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MEMORANDUM

SUBJECT: Review of an amendment request to reduce the refuge required for MON 89034 corn in the Corn Belt. EPA Reg No. 524-575 and 524-576. MRID#: 474748-01. Decision#: 394797. DP Barcode: 354723.

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Action Requested

BPPD[1] has been asked to review an amendment request submitted by Monsanto Company to reduce the required refuge for MON 89034 Bt corn (EPA Reg. No. 524-575) and MON 89034 x MON 88017 Bt corn (524-576) in the Corn Belt. MON 89034 was initially registered with a requirement to plant a 20% refuge in the Corn Belt; Monsanto is proposing to reduce the percent refuge to 5% in these areas. In support of the amendment request, Monsanto submitted data and an analysis of potential resistance risk in a volume titled "Assessment of the Impact of MON 89034 Introduction on Bt Resistance Development in European and Southwestern Corn Borer" (MRID# 474748-01).

Conclusions and Recommendations

1) Monsanto's request to reduce the required non-Bt corn refuge for MON 89034 corn from 20% to 5% is scientifically supported by the submitted cross resistance information and model simulations and should not significantly increase the risk of resistance for

[1] The use of BPPD in this review refers to the BPPD IRM Team consisting of Alan Reynolds and Jeannette Martinez

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European corn borer (ECB) and southwestern corn borer (SWCB). While there are still some uncertainties regarding the refuge reduction (described below in # 3-5), the overall conclusions and recommendations are not affected.

2) BPPD notes that this request pertains only to MON 89034 grown in the U.S. Corn Belt; MON 89034 grown in southern cotton-growing regions (as defined by the terms and conditions of registration) is unaffected by this amendment and must be planted with a 20% non-Bt corn refuge. Although not formally addressed in the submission, the conclusions of this review are also applicable to the lepidopteran refuge portion of the MON 89034 x MON 88017 registration (EPA Reg. No. 524-576).

3) As a condition of registration of MON 89034, Monsanto was required to analyze potential cross resistance in existing Bt corn and Bt cotton products for Cry1A.105, Cry1Fa and Cry1Ac. Monsanto has sufficiently addressed cross resistance for Cry1A.105 and Cry1Fa in this submission, but insufficient analysis was provided for Cry1Ac and Cry1A.105. So that BPPD can fully assess the cross resistance potential of Cry1A.105 with Cry1Ac, it is recommended that Monsanto provide additional information either experimentally (i.e. binding studies or with resistant colonies) or using another analysis.

4) Potential cross resistance between Cry1A.105 with Cry1Ac is an issue primarily for the corn earworm (CEW), which feeds on corn and cotton and could be exposed to both Cry1A.105 (in MON 89034 corn) and Cry1Ac (in Bollgard cotton). However, several factors reduce the likelihood of CEW resistance developing to MON 89034 corn with a 5% refuge: 1) CEW is not as prominent a pest in the Corn Belt as ECB; 2) CEW does not overwinter well in the Corn Belt; 3) CEW is highly polyphagous (feeding on numerous crops and wild hosts) and there may be some degree of natural refuge in the Corn Belt.

5) BPPD noted several limitations to the model simulations used to support the amendment: 1) No model simulations were conducted to compare 5% (proposed) vs. 20% (current) refuge for MON 89034; 2) The model time horizon (30 years) limited comparisons between many of the model scenarios; 3) SWCB scenarios included dose mortality estimates somewhat higher than those suggested by previously-submitted data. While BPPD believes the model analysis would have been improved had these areas been addressed, the impact on the model output would likely not have been great enough to alter the overall conclusions.

6) Since MON 89034 is an expiring registration (expiration date: September 30, 2010), BPPD recommends reevaluating the 5% refuge if warranted by cross resistance data or other information received during this interim period.

Background

MON 89034 and MON 89034 x MON 88017 are plant-incorporated protectants (PIP) that were registered for commercial use on June 10, 2008. Event MON 89034 contains two proteins (Cry1A.105 and Cry2Ab2) that are targeted against lepidopteran corn pests including European corn borer (*Ostrinia nubilalis*, ECB), corn earworm (*Helicoverpa zea*, CEW), southwestern corn borer (*Diatraea grandiosella*, SWCB), and fall armyworm (*Spodoptera frugiperda,* FAW). MON 88017 was registered separately in 2003 and controls corn rootworm (*Diabrotica* sp., CRW).

