pesticides or new unregistered uses of registered pesticides, including PIPs. Under Section 5 of FIFRA, Congress authorizes EPA to issue EUPs in order to allow interested parties the ability to gather sufficient information on a new pesticide or a new use of an existing pesticide to support registration under Section 3 of FIFRA. Before issuing an EUP, the Agency must determine that use of the experimental product, under the conditions proposed in the EUP application, will not result in “unreasonable adverse effects,” as defined in section 2 (bb) of

FIFRA, to human health or the environment.

EUPs are generally required for a small-scale test involving use of a particular pesticide that is conducted on a cumulative total of more than 10 acres of land per pest. An EUP is generally not required for testing at or under 10 acres, because such tests are generally presumed not to involve unreasonable adverse effects. The regulations regarding EUPs allow additional acreage for testing that involves multiple pests that do not occur in the same geographic area or at the same time. This means that testing a PIP to evaluate control of two separate pest species that do not occur in the same geographic vicinity or at the same time may be done on up to 20 acres (i.e., two separate 10 acre test sites) without triggering the requirement for an EUP. On the other hand, if control of the two pests may be evaluated at the same location or at the same time, testing may include no more than 10 acres without triggering the need for an EUP. Part 172.3(c) states generally that an EUP would be required for testing conducted on 10 acres or less under certain circumstances. Specifically, if there is a reasonable expectation that residues of a pesticide could get into food or feed, then a tolerance or exemption from the requirement of a tolerance is required in order for the test to proceed without an EUP, unless all of the plant material potentially containing the residue is destroyed or used for additional experimentation and will not enter the food supply.

II. ISSUES

PIP pesticide residues can be contained in pollen moving from the test plants to surrounding crops or as a result of pollen transfer from a PIP crop to a sexually compatible crop nearby. The accidental mixing of seed or the growth of volunteer plants may also present an opportunity for PIP residues to move. Note that this characteristic of living plants does not necessarily mean that an EUP is required at 10 acres or less, but that tolerance issues must be addressed before the testing commences. Where the testing involves food or feed crops, additional conditions apply to the presumption that small scale field testing is exempt from EUP requirements as described in 40 CFR 172.3(c)(1)(ii), which reads as follows:

“(ii) Any food or feed crops involved in, or affected by, such tests (including, but not limited to, crops subsequently grown on such land which may reasonably be expected to contain residues of the tested pesticides) shall be destroyed or consumed only by experimental animals unless an appropriate tolerance or exemption from a tolerance has been established under the Federal Food, Drug, and Cosmetic Act (FFDCA) for residues of the pesticide.”

Specifically, testing conducted under 10 acres may proceed without an EUP if all food or feed crops involved in or affected by such tests are destroyed or held for further experimentation and prevented from entering the food supply. Alternatively if the developer chooses not to destroy or hold the crops for additional testing, an EUP with appropriate conditions would be required for any testing under 10 acres unless a tolerance for the pesticide residues had been established for any food crops involved in or affected by such tests. The regulations do not provide exclusion from the need for a tolerance for residues resulting from pollen transfer. Nor would such an interpretation be consistent with EPA’s long-standing implementation of this provision. For PIPs, the terms “involved in” or “affected by” would include the presence of a pesticide residue as a result of, for example, pollen transfer from a PIP crop to a sexually compatible crop. For example, a five acre uncontained study using field corn containing a PIP without a tolerance or exemption from the requirement of a tolerance which is planted close

enough to a commercial field of corn (whether containing a PIP or not) such that pollen from the study plot will at least partially fertilize the corn nearby, would require a tolerance (or exemption from the requirement of a tolerance) or an EUP. On the other hand, the same study design would not require a tolerance or exemption or an EUP if sufficient confinement measures (such as those mentioned later in Section III) were instituted.

Note that the spread of pollen from the test corn in the study mentioned above to a sexually incompatible crop such as soybeans would not require a tolerance or tolerance exemption nor an EUP as pollen residues generally quickly break down in the environment. However, the registrants must use sufficient measures to prevent the inadvertent spread of the PIP trait. Therefore, registrants may wish to consult with the Agency for tests under 10 acres to verify that under the test conditions, tolerance and EUP requirements are not triggered.

