UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION PREVENTION

APR 04 2011

**MEMORANDUM**

**SUBJECT:** EPA Review of Monsanto and Dow response and supplemental modeling (dated March 18th, 2011) to address SAP uncertainties for a 5% SmartStax seed blend expressing events MON 89034, TC1507, MON 88017, and DAS-59122-7; PC Codes: 006481 (Cry1F) 006490 (Cry34/35Ab1), 006498 (Cry3Bb1), 006515 (Cry 2Ab2), 006514 (Cry1A.105); MRID# 484234-01.

**TO:** Mike Mendelsohn, Senior Regulatory Action Leader
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division (7511P)

**FROM:** Jeannette Martinez, Ecologist
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division (7511P)

**PEER REVIEW:** Alan Reynolds, Entomologist
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division (7511P)

## ACTION REQUESTED:

BPPD[1] has been asked to review Monsanto and Dow AgroSciences' response (dated March 18, 2011; MRID# 484234-01) to the uncertainties raised by the Scientific Advisory Panel (2011) regarding a 5% SmartStax Refuge-in-the-Bag (RIB) maize targeting lepidoptera. (Note: the corn rootworm component of SSX RIB is not assessed in this document.) BPPD's conclusions and recommendations are detailed in this memorandum.

[1] The use of BPPD refers to the IRM team consisting of Jeannette Martinez and Alan Reynolds.

## CONCLUSIONS AND RECOMMENDATIONS

**Note: The conclusions in this review pertain on to the lepidopteran component of the SmartStax seed blend component. Corn rootworm durability was previously assessed in BPPD (2010).**

1) Based on a review of the Science Advisory Panel (SAP) report (SAP 2011) and revised modeling submitted by Monsanto/Dow, BPPD concludes that a 5% seed blend for SmartStax corn will likely be less durable (perhaps significantly so) than a comparable (5%) block refuge for the product. BPPD notes, however, that a SmartStax 5% seed blend should be more durable than a 20% block refuge for a single toxin Bt corn product or a comparable (5%) seed blend for a two toxin pyramid. Larval movement, potential survival (and selection) of heterozygote genotypes, and loss of refuge effectiveness during the growing season are the primary factors that are likely to reduce durability in seed blends.

2) BPPD has major reservations regarding the modeling approaches taken by Monsanto and Dow (in separate models) to evaluate the SmartStax seed blend. Monsanto and Dow addressed larval movement implicitly (as opposed to an explicit approach as recommended by the SAP) and did not incorporate other important recommendations made by the SAP (i.e., epistatic mechanisms of resistance, density-dependent effects). As detailed in the SAP report, this approach is likely to result in overestimates of durability. For this reason, BPPD is unable to quantify the relative differences in durability estimates between refuge options (i.e., 5% SmartStax seed blend, 5% SmartStax block refuge, and 20% single toxin block refuge).

3) Despite the modeling uncertainties described in #2, BPPD believes that, in general, a three toxin product such as SmartStax should have greater durability than Bt corn products with two or fewer toxins and comparable refuge deployment. In other words, a three toxin seed blend can be expected to be more durable than a two toxin product with the same blend percentage. Similarly, a block refuge for a three toxin product should be more durable than the same block refuge for a two toxin product. This conclusion assumes that the three toxins have high efficacy and low cross-resistance potential. BPPD cautions, however, that the relative gain and loss in durability with multi-toxin pyramids should be evaluated on a case-by-case basis with product-specific data.

4) BPPD agrees with Monsanto/Dow that the SAP's modeling analysis 1) did not include three toxins for lepidoptera (two toxins were modeled for simplicity), 2) incorporated low dose scenarios for the toxins in SmartStax, and 3) neglected to consider effects of non-compliance on block refuge. The first two factors likely resulted in lower durability estimates for both block and blended refuge; the third probably led to an overestimate of durability for block refuges. Monsanto and Dow's revised modeling addressed these three components.

5) Block refuges and seed mixes present different potential risks and benefits for Bt corn resistance management. A summary of these factors is described below:

*Block Refuges*

- **Pros:**
  - Greater durability than other refuge approaches (including seed mixes, strip refuges, and natural refuge) in simulation models;
  - Allows for high production of susceptible insects;
  - Refuges can be managed to preserve yield.

- **Cons:**
  - Random mating may be less likely than with seed mixes or strip refuges if adult movement is limited (though not the case for mobile lepidoptera);
  - Compliance must be monitored (i.e., with a compliance assurance plan);
  - Non-compliance can result in no refuge deployment or inadequate refuge distance from Bt field to assure random mating (more important for high-dose PIPs such as in SmartStax), which can increase the risk of resistance;
  - Refuge may need to be treated with insecticides (potential economic and environmental costs);
  - There have been reports of a lack of available refuge seed in some areas;
  - Planting refuges can incur inconveniences and expenses for growers.