As part of the IRM proposal for MON 89034 corn, Monsanto proposed a 5% lepidopteran structured refuge for non-cotton growing regions instead of the 20% refuge that has been required for all other Bt corn registrations. Monsanto reasoned that the combination of two toxins targeting lepidopteran corn pests with no cross resistance allowed for a reduced refuge with little risk of resistance. BPPD's review of the IRM proposal (BPPD 2007) agreed with much of Monsanto's justification but determined that there were a number of uncertainties in the request for lower refuge. Specifically, there were three areas of concern: 1) Cry1A.105 and Cry2Ab2 dose determination for the major target pests (ECB, CEW, SWCB, and FAW); 2) cross resistance potential between Cry1A.105 and Cry1F and Cry1Ac (toxins expressed in previously-registered PIPs); and 3) species-specific (e.g, ECB and SWCB for the Corn Belt), spatially-explicit, landscape modeling to explore the durability of MON 89034 versus single-protein Bt corn products. Given the uncertainty of the reduced refuge request, EPA registered MON 89034 with a 20% structured refuge requirement, similar to other Bt corn products. Separately, EPA did agree with Monsanto's request to reduce refuge in cotton-growing areas from 50% to 20% (see discussion in BPPD 2007). As a condition of registration, Monsanto was required to address cross resistance in existing Bt corn and Bt cotton products for Cry1A.105, Cry1Fa and Cry1Ac.

Monsanto has subsequently materials to address these three areas of uncertainty as part of a new amendment request for a reduced 5% refuge for non-cotton regions. The response, including a discussion of cross resistance and a new model, is included in a study titled "Assessment of the Impact of MON 89034 Introduction on Bt Resistance Development in European and Southwestern Corn Borer" (MRID# 474748-01).

<u>Monsanto's Proposed Amendment to Support a 5% Refuge for MON 89034</u>

Monsanto's proposal for a 5% refuge with MON 89034 includes two major components: 1) a discussion of the cross resistance potential between the toxins in MON 89034 and 2) a deterministic model to simulate a 5% refuge and the risk of resistance for ECB and SWCB. Each of these sections is described and reviewed individually below.

In lieu of submitting new dose determination data for Cry2Ab2 and Cry1A.105 for the major target pests, Monsanto has used the existing dose information (submitted for the original registration) in the new simulation model. Therefore, Monsanto's response to the dose determination uncertainties (detailed in BPPD 2007) will be discussed and reviewed in the modeling section below.

1) Cross Resistance Potential

MON 89034 contains both Cry1A.105 and Cry2Ab2, which target the same lepidopteran corn pest complex. The Cry1A.105 toxin is a "chimeric" protein containing domains I and II and the C-terminal from Cry1Ac and domain III from Cry1Fa while the Cry2Ab2 protein is the same as that currently expressed in Monsanto's Bollgard II cotton. Monsanto has sufficiently demonstrated that the cross resistance potential between these two proteins should be low, primarily due to differing modes of action (see discussion in BPPD 2007). However, in evaluating new PIP traits, the landscape of previously-registered toxins in the same crop must be taken into account. In addition, for corn PIPs, cotton must also be considered because one of the key target pests, corn earworm (also referred to as cotton bollworm, CBW, when a pest on cotton), is a pest of both crops. As a condition of registration, Monsanto was required to address cross resistance in existing Bt corn and Bt cotton products for Cry1A.105, Cry1Fa and Cry1Ac.

Monsanto's amendment submission for MON 89034 contained a discussion of cross resistance including an analysis of previous studies as well as a summary of recently developed data. Analysis of existing data was conducted for four toxin combinations: 1) Cry1Ab vs. Cry1Ac; 2) Cry1F vs. Cry1Ab and Cry1Ac; 3) Cry2Ab2 vs. Cry1 proteins; and 4) Cry1A.105 vs. Cry1Ab and Cry1Ac. New data were presented for comparisons between Cry1A.105 and Cry2Ab2 vs. Cry1F.

<u>Cry1Ab vs. Cry1Ac</u>: Based on a literature review of binding studies with numerous lepidopteran species, Cry1Ac is known to have strong cross resistance with Cry1Ab. Both toxins share a high affinity binding site in ECB, CEW/CBW, SWCB, FAW, and others (references cited in MRID# 474748-01).

