June 25, 2002

MEMORANDUM

SUBJECT:	Review of efficacy and insecticidal activity data for Event MON 863: Corn Rootworm Protected Corn (Vector ZMIR13L); Permit No. 524-LEI; Chemical No 006484; DP Barcode No D275903, D280086; Case No 065182; MRID # 453613-03, 455382-07, 455382-08.
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FROM:	Alan Reynolds, Entomologist /s/ Microbial Pesticides Branch Biopesticides and Pollution Prevention Division, 7511C
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Action Requested:

BPPD has been asked to review efficacy and insecticidal activity data submitted by Monsanto Co. in support of a registration application for MON 863 Corn Rootworm Protected field corn (EPA Reg. No. 534-LEI). Event MON 863 (vector ZMIR13L) contains the Cry3Bb *Bt* gene that produces an insecticidal crystal protein and a *nptII* marker gene. MON 863 is targeted against corn rootworm beetles (*Diabrotica* spp.), a major pest of corn in the U.S. The submitted data consists of three studies: 1) Insecticidal Spectrum of Activity for Cry3Bb Protein *in vitro* (MRID # 455382-07); 2) Comparing the Efficacy of MON 853 and MON 863 to Three Corn Rootworm Species, Northern Corn Rootworm (*Diabrotica barberi*), Southern Corn Rootworm (*D. undecimpunctata howardi*), and Western Corn Rootworm (*D. virgifera virgifera*) (MRID # 455382-08); 3) Efficacy of MON 863 Against Corn Rootworm and Comparison to Insecticide Treatments - Results of Year 2000 Field Trials (MRID # 453613-03). It should be noted that this review will addresses only these three studies and that other benefits and non-target data will be considered separately.

BPPD Conclusions:

1) Insecticidial spectrum studies showed that, of the Coleopteran, Lepidopteran, Hymenopteran, Neuropteran, and Collembola species tested, only beetles from the family Chrysomelidae (Colorado potato beetle and western corn rootworm) were shown to be susceptible to the Cry3Bb

protein. Additional species of corn rootworm, including southern, northern, and Mexican corn rootworm, were not tested. It is noted, however, that a complete review of Cry3Bb effects to non-target, beneficial, and endangered insect species has been conducted separately (see R. Rose memo to M. Mendelsohn, 5/20/02).

2) Comparative efficacy studies showed that both MON 863 and MON 853 (a non-commercialized hybrid expressing Cry3Bb) experienced significantly less root damage from southern, northern, and western corn rootworm than a non-transformed control hybrid. MON 863 also had significantly less root damage from western and northern rootworm than MON 853 (there was no significant difference in southern rootworm damage). In all treatments, damage from southern corn rootworm exceed that from northern and western rootworms.

3) In comparative efficacy studies, MON 863 prevented root damage from rootworm feeding as well or better than commonly used rootworm soil insecticides including Force 3G (tefluthrin), Counter CR (terbufos), and Lorsban 15G (chlorpyrifos). Root damage ratings for MON 863 were typically between 1.2 and 2.0, a high level of control relative to untreated control hybrids. It should be noted that efficacy data such as these (and other studies) will also be reviewed and considered as part of a full benefits assessment for MON 863.

Insecticidal Spectrum of Activity for Cry3Bb Protein in vitro (MRID # 455382-07)

Monsanto has conducted diet bioassays to determine susceptibility to Cry3Bb for a number of insect species. The insects tested included representative species from Coleoptera (families Chrysomelidae, Bruchidae, Coccinellidae, Tenebrionidae, and Curculionidae), Lepidoptera (Pyralidae and Noctuidae), Hymenoptera (Apidae and Pteromalidae), Neuroptera (Chrysopidae), and Collembola (Isotomidae).

The assays were performed with purified Cry3Bb1 (variant 11231) protein obtained from recombinant strains of *Bacillus thuringiensis*. The protein differs from “wild type” Cry3Bb protein by four amino acids. It is unclear from the study report whether the tested Cry3Bb variant is the same as the Cry3Bb protein transformed into the MON 863 corn hybrid. All of the diet bioassays were conducted at Monsanto’s laboratories or at contract laboratories.

