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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)
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AUGUST 27-29, 2002
FIFRA SAP WEB SITE http://www.epa.gov/scipoly/sap/
OPP Docket Telephone: (703)305-5805

TUESDAY, AUGUST 27, 2002
SHERATON CRYSTAL CITY HOTEL
1800 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202
703-486-1111

Corn Rootworm Plant-incorporated Protectant Non-target Insect and Insect Resistance Management Issues

Charge/questions to the Panel

Non-target Effects

Monsanto Company has applied to EPA for registration of their corn rootworm plant-incorporated protectant (PIP) product. As part of their application, Monsanto has submitted studies on effects of the PIP to non-target invertebrates and soil fate studies. Some of these studies are ones typically required for PIPs and some are unique to this product which is intended to control a soil rather than foliar insect pest. EPA has evaluated 13 studies as part of its assessment of potential impact on non-target invertebrates and soil fate. These studies, along with EPA's reviews and preliminary risk assessment, have been provided to the Panel members and made available to the public through the Office of Pesticide Programs Public Docket. EPA requests the Scientific Advisory Panel to provide guidance to the Agency on the following questions related to its preliminary risk assessment for non-target invertebrates and soil fate.

Question 1: Single Species Testing vs Field Data Approach

In October 2000, the FIFRA Scientific Advisory Panel recommended that non-target testing be focused on species exposed to the crop being registered. The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic corn fields are beneficial insects feeding on corn pollen and soil invertebrates, particularly Coleoptera. In lieu of extensive and difficult single species soil coleopteran toxicity testing followed by an extrapolation from the results to a community risk assessment, direct field data on coleopteran insect effects and abundance were received and evaluated.

A) Please comment on the relative strengths and weaknesses of such field data vs. laboratory feeding studies performed on a limited number indicator organisms, for

purposes of hazard assessment.

The Agency believes that a complete census of the invertebrate community would be costly and unlikely to be useful for Bt proteins which are usually target specific groups of invertebrates.

B) The Panel is requested to comment on the logistics, validity, cost and expected scientific gain, if any, of conducting a census of the invertebrate community vs concentrating the studies on specific indicator organisms. In addition, please comment on suggested indicator groups such as Carabids and Staphyllinids in the case of Cry3Bb1, that would be most likely to provide the Agency with meaningful data for assessing the potential hazards to non-target invertebrates from corn rootworm PIPs.

Question 2: Duration of Field Abundance Studies

A two-season field invertebrate abundance study indicates that MON 863 corn does not have a negative impact on the abundance of non-target invertebrates. Data also indicated that planting event MON 863 results in less impact on non-target invertebrate than conventional pest management practices.

Please comment on the adequacy of the 2 year field abundance study for making a determination of the potential risks from commercial use of event MON 863.

Question 3: Green Lacewing Larva Test

The Agency accepts data on lacewing larvae fed on a Cry protein-coated moth egg diet. The testing is performed with a concurrent positive control which incorporates arsenate into the moth egg diet. However, there are published comments that this protocol does not expose the larvae to the test substance because the larvae pierce the eggs and feed on the egg fluids, thus not getting exposure to the Cry protein which coats the outside of the eggs. Tritrophic studies using a diet of aphids fed on Bt corn plants have been suggested as more valid approach. This may not be a solution to the problem, because the lacewing larvae are also said to feed on the aphid body fluids which do not contain the Cry proteins. The Cry proteins are confined to the digestive tract of the aphid.

The Agency solicits the Panel's comments on an appropriate design for evaluating the toxicity of Cry3Bb1 proteins to lacewing larvae.

Question 4: Soil Degradation/Accumulation of Cry3Bb1

The reviewed data indicate that Cry3Bb1 protein in plant tissue degrades rapidly in sandy loam soil. However, corn is not necessarily grown in sandy loam soil in all regions. Corn is grown in other soil types such as clay loam and silt loam soils in various regions of the U.S. Cry protein has also been shown to bind to clay soils. Therefore, it is may be desirable that soil

degradation and persistence studies be conducted in other common agricultural soils, perhaps for 3 years.

A) The Panel is requested to comment on the advisability of testing additional soil types and for having soil persistence studies for up to 3 years.