<u>Cry1F vs. Cry1Ab and Cry1Ac</u>: Cry1F also shares a binding site with Cry1Ab/Cry1Ac, though the level of cross resistance between Cry1F and Cry1A is not as strong as Cry1Ab vs. Cry1Ac. ECB resistant to Cry1Ab have been shown to be partially resistant to Cry1F although Cry1F resistant ECB were not cross resistant to Cry1Ab and only slightly resistant to Cry1Ac. Similar trends have also been shown with tobacco budworm (*Heliothis virescens*, TBW) (references cited in MRID# 474748-01). Overall, Cry1F can be considered partially cross resistant to Cry1Ab and Cry1Ac. The availability of binding sites may explain the partial cross resistance: Cry1Ab and Cry1Ac could have more different sites to bind with than Cry1F so that resistance to Cry1F still allows for some binding of Cry1Ab or Cry1Ac.

<u>Cry2Ab vs. Cry1 proteins</u>: A literature review suggests that Cry2Ab has no cross resistance potential with any of the currently registered Cry1 proteins including Cry1Ab and Cry1Ac. Studies have been conducted with numerous cotton pests including CEW, TBW, pink bollworm (*Pectinophora gossypiella*, PBW), and *Helicoverpa armigera* that revealed no shared binding sites between Cry2A and Cry1Ab or Cry1Ac proteins. Additional studies with Cry1Ac-resistant TBW, CEW/CBW, and PBW found no cross resistance with Cry2Ab (references cited in MRID# 474748-01). Previously submitted data by Monsanto for MON 89034 (Head 2006; reviewed in BPPD 2007) demonstrated that Cry1Ab-resistant ECB were not found to be cross resistant with Cry2Ab while Cry2Ab2-resistant *H. armigera* were not cross resistant with Cry1A.105 or Cry1Ac.

Cry1A.105 vs. Cry1Ab and Cry1Ac: For Cry1Ab, a previously submitted binding study with ECB (Head 2006; reviewed in BPPD 2007) showed that the protein has a distinct binding site from Cry1A.105. This was confirmed by studies with Cry1Ab-resistant ECB and sugarcane borer (*Diatraea saccharalis*, SCB) that showed no cross resistance with Cry1A.105. Monsanto argues that due to similar characteristics between Cry1Ab and Cry1Ac (i.e. mode of action), it is reasonable to assume that Cry1Ac should not be cross resistant with Cry1A.105. However, no binding studies or experiments with resistant colonies were described to verify that assumption.

Cry1A.105 and Cry2Ab2 vs. Cry1F: New data were cited by Monsanto (Schlenz et al. 2008) to assess the cross resistance potential between Cry1A.105/Cry2Ab2 and Cry1F using Cry1F-resistant ECB and FAW colonies. Artificial diet bioassays were used to test Cry1A.105, Cry2Ab2, and control groups against ECB and FAW colonies previously selected for high-level Cry1F resistance as well as unselected control colonies. A range of five concentrations was used and the test was conducted over a seven day period to determine growth inhibition (GI_{50}) for each colony. The results showed that, as expected, Cry1F-resistant ECB and FAW were not cross resistant with Cry2Ab2 -- the GI_{50} resistance ratios (Cry1F-resistant : Cry1F-susceptible) were 1.4 for ECB and 0.11 for FAW. With Cry1A.105, the GI_{50} resistance ratios were > 3.9 for ECB and 7.0 for FAW, indicating low level cross resistance.

Table 1: Cross resistance potential of MON 89034 (Cry1A.105 and Cry2Ab2) with previously registered Bt corn toxins.

	Bt toxins in MON 89034	
Existing Bt toxins	**Cry1A.105**	**Cry2Ab2**
Cry1Ab	No cross resistance (ECB, SCB)	No cross resistance (ECB)
Cry1Ac	Unlikely cross resistance, but unverified experimentally	No cross resistance (TBW, PBW, CEW/CBW)
Cry1F	Low level cross resistance (ECB, FAW)	No cross resistance (ECB, FAW)

<u>BPPD Review - Cross Resistance</u>

BPPD agrees with Monsanto's characterization of the cross resistance potential for the Cry1A.105 and Cry2Ab2 toxins with 1) each other (previously demonstrated in Head 2006), 2) Cry1F, and 3) Cry1Ab. Binding and resistant colony work conducted by Monsanto and other researchers clearly show that no cross resistance can be expected between Cry1A.105, Cry2Ab2 and Cry1Ab (see Table 1 above). New data referenced in Monsanto's amendment request also experimentally demonstrate the cross resistance potential between Cry1F and Cry2Ab2 (no cross resistance) and Cry1A.105 (low cross resistance).