*Seed Blends*

- **Pros:**
  - Non-compliance is not an issue -- all seed bags are assumed to have the same amount of refuge seed (± standard error);
  - A compliance monitoring program should not be necessary (cost/resource savings);
  - No separate refuge management and insecticide use are needed;
  - Ease of use for growers.

- **Cons:**
  - Lower durability (perhaps substantially) than block refuges in simulation modeling, particularly for pests with high adult dispersal and larval plant-to-plant movement (resistance driven by heterozygous genotypes);
  - Potentially lower "effective" refuge due to damage to non-Bt plants and/or Bt pollination within the growing season, reduced larval movement from Bt onto non-Bt plants, and within-plant density-dependent mortality;
  - Possible yield loss due to lodging of refuge plants within the Bt field, particularly with higher (>10%) blend percentages;
  - Difficulty detecting "unexpected pest damage" (a key component of resistance monitoring).

5) **Recommendations for additional information.** To improve BPPD's ability to assess the risks of resistance for a SmartStax seed blend, BPPD recommends that Monsanto and Dow address the following topics and uncertainties:

- Revised modeling incorporating the structural elements recommended by the SAP (i.e., explicit larval movement, switch from a frequency-based model to one including density-dependent larval mortality, epistatic mechanisms for resistance in target pests) with separate analyses for SWCB and ECB. Non-uniform oviposition should be modeled for both ECB and SWCB, especially (but not only) for the second generation of adults which will more likely lay eggs on Bt rather than on damaged (or crowded out) non-Bt refuge plants in seed blends.
- Biological research on adult movement (related to mating and movement from refuges), larval movement, larval feeding (i.e., selective feeding within corn ears or on pollen), survival of heterozygote genotypes on SmartStax (markers may need to be determined for heterozygotes), and the potential for epistatic mechanisms of resistance (particularly with older instars).

## I. BACKGROUND

The Agency held a Scientific Advisory Panel meeting on December 8-9, 2010 to address BPPD's risk assessment of 5% SmartStax RIB, a multi-toxin double pyramid targeting above ground (Lepidoptera) and below ground (Coleoptera) pests of maize. In the Agency's risk assessment, BPPD evaluated Monsanto's modeling for Lepidoptera pests and Dow's modeling for corn rootworm; additionally, BPPD collaborated with EPA/ORD in an independent modeling effort to evaluate the applicants' proposal (BPPD 2010). The SAP provided its written report to the Agency on March 3, 2011 and expressed concern about the risk of resistance by the European corn borer (ECB) and southwestern corn borer (SWCB) in a 5% SmartStax seed blend compared to a 5% structured refuge (approved by the Agency in 2009). Their overall conclusion was that a 5% SmartStax RIB strategy would be substantially less durable than a 5% SmartStax (SSX) structured refuge and that there was "insufficient scientific basis for supporting the SSX RIB as an effective IRM strategy" for ECB and SWCB. The following is a BPPD summary of the SAP's main concerns about Monsanto's assumptions and parameter values chosen in their model for European corn borer and southwestern corn borer:

A. Structural equations to model larval movement were not included in the model, and the approximations used by the applicant were structured to minimize the effect of larval movement on the rate of resistance evolution. The durability of 5% SSX RIB was overestimated by modeling larval movement implicitly and by not considering different larval-movement hypotheses (NBI, NBP, BNI, and BNP). Larval movement in Bt/non-Bt seed mixtures may lead to greater heterozygote survival, which in turn would speed up resistance evolution.

B. No cross-resistance was incorporated into the model between Cry1F and Cry1A.105 and Cry2Ab when the applicants' data (Schlenz et al. 2008) indicated some level of epistasis and cross-resistance between Cry1F and the other two toxins.

C. Other forms of epistasis (expression of a gene is suppressed by a gene at another locus) were not considered; rather the applicant assumed that survival of genotypes was multiplicative for all three loci (least conservative assumption because heterozygote survival was, therefore, low). Other forms of epistasis should have been explored for ECB and SWCB such as, for example (but not only), "developmentally restricted expression of low levels of Cry-protease where older larvae survive Bt exposure when moving from non-Bt onto Bt". Additionally, resistance at all loci could be determined by the most rapidly evolving locus, which could drag other resistant loci along and, thereby, increase the rate of resistance evolution.