B) What soil types would need to be tested and what duration is needed for soil persistence studies.

The soil fate studies submitted to EPA describe DT_{50} (time to 50% degradation of the Bt protein in soil) and DT_{90} (time to 90% degradation of the Bt protein in soil) for Cry3Bb1 protein in sandy loam soil based on ELISA test are 2.76 and 9.16 days. However, the value of these results are not necessarily correlated with activity in insect guts because it is unknown if the extractable protein in the ELISA test was functional or non-functional. The DT_{50} and DT_{90} determined by insect bioassays with CPB were 2.37 and 7.87 days respectively.

C) Are these studies truly expressing the time to 50% or 90% degradation of Bt protein in the soil or whether they are only determining the level of detection of Cry3Bb1 protein in the soil. Discuss the acceptability of these studies for a preliminary risk assessment to evaluate the fate of Cry3Bb1 in soil.

D) What if any difference would it make in the values of these ELISA based studies if clay particles to which the Cry3Bb1 protein might bind are present in the soil being tested? What measures should be taken to ensure that the test is not measuring inactive protein fragments?

Question 5: Preliminary Risk Assessment for Non-target Invertebrates and Soil Fate

The Agency's preliminary risk assessment based on single species laboratory toxicity studies on adult and larval lady beetles, green lacewing larvae, a parasitic hymenopteran, adult and larval honey bees, Collembola, earthworm, the monarch butterfly, field invertebrate census evaluations, and a soil persistence study indicates no unreasonable adverse effects on the invertebrate fauna of the corn field.

Please comment on the Agency's non-target invertebrate and soil fate assessment?

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Insect Resistance Management

Monsanto Company submitted an application to EPA for the registration of *Bacillus thuringiensis* (Bt) Cry3Bb1 protein and the genetic material (Vector ZMIR13L) necessary for its production in corn. Corn expressing the Cry3Bb1 protein is intended to provide protection against the corn rootworm (CRW, *Diabrotica* spp.). This product has been designated event MON 863 by Monsanto. EPA has determined that an insect resistance management (IRM) plan is necessary for this product. At EPA's request for a IRM plan, Monsanto designed a plan intended to be both scientifically valid for resistance risk mitigation and feasible for growers to understand and implement. EPA's preliminary assessment of the Monsanto IRM plan for MON 863 has determined that further data and evaluation is needed to develop a robust, practical, long-term IRM plan. The proposed plan submitted by Monsanto might be used for 3 years while in-field testing and evaluation is conducted to develop a IRM plan which might be used for 10 or more years. In order to develop such a long-term IRM plan, grain growers, and researchers need to be able to grow MON 863 corn for a period of time so that important information can be generated including how an IRM plan can be effective in areas where MON 863 is used alone and in areas where MON 810 (used for control of certain lepidopteran pests such as European corn borer) is combined with MON 863. EPA requests the Scientific Advisory Panel to provide guidance to the Agency on the following questions related the Agency's assessment of the interim IRM plan and information that needs to be generated to develop a long-term IRM plan for corn rootworm plant-incorporated protectant (PIP).

Question 1: Pest Biology Research

Pest biology is important to refuge placement since the goal is to encourage random mating between pests emerging from the transgenic and non-transgenic corn fields. Knowledge of corn rootworm (CRW) biology, dispersal characteristics, host range, feeding habits and history of insecticide resistance is important in developing an IRM strategy. Most information provided to the Agency thus far relates to western corn rootworm (WCRW) and limited information was provided on northern corn rootworm (NCRW). The Mexican corn rootworm (MCRW) is only briefly discussed and the southern corn rootworm (SCRW) is not considered in Monsanto's IRM proposal.

The Panel is requested to comment on the Agency's conclusion that additional information is needed on various aspects of CRW pest biology as it relates to a long-term IRM strategy. Specifically, discuss:

A) Whether an IRM strategy designed for WCRW (and NCRW) is applicable to other corn rootworm species? How much species-specific data is needed vs. how much can the Agency rely on existing data for WCRW and NCRW to predict what would be an adequate IRM plan for SCRW and MCRW?