However, BPPD still has reservations about Cry1Ac. While Monsanto has made the case that Cry1Ac should be expected to behave like Cry1Ab due to a similar mode of action, no experimental data (i.e. binding studies or bioassays with resistant insect colonies) were provided either in the original MON 89034 IRM submission (Head 2006) or the follow-up amendment request (MRID# 474748-01). BPPD notes that Cry1A.105 (a chimeric protein) contains domains I and II and the C-terminal from Cry1Ac. Cross-resistance could result when proteins share key structural features, which allows one resistance mechanism to confer resistance to more than one protein (Tabashnik, 1994; Gould et al., 1995).

BPPD recognizes that at the present time there are no registered Bt corn products containing Cry1Ac. Therefore, exposure to ECB and SWCB to Cry1Ac is unlikely, as neither is known as a cotton pest. FAW may occasionally feed on cotton, but favor corn and is also unlikely to have much exposure to Cry1Ac. On the other hand, successive generations of CEW may feed on both corn and cotton during the same growing season. This could result in a potential "double" exposure to Bt cotton (including Cry1Ab) and Bt corn (including Cry1A.105) and increased selection pressure for resistance, particularly if there is a risk of cross resistance.

Given that Monsanto has proposed to substantially reduce refuge for MON 89034 from 20% to 5%, cross resistance is an important consideration even for Cry1Ac. Although improbable, BPPD cannot rule out that a CEW/CBW population could develop Cry1Ac resistance in cotton and then encounter MON 89034 corn. [Tabashnik et al. (2008) have argued that Cry1Ac resistance has already evolved in CBW in the south, although this conclusion has been disputed (Moar et al. 2008).] Should there be a degree of cross resistance between Cry1Ac and Cry1A.105, MON 89034 might functionally have only Cry2Ab2 remaining as an effective toxin against CEW. With a reduced refuge (5%), selection pressure could be increased for resistance to MON 89034 and Cry2Ab2 (which also is expressed in Bollgard II cotton). So that BPPD can fully assess the cross resistance potential of Cry1A.105 with Cry1Ac in CEW/CBW, it is recommended that Monsanto provide additional information either experimentally (i.e. binding studies or with resistant colonies) or using another analysis. Alternatively, Monsanto could revise the CEW model submitted with the original MON 89034 IRM plan (Head 2006) to support 20% refuge in cotton-growing regions. This model simulated CEW resistance to MON 89034 and assumed complete cross resistance between Cry1A.105 and Cry1Ac; the model could be adapted to evaluate a 5% refuge in the Corn Belt with similar assumptions.

2) Modeling

As part of the review of Monsanto's initial IRM plan for MON 89034, BPPD identified the need for additional species-specific (e.g, ECB and SWCB for the Corn Belt), spatially-explicit, landscape modeling to explore the durability of MON 89034 versus single-protein Bt corn products (BPPD 2007). Previously, Monsanto had cited the modeling work of Roush (1998) to demonstrate that a 5% refuge was justified with a two toxin pyramided product. Roush's model has a number of key assumptions, particularly in terms of the toxin expression level in pyramided product. For homozygote susceptible insects, the model assumes 95% mortality and 70% mortality for heterozygotes (with one resistance allele) for each toxin. However, the dose information provided by Monsanto for MON 89034 was not sufficient to demonstrate that each protein would kill 95% of the homozygous susceptible insects and 70% of the heterozygotes (see BPPD 2007). BPPD recommended that Monsanto further characterize the dose expression for the MON 89034 toxins for the major target pests of the Corn Belt (ECB and SWCB). Given the dose uncertainties, BPPD could not at the time of registration support the use of Roush's model to justify a lower 5% refuge for MON 89034 (BPPD 2007).

Rather than re-run dose studies for Cry1A.105 or Cry2Ab2, Monsanto created a deterministic model for ECB and SWCB using dose mortality estimates consistent with the previously conducted studies. The model (Gustafson and Head 2008; contained in MRID# 474748-01) included the toxins from other registered Bt corn products (Cry1Ab, Cry1F) and has a number of assumptions and parameters:

- Dose mortality for ECB: 99.9% for Cry1 (Cry1Ab, Cry1F, Cry1A.105) and Cry2Ab2 toxins (one mortality scenario was modeled);
- Dose mortality for SWCB: 99 - 99.5% for Cry1 and 85 - 95% for Cry2Ab2 (six dose mortality scenarios were modeled);
- Complete resistance to Cry2Ab2 and Cry1A.105 (i.e. survival probability of heterozygote resistant individuals = 1) with no fitness costs;
- Heterozygotes (i.e. with one resistance allele) survival probability is twice that for homozygote susceptible insects;
- Three cross resistance scenarios: 1) Cry1A.105 and Cry1Ab fully cross resistant (but not Cry1F) (the "base case" scenario); 2) Cry1A.105 and Cry1F fully cross resistant (but not Cry1Ab) (alternate "base case" scenario), and 3) Cry1A.105, Cry1Ab, and Cry1F all fully cross resistant (worst case scenario);
- All resistance alleles (Cry1, Cry1A.105, and Cry2Ab2) have initial frequencies of 0.005. Cry1Ab and Cry1F are modeled as one output (i.e. estimated time to resistance for Yieldgard/Herculex);
- MON 89034 was assumed to have a refuge of 5%; other single gene products (Yieldgard and Herculex) were assumed to have 20% refuge;
- ECB and SWCB have no natural refuge (i.e. wild hosts or other cultivated crops that could serve as a source of susceptible insects) and have two generations per year on corn;
- A range of market share adoption values for MON 89034 and other products (Herculex and Yieldgard) were included in the model simulations. MKT 1 = 100% MON 89034; MKT 2 = 50% MON 89034, 25% MON 810, 25% TC1507; MKT 3 = 0% MON 89034, 50% MON 810, 50% TC1507.

Most of the assumptions above are conservative estimates, with the possible exception of the dose mortality parameters for SWCB (see discussion in the BPPD review section). Simulations were run with both ECB and SWCB to estimate the time to resistance (in years; up to a maximum of 30 years) and resistance allele frequency for each of the three cross resistance scenarios described above. Within each cross resistance scenario, model runs were conducted for three different market adoption contingencies of MON 89034, MON 810 (Cry1Ab Yieldgard) and TC1507 (Cry1F Herculex).

ECB Results

For ECB, the results of the model runs were relatively consistent among the different cross resistance and market adoption scenarios. In almost all cases, the durability of the MON 89034 toxins (Cry1A.105 and Cry2Ab2; assuming a 5% refuge) exceeded the 30 year time frame of the model. Only in the "worst case" cross resistance scenario (i.e., all three toxins cross resistant) was the durability of Cry1A.105 less than 30 years (29 years) for ECB -- Cry2Ab2 remained effective in all model simulations (> 30 years). For the other Cry1 toxins (Cry1Ab and Cry1F) that are expressed in other Bt corn products, resistance developed in less than 30 years for some of the cross resistance and market adoption scenarios. In the "base case" (Cry1Ab and Cry1A.105 cross resistant), the durability of Cry1Ab/Cry1F lasted 26 years (0% MON 89034, 50% MON 810, 50% TC1507) and 29 years (50% MON 89034, 25% MON 810, 25% TC1507). However, for the alternate base case (Cry1F and Cry1A.105 cross resistance), resistance to Cry1Ab/Cry1F did not evolve within 30 years. In the worst case scenario (all three toxins cross resistant), resistance to Cry1Ab/Cry1F developed in 29 years.

SWCB Results

For SWCB, more model simulations were run to account for a range of dose mortalities. Overall, durability of the traits was affected by the dose mortality scenarios -- the simulations with lower dose mortality frequently resulted in fewer years to resistance in Cry1A.105 and Cry1F than those with higher dose mortalities. As with ECB, Cry2Ab2 remained durable (> 30 years) in all but one of the simulations regardless of the cross resistance or market adoption scenario.

For the "base case" cross resistance scenario, the time to resistance was lowest in the market adoption scheme (MKT 3) without MON 89034 (50% MON 810, 50% TC1507) ranging from 17 years (lower dose mortalities for Cry1 and Cry2Ab2 toxins) to 20.5 years (higher dose mortalities). Once MON 89034 was added to the model (MKT 1 and 2), the time to resistance with the Cry1 toxins increased by 2 -2.5 years for all simulations. Cry1A.105 and Cry2Ab2 did not evolve resistance in any of the model runs for MKT 2, although there were two instances with MKT 1 (100% MON 89034) in which resistance evolved within 30 years. In both of these cases, lower dose mortality values for SWCB (85% for Cry2Ab2; 99% for Cry1A.105) were included in the model.

Time to resistance in the "alternate base case" (Cry1F and Cry1A.105 cross resistant) was > 30 years in almost all cases. Only in the simulation that incorporated the lowest dose mortality values (85% for Cry2Ab2 and 99% for Cry1A.105) did resistance evolve to one of the toxins (28.5 years for Cry1A.105).