Diet bioassays for Coleoptera (Colorado potato beetle, western corn rootworm, cotton boll weevil) and Lepidoptera (European corn borer, corn earworm) were performed using serial dilutions of 6-8 concentrations (typically 1 to 200 ppm) in accordance with a Monsanto SOP. Assays for Coleoptera (ladybird beetle), Hymenoptera (honey bee, parasitoid wasp), Neuroptera (green lacewing), and Collembola were preformed by a contractor (GLP) using a single concentration, based on the maximum anticipated exposure in the environment. For honey bee larvae, the dose was delivered via deionized water injected into hive cells and for adult bees the dose was dissolved into a sugar feeding solution. For the parasitoid wasp and ladybird beetle, the Cry3Bb dose was incorporated into a honey mixture for adult feeding. Green lacewings were tested using a mixture of the Cry3Bb dose and moth eggs fed to larvae. Collembola were tested using lyophilized Cry3Bb leaf tissue mixed with yeast. Testing of other Coleoptera (cowpea weevil, red flour beetle, rice weevil, and pepper weevil) was done by a contractor (non-GLP) using single, maximum concentration doses of Cry3Bb. Where significant toxicity was observed, an LC_{50} was calculated using a nonlinear regression model.

The results from the susceptibility tests are contained in the following table:

Order/Family	Genus/Species	LC_{50} (µg/mL diet)	Max. Conc. (µg/mL)
Coleoptera/Chrysomelidae	*Leptinotarsa decemlineata* Colorado Potato Beetle	2.7	-
Coleoptera/Chrysomelidae	*Diabrotica virgifera* Western Corn Rootworm	75	-
Coleoptera/Bruchidae	*Callosobruchus maculatus* Cowpea Weevil	>200	200
Coleoptera/Coccinellidae	*Hippodamia convergens* Ladybird Beetle	>8000	8000
Coleoptera/Tenebrionidae	*Tribolium castaneum* Red Flour Beetle	>200	200
Coleoptera/Curculionidae	*Anthonomus grandis* Cotton Boll Weevil	>50	50
Coleoptera/Curculionidae	*Anthonomus eugenii* Pepper Weevil	>200	200
Coleoptera/Curculionidae	*Sitophilus oryzae* Rice Weevil	>200	200
Lepidoptera/Noctuidae	*Helicoverpa zea* Corn Earworm	>200	200
Lepidoptera/Crambidae	*Ostrinia nubilalis* European Corn Borer	>200	200
Hymenoptera/Pteromalidae	*Nasonia vitripennis*	>200	400
Hymenoptera/Aphidae	*Apis mellifera* Honey Bee	>360	360
Neuroptera/Chrysopidae	*Chrysoperla carnea* Green Lacewing	>8000	8000
Collembola/Isotomidae	*Folsomia candida*	>870	870

The results from the susceptibility assays show that toxicity was only observed within the Chrysomelid family of beetles (Colorado potato beetle and western corn rootworm). Additional species of corn rootworm, including southern, northern, and Mexican corn rootworm, were not tested (MON 863 is claimed to be effective in controlling corn rootworm (*Diabrotica* spp.) larvae). Other assayed insects, including species from Lepidoptera, Neuroptera, Collembola, Hymenoptera, and the additional families of Coleoptera did not exhibit any susceptibility to Cry3Bb at the tested doses.

While the test included several beneficial species (ladybird beetle, green lacewing, honey bee, and *Nasonia vitripennis* parasitic wasp), these data do not represent a complete assessment of effects to beneficial insects (non-target effects, including beneficial species, have been reviewed separately -- see R. Rose memo to M. Mendelsohn, 5/20/02).

It is also noted that there are a number of endangered beetle species that are of possible concern with the widespread planting of MON 863 corn. These beetle include the American burying beetle (family: Silphidae), delta green ground beetle (Carabidae), and valley elderberry longhorn beetle (Cerambycidae) (previously approved EUPs for MON 863 have been restricted to prevent exposure to these beetles). The submitted data did not include any assays from other species in the Silphidae, Carabidae, and Cerambycidae families. However, it is noted that a separate assessment of MON 863 corn effects on endangered species has been completed (see R. Rose memo to M. Mendelsohn, 5/20/02).