III. RESIDUE CONFINEMENT IN SMALL SCALE FIELD TESTING

Testing at 10 acres or less does not require an EUP if a tolerance exists for residues of the PIP involved. Additionally, the presumption that an EUP is not required may still pertain when the PIP does not have a tolerance providing that the test is conducted in a manner that will provide for the destruction of all crop material containing PIP residues or such crop material is fed to experimental animals not entering the food supply, and that the test design precludes potential contamination of the food supply by the PIP from gene transfer or other routes such as seed mixing.

Several methods exist that may reduce the probability for PIP residues to enter the food and feed supply from small scale field tests. These include procedures to control reproduction, pollen movement, and seed movement through identity preservation, and quality assurance/quality control mechanisms related to the identification of seeds and other propagative materials.

A. Procedures to Control Reproduction and Pollen Movement

1. Spatial isolation of test plants. This may involve considerable distance as pollen from some species may move long distances.
2. Physical isolation such as exclusion barriers to pollination such as bagging corn or the planting of crops to intercept pollen.
3. Reproductive isolation such as emasculation or sterilization of plants.
4. Temporal isolation to ensure that potentially receptive plants are not sexually synchronous with test plants.
5. Elimination of pollinating insects either through physical barriers such as netting or through the use of insecticide applications.

Usually several techniques are used in combination to ensure that no detectable residues of unapproved PIP proteins can result from gene transfer. Since the appropriate techniques and isolation distances vary from crop to crop, questions may arise related to test design -- specifically with respect to reproduction and pollen movement. In these cases, it is suggested that developers and researchers involved in the small-scale field testing of PIPs consult with EPA.

B. Identity Preservation

The mixing of seeds used during or derived from testing, along with any similar propagative or food/feed materials with materials, (i.e., grain or seeds) to be used for food or feed can be prevented by the development of a strict and thorough system of identity preservation. Several items are important steps in such a program:

1. Labeling of propagative plant materials including seeds before, during and after the study.
2. Written plans and records related to planting locations and the compatibility of plantings within the distance of potential gene transfer.
3. Written records and documentation with respect to activities to prevent gene transfer such as emasculation or the installation of barriers.
4. Procedures to check potential compatible recipients and/or their seeds or other propagative materials for the presence of unintended events regardless of any prior mechanisms to prevent pollen spread.
5. Tracking systems to ensure that seeds and plant materials held for further experimentation are not unintentionally commingled with other similar plant materials also held at the facility, or other facilities where they may be sent for testing.

Note that the test procedures to detect PIP residues are extremely sensitive and that methods for assuring phenotypic purity may not be adequate to address low-level PIP contamination. Laboratory analysis for inadvertent presence of additional events may therefore be an important step in QA/QC procedures.

IV. IMPORTANT CONSIDERATIONS IN TOLERANCE DETERMINATIONS

Sometimes the actions necessary to prevent low-level presence are not compatible with the test design for a particular study, and the researcher(s) will need to apply for a tolerance. When a tolerance is needed for an EUP or registration action, information is submitted that allows the Agency to evaluate the potential of the PIP to have effects on human health. The Agency must consider whether any part of the PIP is a potential allergen, toxin, or has any other deleterious effect. To date, tolerance requests for PIPs have been limited to pesticidal and inert proteins and the genetic material necessary for their production. In July 2001 (66 FR 37817), EPA issued a tolerance exemption for nucleic acids; this tolerance exemption applies to residues of nucleic acids that are part of the PIP. For the tolerances approved thus far, the EPA has received several types of data to support the finding of a reasonable certainty that no harm will result from the aggregate exposure to the protein portion of a PIP. The information is intended to show that: 1) the protein behaves as would be expected of a protein ordinarily found in the diet;

2) the protein is not structurally related to any known allergen or protein toxin, and, if appropriate; 3) the protein does not show any oral toxicity when administered at high doses. Data submitted to make these determinations thus far have consisted of characterization of the introduced gene(s) and protein, an *in vitro* digestion assay, amino acid sequence homology comparisons of the introduced protein with known allergens and toxins and, in many cases, an acute oral toxicity test. Details on these studies follow:

A. Characterization. Applicants have provided data with sufficient information (e.g. sequence data, Southern and Northern blots) regarding the DNA that is inserted into the plant and the protein expressed, molecular weight, post-translational modification, expression, stability of the protein to heat, genetic stability and biological activity of the protein. Characterization data are critical to understanding the way in which the PIP was made and the unique nature and potential exposure of each PIP. Characterization data provide information on the specific transformation systems used for each product, the actual DNA that is inserted into the plant, and protein expression levels for various plant tissues.