In the "worst case" (Cry1Ab, Cry1F and Cry1A.105 are all cross resistant), resistance developed in all scenarios for both the Cry1 toxins and Cry1A.105. Conversely, Cry2Ab2 remained durable (> 30 years) for all of the simulations. Time to resistance in the Cry1 and Cry1A.105 toxins was lowest (17 years) in the model run using the lower SWCB dose mortality values (85% for Cry2Ab2 and 99% for Cry1A.105). Resistance also evolved for case with the higher dose mortality values, ranging up to 22 years for each toxin. A truncated summary of the results for all of the model simulations is contained in Table 2 below -- the complete results of the modeling are detailed in Tables 5 and 6 in Monsanto's submission (MRID# 474748-01).

Table 2: Results of Monsanto's model simulations of MON 89034 (5% refuge), MON 810, TC1507 (20% refuge) expressed in years to resistance (30 year maximum). Derived from data reported in MRID# 474748-01.

Pest		Cross resistance scenario				
		Base case[1]			Alt. base case[2]	Worst case[3]
		MKT 1	MKT 2	MKT 3		
ECB	Cry1A.105	>30	>30	N/A	>30	29
	Cry2Ab2	>30	>30	N/A	>30	>30
	Cry1Ab/Cry1F	N/A	29	26	>30	29
SWCB	Cry1A.105	22.5 - >30	>30	N/A	28.5 - >30	17 - 22
	Cry2Ab2	25 - >30	>30	N/A	>30	>30
	Cry1Ab/Cry1F	N/A	19 - 23	17 - 20.5	>30	17 - 22

[1] Base case = Cry1Ab and Cry1A.105 cross resistant; three different marketing scenarios included (Mkt 1 = 100% MON 89034, 0% MON 810/TC1507; Mkt 2 = 50% MON 89034, 25/25% MON 810/TC1507; Mkt 3 = 0% MON 89034, 50/50% MON 810/TC1507).
[2] Alt. base case = Cry1F and Cry1A.105 cross resistant (only Mkt 2 simulated).
[3] Worst case = Cry1A.105, Cry1Ab, and Cry1F all fully cross resistant (only Mkt 2 simulated).

Based on the model work, Monsanto concluded that the durability of the MON 89034 proteins (Cry1A.105 and Cry2Ab2) will remain strong for both ECB and SWCB. With a 5% refuge, Monsanto predicts that MON 89034 will have at least 22 years durability even under the "worst case" model assumptions. The durability of Cry2Ab2 in the model was particularly robust in almost all simulations for ECB and SWCB (only one simulation predicted less than 30 years durability). Resistance to Cry1A.105 was also rare in most simulations, although the "worst case" modeling (assuming complete cross resistance with Cry1Ab and Cry1F) showed resistance developing in less than 30 years. Monsanto also noted that in the simulations with different market adoption scenarios, the addition of MON 89034 increased the time to resistance for the previously registered Cry1 toxins (Cry1Ab and Cry1F).

<u>BPPD Review - Modeling</u>

BPPD agrees with Monsanto's overall conclusions that the model simulations demonstrate the effectiveness in delaying resistance of MON 89034 and provide support for the use of a 5% refuge in the Corn Belt. However, BPPD notes that some of the parameters and assumptions of the model could be revised to improve and expand the overall analysis.

For ECB, the model clearly predicts that resistance is unlikely to evolve to Cry1A.105, Cry2Ab2, or the previously-registered Cry1 toxins. Even under the worst case scenario that assumed complete cross resistance, the durability of all toxins was at least 29 years. Presumably, a large reason for this is the high dose mortality of the MON 89034 toxins against ECB. Previous mortality studies submitted by Monsanto (reviewed in BPPD 2007) showed that the Cry1A.105 and Cry2Ab2 proteins in MON 89034 each provide essentially 100% control of ECB (Monsanto assumed 99.9% mortality for each toxin in the model).

For SWCB, the model predictions were more varied, largely due to the different simulations run with the range of dose mortality assumptions. Not surprisingly, the simulations that were run with the lower mortality estimates (i.e. 85% for Cry2Ab and/or 99.0% for Cry1) resulted in less time to resistance than those using the higher dose values. In the worst case simulations with the lower dose estimates, SWCB resistance evolved in 17 years to both Cry1A.105 and Cry1Ab/Cry1F while with the higher doses resistance took 21 or 22 years to develop. As with ECB, Cry2Ab2 remained durable (>30 years) for almost all of the simulations.