D. Non-uniform oviposition of 2^nd^ generation ECB and SWCB in seed blends should favor Bt plants because adult females could distinguish between damaged (non-Bt) and protected (Bt) plants. This selective oviposition behavior based on unsuitable non-Bt host plants will reduce the effective refuge in a seed blend compared to a structured refuge. For 1^st^ generation ECB and SWCB, non-uniform oviposition is also a probability, especially when the refuge plants incurred root damage from corn rootworm (CRW) and subsequently experience crowding out by faster growing (CRW protected) Bt plants.

E. Strong density-dependence occurs in SWCB, and this aspect was not incorporated into the applicant's model to estimate resistance evolution. "Soft selection" might be operating in this species in contrast to viability selection: "larvae that win out in cannibalistic encounters in the presence of Bt are likely to be those that have a slight fitness advantage from being more resistant to Bt".

F. The Panel recommended that emphasis in modeling assessments of stacked cultivars should be placed on durability for the pest that shows the greatest potential rate of resistance evolution. The Panel suspected that this may be SWCB for SmartStax.

The panel suggested that the current industry and EPA models be revised (or new models created) to address the factors that led to overestimates of durability. In particular, the panel recommended that new modeling focus on improving the parameters for survival of genotypes (especially heterozygotes) in a pyramided toxin environment. Further, the panel indicated that modeling on a regional scale may be suitable to investigate the effects of region-wide pest population suppression from a seed blend deployment.

The panel recommended additional research regarding dispersal/movement of adults, effects of plant-to-plant movement on larvae, survival of different genotypes on Bt toxins (particularly heterozygotes), and effects of kernel pollination effects on corn earworm (CEW) refuge.

The panel suggested that seed blends could be implemented with a phase-in approach in which the seed blend percentage was lowered as data were developed (i.e., resistance monitoring and population density). No specific blend percentage numbers were recommended by the panel for this approach. It was also suggested that the resistance management plan have a well-defined trigger for remedial action (in the event that resistance develops).

For corn rootworm, the SAP concluded that seed blend and block durability for SSX would be comparable. For corn earworm and SSX RIB, the panel concluded that there were serious risks to both cotton and corn due to pollination concerns of corn ears in a seed blend environment. The panel was unable to quantify the role of selection on the rate of resistance evolution in CEW associated with a SSX RIB in the Corn Belt and migration between the northern corn growing and southern cotton growing regions at the time.

Monsanto and Dow AgroSciences were charged with addressing the SAPs concerns and submitting a written response to BPPD. The following section is a summary of Monsanto/Dow's response dated March 18, 2011.

## II. MONSANTO AND DOW RESPONSE TO SAP (2010) CONCERNS AND UNCERTAINTIES

The following is a summary of Monsanto's and Dow AgroSciences' understanding of the SAP's concerns with modeling for ECB and SWCB. The applicants indicated in their response to the SAP report that they agreed with the Panel's general findings:

a) A structured refuge is generally more durable than a blended refuge of the same percentage for a single-trait product, two-trait pyramid or a three-trait pyramid, assuming 100% grower compliance with the required planting of a structured refuge across the landscape;
b) The potential that larval movement and survival of heterozygotes in a 5% seed blend could reduce durability compared to a 5% structured refuge for the same product;
c) The potential that epistasis and cross-resistance could reduce the durability of two-trait and three-trait pyramids for both the blended and structured refuge scenarios;
d) Density-dependent mortality manifested as cannibalism by the southwestern corn borer could lead to selection for resistance if heterozygote larvae grow faster and larger than susceptible larvae; and
e) Consideration of dose values lower than those used in models by EPA/ORD and Monsanto would increase the survival of heterozygotes and reduce the durability of the seed blend compared to a structured refuge.

The applicants provided supplemental modeling to analyze how the SAP's findings affected a 5% SSX RIB and model output compared relative to other IRM strategies. Monsanto and Dow AgroSciences used the 20% structured refuge for a single Bt PIP as the benchmark.

Response to SAP findings (structured refuge vs. seed blends): Assuming high grower compliance with structured refuge requirements, the applicants agreed with the SAP's conclusions but emphasized that the durability of structured refuges was decreased with realistic values for non-compliance as was seen in Monsanto's and EPA/ORD's model. With non-compliance incorporated into the model, the difference in durability between structured refuge and seed blend was reduced. The applicants stated that in cases of localized non-compliance (localized, zero compliance with refuge plantings as per anecdotal reports; see Appendix B for Dow's modeling and Figure 3 below) seed blends were a superior strategy to blocks. The effect of non-compliance was explored and provided to the Agency in Appendix A of Monsanto's supplemental modeling and Appendix B of Dow AgroSciences' supplemental modeling. In addition, Monsanto also emphasized that a three gene pyramid was highly durable and much more durable (under low efficacy assumptions) than a single trait PIP with a 20% block refuge (Figure 1), which SSX RIB aims to replace. Low efficacy assumptions for SSX RIB were: fitness of susceptible genotype was 0.02, 0.05, and 0.10 (min, mode, max in PERT analysis), and dominance values for the resistance genes were 0.2, 0.05, and 0.4 (min, mode, max).