B. *In vitro* digestibility assay. EPA looks at the *in vitro* digestibility test to determine whether the protein is unstable in the presence of simulated human digestive fluids and whether it is unusually persistent in the average human digestive tract. This test provides information on the potential of the protein to be a food allergen.

C. Amino acid homology. Amino acid sequence comparisons using protein databases are typically performed to identify similarities with known toxins or allergens.

D. Acute oral toxicity. In certain instances, acute toxicity testing data have been provided. This testing relies on the fact that toxic proteins generally express toxicity at relatively low doses. Therefore when a protein has no apparent effects in the acute oral toxicity test, particularly at relatively high doses, the protein is considered non-toxic. The acute oral toxicity test is done at maximum-hazard dose levels using purified protein of the plant-incorporated protectant as a test substance. If the purified protein is derived from microbial production, then data demonstrating equivalence to the protein as expressed in the plant has typically been submitted.

V. SCOPE OF POLICY

This PR Notice describes certain requirements set forth by FIFRA and FFDCA along with associated regulations and provides general guidance to EPA and affected parties. While the requirements in FIFRA and its regulation are binding on EPA and other affected parties, this PR Notice is intended to provide guidance to EPA, applicants, registrants and the public. As guidance, this policy is not binding on either EPA or any outside parties, and EPA may depart from the guidance where circumstances warrant and without prior notice. Registrants and applicants may propose alternatives to the recommendation in this PR Notice, and the EPA will assess them on a case-by-case basis.

VI. PAPERWORK REDUCTION ACT NOTICE

The information collection activities associated with the application process for EUPs that are also described in this PR Notice are already approved by the Office of Management and Budget (OMB) under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.* The

corresponding Information Collection Request (ICR) document has been assigned EPA ICR number 0276, and is approved OMB control number 2070-0040. Under that ICR, the total estimated annual respondent paperwork burden associated with the application process for EUPs is 10.10 hours per application. A copy of the most recent version of this ICR is available under Docket ID No. EPA-HQ-OPP-2006-0632 at www.regulations.gov. Under the PRA, “burden” means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. For this collection, it is the time reading the regulations, planning the necessary data collection activities, conducting tests, analyzing data, generating reports and completing other required paperwork, and storing, filing, and maintaining the data.

Under the PRA, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. The OMB control numbers for EPA's regulations codified in Chapter 40 of the CFR, after appearing in the preamble of the final rule, are listed in 40 CFR part 9, are displayed either by publication in the Federal Register or by other appropriate means, such as on the related collection instrument or form, if applicable. The display of OMB control numbers in certain EPA regulations is consolidated in 40 CFR part 9. For the ICR activity contained in this PR Notice, EPA is displaying the applicable OMB control number in the PR Notice above, and the applicable OMB control number also appears on the EUP application.

VII. FOR FURTHER INFORMATION

The Agency recognizes the difficulty of completely containing living organisms by seeking to avoid pollen drift and cross pollination or of commingling of seeds or grain or other living plant parts after harvest. Therefore, potential applicants desiring additional information or confirmation that confinement measures are adequate may consult with EPA in order to discuss these topics and/or making applications for tolerances, tolerance exemptions, or EUPs. Applicants can request written responses to specific questions if needed. The appropriate contact for such consultation is:

Patricia Moe, Team Leader
Microbial Pesticides Branch (7511P)
Office of Pesticide Programs
U.S. Environmental Protection Agency
1200 Pennsylvania Ave. NW
Washington, DC 20460
Telephone number (703) 305-0744
Email: moe.patricia@epa.gov

Debra Edwards, PhD, Director
Office of Pesticide Programs