A number of factors appeared to influence the model results. BPPD agrees with Monsanto that the addition of MON 89034 in the simulations testing various market adoption scenarios delayed resistance in the other previously-registered Cry1 toxins. Likely, these results were due to less selection pressure on each individual toxin because of a diverse mosaic of toxins in the landscape. Cross resistance was also an important variable. Monsanto's "base case" for cross resistance assumed cross resistance between Cry1Ab and Cry1A.105. This resulted in resistance always developing in Cry1Ab/Cry1F (i.e. within 30 years), although Cry1A.105 and Cry2Ab2 durability remained strong. On the other hand, when cross resistance between Cry1A.105 and Cry1F was assumed, resistance rarely developed in either the MON 89034 toxins or the existing Cry1 toxins. In the worst case scenario (all three toxins cross resistant), the durability of Cry1A.105 to SWCB was clearly impacted relative to the other cross resistance simulations. Conversely, Cry2Ab remained durable in almost all cases regardless of the varying assumptions and scenarios included in the model. Since Cry2Ab is not cross resistant to the Cry1 toxins, this result was not unexpected.

BPPD generally agrees with Monsanto that conservative assumptions were used in the model. However, BPPD notes that several of the parameters could have been expanded or have included an additional degree of conservatism or additional refinement to improve the model analysis. For example, Monsanto's simulations assumed a 5% refuge for MON 89034 (while maintaining the 20% refuge for the other Bt toxins). Although MON 89034 is currently registered with a requirement for a 20% refuge, simulations were not run with the larger refuge size. Separate simulations with 5% and 20% MON 89034 refuges would have been useful for comparative purposes. To illustrate using the SWCB "base case" (with the three different marketing adoption cases), with no MON 89034 adoption resistance to the Cry1 toxins occurred in 17 - 20.5 years. When MON 89034 with a 5% refuge was included, the time to Cry1 resistance was 19 - 23 years --

indicating that the addition of MON 89034 provides some delay in resistance development (2 - 2.5 years). It would have been interesting to observe the impact of adoption of MON 89034 with a 20% refuge on Cry1 resistance. In all likelihood, the time to resistance would be increased, although the magnitude of such an increase is unknown. Had the difference been small, it could be argued that there is little value gained in having a 20% refuge versus a 5% refuge.

The model time frame (maximum 30 years) was another limiting parameter. Many of the simulations resulted in no resistance within the 30 year time period of the model, so it was difficult to discern the effects of certain variables (i.e. cross resistance, market adoption, dose mortality) between model runs. Had the time horizon been extended (e.g. to 50 years), differences between the various model scenarios may have been apparent.

For the SWCB simulations, Monsanto used dose mortality range of 85-95% for Cry2Ab2 and 99-99.5% for Cry1 toxins. Based on the dose data submitted for the registration of MON 89034 (reviewed in BPPD 2007), BPPD believes these estimates to be somewhat high. For example, dose data for Cry2Ab2 and SWCB suggested a mortality range of 80-90%. The Cry1A.105 protein in MON 89034 provided approximately 95% control in mortality assays, though the other registered Cry1 proteins (Cry1Ab and Cry1F) may provide closer to 99% of SWCB. Had the model simulations been run with these more conservative dose estimates, it is likely the time to resistance would have been reduced in some scenarios. The extent of this effect is unknown, although BPPD notes that the differences between the lower Cry2Ab2 dose (85%) and the highest dose (95%) in the range appeared to be negligible in the model runs (i.e. no differences in years to resistance).

BPPD Review - Overall Proposal to Reduce Refuge

Taken together, Monsanto's cross resistance and modeling work provides justification for reducing the MON 89034 structured refuge requirement in the Corn Belt from 20% to 5% non-Bt corn. Key elements of support include a lack of cross resistance between Cry2Ab2 and Cry1 proteins and model simulations which demonstrate strong durability of Cry1A.105 and Cry2Ab2 under a variety of dose, market adoption, and cross resistance scenarios. Reducing the refuge to 5% is unlikely to increase the selection pressure for resistance in either MON 89034 or the other previously-registered Cry1Ab or Cry1F corn hybrids.