B) Whether, and if so what, additional research regarding male and female adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges?

C) Whether, and if so what, more information is needed on mating habits, ovipositional patterns, number of times a female can mate and fecundity as it relates to refuge structure and placement?

D) How should CRW extended diapause and oviposition outside of corn (e.g., soybean rotation) be used to evaluate the effectiveness of IRM plans?

Question 2: Dose.

Determining the level of dose is crucial to size and structure of a refuge needed to delay CRW resistance to Cry3Bb proteins. In the February 1998 Scientific Advisory Panel meeting, a high dose for lepidopteran-active Bt proteins was defined as 25 times the amount of Bt delta-endotoxin necessary to kill susceptible individuals. Based on Monsanto's modified version of a model by Caprio, a moderate dose is defined as 30% survival of larvae and a low dose as 50% survival. Data provided by Monsanto shows 17% to 62% survival of larvae. EPA believes that a 17% to 62% survival of larval CRW constitutes a low to moderate dose of Cry3Bb1 protein in MON 863 corn.

A) The Panel is requested to comment on EPA's determination that MON 863 expresses a low to moderate dose for CRW. The Panel is requested to provide guidance on definitions of a high, moderate and low dose for a corn rootworm-protected Bt corn product.

B) What techniques should be used to determine dose for Cry3Bb1?

As a part of this discussion the Panel might want to consider the definition of high dose provided by the February 1998 SAP noting that for Bt corn, the pests are above ground feeding lepidopteran insects. The relevant excerpt from the Panel's report is provided below.

> The Subpanel discussed ways to define and measure "high dose" in plants. It was agreed that the definition of high dose as "25 times the toxin concentration needed to kill susceptible larvae" was reasonable based on current empirical data. However, the

Subpanel recognized that it is conceivable that a heterozygot may develop with higher than 25-fold resistance.

The major problem identified by the Subpanel was in determining if the 25-fold level was achieved in a specified cultivar. After much discussion, it was concluded that there were at least 5 imperfect ways to assess this 25-fold level, and that some approaches were more appropriate for specific crop pests. The Subpanel concluded that a cultivar could be considered to provide a high dose if two of the five approaches described here indicated presences of a high dose.

The five approaches are:

(1) Serial dilution bioassay with artificial diet containing lyophilized tissues of Bt plants (tissue from non-Bt plants serving as controls);

(2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar (determined by quantitative ELISA or some more reliable technique);

(3) Survey large numbers of commercial plants on sentinel plots in the field (e.g. sentinel sweet corn method) to make sure that the cultivar is at the LD99.99 or higher to assure that 95% of heterozygotes would probably be killed. With this approach Bt sweet corn hybrids are used to attract high densities of ECB and cotton bollworm (*Helicoverpa zea*) (Boddie)) (CBW/CEW) moths, sampling can be limited to sweet corn ears in the Bt plot (ca. 1/4-1/2 acre block), and a frequency of resistance phenotypes can be estimated as the ratio of density of larvae/plant in Bt sweet corn to density of larvae/plant in an adjacent planting of non-Bt sweet corn (Andow and Hutchison, 1998; Hutchison, unpublished data).

(4) Similar to (3) above, but would use controlled infestation with a laboratory strain of the pest that had an LD50 value similar to field strains;

(5) Determine if an older instar of the targeted pest could be found with an LD50 that was about 25-fold higher than that of the neonate larvae. If so, that stage could be tested on the crop plants to determine if 95% or more of the older stage larvae were killed.

Question 3: Models.

Simulation models are one of the tools used to evaluate IRM strategies to delay resistance. Assumptions in resistance models are based on aspects of pest biology including CRW survival and fitness. EPA has used predictive models to compare IRM strategies for Bt crops. Because models cannot be validated without actual field resistance, models have limitations and the information gained from the use of models is only a part of the weight of evidence used by EPA in assessing the risks of resistance development. It was the consensus of

the October, 2000 FIFRA SAP that models were an important tool in determining appropriate Bt crop IRM strategies. They agreed that models were "the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options."

A) The Panel is asked to comment on the product duration or longevity of corn rootworm susceptibility considered in CRW IRM models.