Comparing the Efficacy of MON 853 and MON 863 to Three Corn Rootworm Species, Northern Corn Rootworm (*Diabrotica barberi*), Southern Corn Rootworm (*D. undecimpunctata howardi*), and Western Corn Rootworm (*D. virgifera virgifera*) (MRID # 455382-08)

In this experiment, Monsanto compared the relative efficacy of two transformed corn hybrids expressing the Cry3Bb protein (MON 853 and MON 863) in preventing damage from three species of corn rootworm larvae. This was accomplished by artificially infesting potted corn plants (treatments consisting of the two transformed hybrid and a non-transformed control hybrid) with eggs from each of three rootworm species. Each plant (in the V2 stage) was infested with approximately 800 eggs (6-8 plants per treatment were used). Root damage was scored using the Iowa Root Damage Rating (RDR) index (1 = no damage, 6 = extensive damage) after 3-4 weeks of larval feeding.

Results from the study showed that both MON 853 and MON 863 experienced significantly less root damage from all three rootworm species than the non-transformed control hybrid. In terms of western and northern corn rootworm damage, MON 863 had significantly less root damage (<2 RDR) than MON 853 (- 2.3 RDR). For southern corn rootworm, there was no significant difference between MON 853 and MON 863 (RDR - 3.5 - 3.8). Southern corn rootworm damage was greater than western or northern corn rootworm damage for all treatments. It is noted that, at present, Monsanto has not proposed to register MON 853.

Efficacy of MON 863 Against Corn Rootworm and Comparison to Insecticide Treatments - Results of Year 2000 Field Trials (MRID # 453613-03)

In this experiment, Monsanto evaluated the relative effectiveness of MON 863 and conventional pesticide treatments at preventing damage from corn rootworm feeding in field efficacy trials. The pesticides tested (all soil insecticides) included Force 3G (tefluthrin), Counter CR (terbufos), and Lorsban 15G (chlorpyrifos).

The study consisted of three separate field experiments, all of which utilized similar growth stage MON 863 hybrid and a non-transgenic control hybrid (negative MON 863 isoline). In each of the experiments, treatments were deployed using a randomized block design and were scored for root damage in late July. Root damage was assessed using the Iowa Root Damage Rating (RDR) index (1 = no damage, 6 = extensive damage, >3 = economic threshold). For the first experiment (conducted at seven different locations), treatments (MON 863, control, Force 3G, Counter CR, and Lorsban 15G) were deployed as four-row strips (4 replicates per treatment). Each plot was artificially infested with 800 rootworm eggs/foot (species not specified). In the second experiment (conducted at eight different locations), MON 863 was evaluated against Force 3G treatment and an untreated control. Treatments were deployed as single rows and were artificially infested with 1600 rootworm eggs/foot (species not specified). In the third experiment (conducted at nine test sites), treatments (MON 863, control, Force 3G, Counter CR, and Lorsban 15G) were planted in four-row strips in continuous corn acres or a corn/pumpkin trap crop (no artificial rootworm infestation was used). For all tests, RDR damage was analyzed via analysis of variance and t-tests to determine significant differences between treatments. Also, a "consistency rating" was calculated for each experiment by determining the percentage of root damage in a treatment that is below the economic threshold (RDR = 3) when the corresponding control treatment root damage is above the threshold.

The results of the first experiment showed that when summed across all test locations, MON 863 (RDR = 2.02), Force 3G (2.40), Counter CR (2.26), Lorsban 15G (2.40) experienced significantly less root damage than the untreated control (3.91), although there was no significant difference between MON 863 and the insecticide treatments. However, at three of the seven locations, MON 863 had significantly less root damage than all of the other insecticide treatments. For the second experiment, when summed across all eight test sites, MON 863 (RDR = 1.41) and Force 3G treatment (1.91) showed significantly less root damage than the untreated control (3.27). There was no significant difference between MON 863 and Force 3G, although root damage for MON 863 was significantly less than that for Force 3G at five of the test sites. In the third experiment, MON 863 experienced significantly less root damage (RDR = 1.72, summed over all nine locations) than any of the insecticide treatments or the control (all insecticide treatments had significantly less damage than the control). For all three experiments, the "consistency rating" for MON 863 was close to 100%, meaning that damage in MON 863 hybrids was almost always kept below the economic threshold when the control treatment showed damage exceeding the threshold.

Taken together, the results show that MON 863 prevented root damage from rootworm feeding as well or better than rootworm soil insecticides. Root damage ratings for MON 863 were typically between 1.2 and 2.0, a high level of control relative to untreated control hybrids. In addition, the results were generally consistent from location-to-location (test sites included plots in six separate corn-growing states).

It should be noted that efficacy data such as these (and other studies) will also be reviewed and considered as part of a full benefits assessment for MON 863.