Despite a good case for a refuge reduction, BPPD notes that there are still some limitations and uncertainties in the analysis that could be addressed to provide additional support for the proposal. These areas include:

- Cross resistance between Cry1Ac and Cry1A.105. Cry1Ac is registered in Bt cotton products and the chimeric protein Cry1A.105 has two Cry1Ac domains. CEW feed on both corn and cotton and successive generations may have exposure to both Cry1A.105 and Cry1Ac during the same growing season;

- No model simulations were conducted to compare 5% vs. 20% refuge for MON 89034; the model assumed a 5% refuge for MON 89034;
- The model time horizon was limited to 30 years. Many of the model runs did not evolve resistance during this time precluding comparisons between some of the scenarios;
- SWCB model simulations included dose mortality estimates somewhat higher than those suggested by previously-submitted data. For Cry2Ab2, mortality ranged from 80 to 90% in dose testing submitted for MON 89034 (instead of 85-95% used in the model). Cry1A.105 caused 95% mortality in submitted dose studies, though a range of 99-99.5% was used in the model.

As a condition of registration of MON 89034, Monsanto was required to address cross resistance in existing Bt corn and Bt cotton products for Cry1A.105, Cry1Fa and Cry1Ac. Monsanto has sufficiently addressed cross resistance for Cry1A.105 and Cry1Fa, but there are lingering questions regarding Cry1Ac and Cry1A.105. The amendment submission included only a circumstantial discussion of Cry1Ac cross resistance with an assumption that the protein will behave similarly to Cry1Ab. However, since Cry1A.105 contains domains I and II and the C-terminal from Cry1Ac, BPPD is still concerned about the potential for cross resistance. As such, BPPD recommends additional work (as described in the cross resistance section above) to satisfy the condition of registration. Should additional cross resistance work (as previously described) demonstrate little or no cross resistance potential between Cry1A.105 and Cry1Ac, further support could be provided for the use of a 5% refuge in the Corn Belt.

In terms of resistance risk for MON 89034, cross resistance between Cry1Ac and Cry1A.105 is an issue primarily for CEW. This insect is known to feed on both corn and cotton during the same growing season and could be exposed to Cry1A.105 (in corn) and then Cry1Ac (in Bollgard cotton) later in the growing season. Theoretically, CEW could develop resistance to Cry1Ac due to exposure in cotton -- should there be a degree of cross resistance between Cry1Ac and Cry1A.105, MON 89034 could functionally have only Cry2Ab2 remaining as an effective toxin against CEW. With a reduced refuge (5%), selection pressure could be increased for resistance to MON 89034 and Cry2Ab2 (which also is expressed in Bollgard II cotton). While these are legitimate concerns (and reason for additional analysis), BPPD notes that there are several mitigating factors that reduce the overall resistance risk for CEW and MON 89034. First, CEW is generally a lesser pest in the Corn Belt than ECB (and in some areas SWCB), primarily due to poor overwintering capability in much of the Corn Belt (i.e. north of Virginia, Tennessee, and Missouri). Therefore, selection pressure for resistance will likely be less for CEW than ECB which does overwinter in the Corn Belt. On the other hand, in cotton-growing regions south of the Corn Belt where CEW can overwinter, conditions for resistance development may be more probable. In these areas, a 20% refuge (approved with the initial registration of MON 89034) will still be required. Along these lines, in Monsanto's original MON 89034 IRM submission, modeling was conducted to support the use of a 20% refuge for CEW in southern cotton-growing regions (see discussion in BPPD 2007).

A second mitigating factor is that CEW is a highly polyphagous insect and is known to feed on a wide variety of plants including weeds, wild hosts, and other cultivated crops (unlike ECB and SWCB which feed primarily on corn). Analysis conducted for Bollgard II cotton determined that a natural refuge is present for CEW (CBW) in cotton growing areas in the southeastern U.S. (see BPPD 2004 and 2006). It is likely that in the Corn Belt, there is also at least some degree of natural refuge that could supplement a 5% structured refuge to help reduce the overall selection pressure on CEW and MON 89034. BPPD emphasizes that natural refuge for CEW has been quantified only in cotton-growing regions and that host utilization patterns in the Corn Belt are speculative.

The other modeling parameter uncertainties detailed above are relatively minor, though a more expanded model analysis could have provided stronger support for the proposal. Separate model runs with 5% and 20% MON 89034 refuges would have been useful to compare potential differences in times to resistance. Although since most of the simulations did not result in resistance within 30 years, any differences would have been difficult to detect. Expanding the time horizon of the model (e.g. from 30 years to 60 years) possibly could have fleshed out variation between model scenarios and provided a more thorough basis for comparison. Finally, BPPD would have preferred if Monsanto had used the more conservative estimates of SWCB dose mortality (based on the MON 89034 dose data), though the impact on the model output would likely have been relatively small.

MON 89034 is an expiring registration (expiration date: September 30, 2010) and BPPD recommends reevaluating 5% refuge if warranted by cross resistance data or other information during this interim period.

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