B) Considering EPA's evaluation of the three models addressed in the Monsanto submission, discuss the applicability of each of the models for assessing the likelihood of CRW developing resistance to Cry3Bb1.

C) Please comment on the appropriateness of the following input parameters of these simulation models for CRW-protected field corn: Resistance allele frequency, dominance of the heterozygote, movement of the males and females, mating and ovipositional behavior, and other genetic and behavioral parameters.

D) How does insecticide use in the refuge and/or Bt fields affect the predictions of time to resistance?

Question 4: Refuges.

Refuges are planted to delay potential pest resistance to a Bt crop. Planting non-Bt corn within or near Bt corn fields will provide CRW offspring that will remain susceptible to the Cry3Bb proteins. The refuge should be structured to provide an adequate number of susceptible individuals that are available to mate with potentially resistant individuals and dilute resistance alleles in the field. Based on current information on CRW biology, MON 863 dose, simulation models, hybrid availability and adoption rate, a 20% refuge should be adequate on an interim basis to produce enough CRW adults to delay resistance. EPA has concluded that it is acceptable to plant refuges as continuous blocks or in-field row-strips. Based on the only available currently published paper, in-field strips should consist of at least 6 to 12 consecutive rows planted within 9 to 18 m of the center of the transgenic corn field.

EPA has concluded that a 20% refuge is adequate to delay resistance during a three-year period.

A) Please comment on whether this refuge strategy is adequate to delay resistance?

B) Because the current plan being evaluated is based on limited data and is an interim plan, limitations to the total number of acres MON 863 might be considered. If so, should the limitations be on acres planted per state or per county or on another basis during the time an interim IRM plan is in place?

C) The Panel is asked to comment on the adequacy of in-field row-strips and/or

immediately adjacent blocks to delay resistance during a three-year period and whether one method or another is preferred.

D) The Panel is requested to comment on the width of the in-field strips. As an example, the Agency is aware that at least 6 to 12 consecutive rows have been discussed in the following paper: Onstad, D. W., C. A. Guse, J. L. Spencer, E. Levine and M. E. Gray. 2001. Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 94(2): 529-540.

E) Please comment on EPA's conclusion that alternate hosts should not be considered and refuges should only consist of non-Bt corn that are similar hybrids to the Bt corn.

F) The Panel is requested to comment on whether, and if so under what conditions, insecticides could be used in the refuge.

Question 5: Monitoring.

A resistance monitoring strategy for Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. EPA believes the mechanism of potential resistance of CRW to MON 863 should be determined to develop an appropriate long-term IRM strategy. EPA has concluded that CRW resistance is necessary to determine the mechanism and genetics of resistance to Cry3Bb1. Therefore, colonies resistant to Bt should be established and evaluated in the laboratory during the initial three years MON 863 is grown commercially.

Please comment on the Agency's conclusions regarding refinements to Monsanto's resistance monitoring program. In your response, please consider the following factors: how should CRW resistance should be monitored; the value of developing resistant colonies of CRW to determine the mechanism and genetics of resistance; insect rearing for CRW spp. and whether one colony in more than one laboratory should be established.

Question 6: Mitigation/Remedial Action.

Remedial action plans are a potential response measure should resistance develop to Bt crops. Since resistance may develop in "localized" pest populations, it may be possible to contain the resistance outbreak before it becomes widespread. There is a concern regarding Monsanto's proposed outline of detecting and confirming resistance. Monsanto suggests that they will initiate mitigation measures when unexpected levels of CRW damage occur. However, Monsanto does not describe what is meant by unexpected levels of damage. Some level of damage is expected since there is not a high dose of MON 863 expressed to control the CRW

and research has shown that some level of "grazing" will occur. Monsanto also suggested using a root damage rating scale to determine unexpected levels of damage. However, this method may not be appropriate for CRW protected Bt corn.

A) The Panel is requested to discuss an appropriate method of determining suspected and confirmed resistance for CRW including recommendations as to how suspected resistance or unexpected damage may be identified.

B) Please discuss whether root ratings are an appropriate indicator of suspected resistance. If so, how could a typical farmer use root ratings to identify suspected resistance.