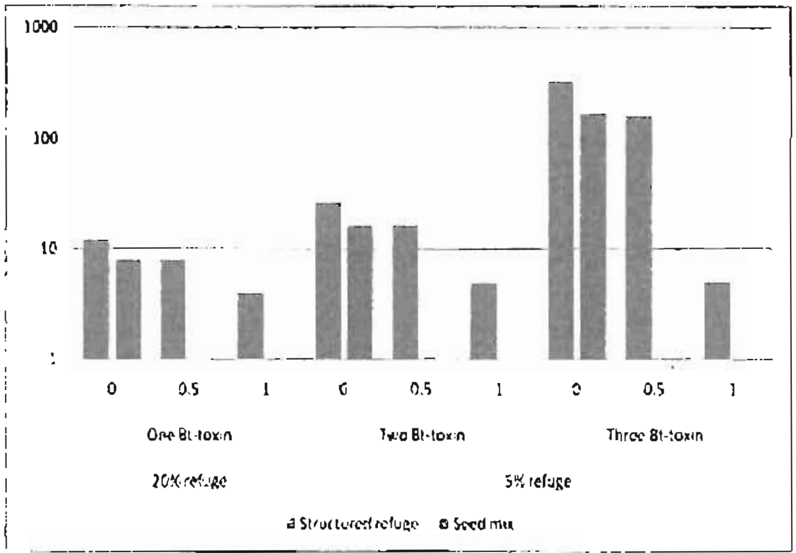


Figure 1 Median number of generations to resistance for one, two, and three Bt toxin products under low efficacy scenario and with three levels of non-compliance 0%, 50%, and 100% (extracted from Monsanto/Dow's submission, Appendix A)

Response to SAP findings (larval movement and selection/survival of heterozygote genotypes): Monsanto and Dow concurred with the SAP that larval movement can decrease the durability of SSX. First, larval movement in a seed blend would reduce the effective refuge because more larvae move from non-Bt plants to surrounding Bt than from Bt to non-Bt. Monsanto addressed this by incorporating conservative estimates of effective refuge (1% and 2.5% in addition to 5%). Second, larval movement of older heterozygous individuals would lead to greater survival than movement of susceptible genotypes. Monsanto stated that it was important to consider all differential mortality factors when considering these effects. Insects that were resistant to one or two toxins still suffered mortality from the third toxin. Any fitness differential only occurred for homozygous resistant genotypes at all three loci (a very rare event). Monsanto's and Dow's field data showed that successful movement from refuge to SSX plants did not occur.

Monsanto and Dow stated that the Panel modeled a full range of larval movement and survival scenarios but did not focus their analysis on the biologically plausible scenarios based on the product, pest biology, and field observations. Low larval movement and survival were supported by field data, and higher levels of either movement or survival were not. The SAP model showed that a SSX RIB was only slightly less durable than a structured refuge when larval movement and survival of heterozygote larvae was low.

Dow provided additional modeling (deterministic, spatially implicit, uniform oviposition, frequency based dynamics) to include worst-case fitness differential parameters into a three gene model to replicate the worst-case movement/survival scenarios by the SAP's two gene model. Dow also included potential cross-resistance between two traits in SSX RIB (varied SS-fitness from 0-0.25 on one trait if individual had RR-genotype on second trait- vice versa). Initial resistance allele frequency was 0.005 for all three loci; dominance was 0.05 in structured refuges and 0.5 in seed blends; genotypic fitness was the product of each locus specific fitness value. Dominance was increased from 0.05 to 0.5. Dow reports that such worst-case assumptions made a two-gene pyramid with a 5% refuge (as modeled by the SAP) slightly more durable (25 generations) than a single gene with a 20% structured refuge (17 generations). The results for a three-gene pyramid suggested that durability was extended by 500-fold (no resistance in 1000 generations). When a three-gene pyramid with cross resistance was modeled, then durability was intermediate between the three and two-trait products in absence of cross-resistance. In contrast, SmartStax without a refuge component evolved resistance in just 2 generations (representing worst-case of local non-compliance with 5% structured refuge leading to local resistance evolution).

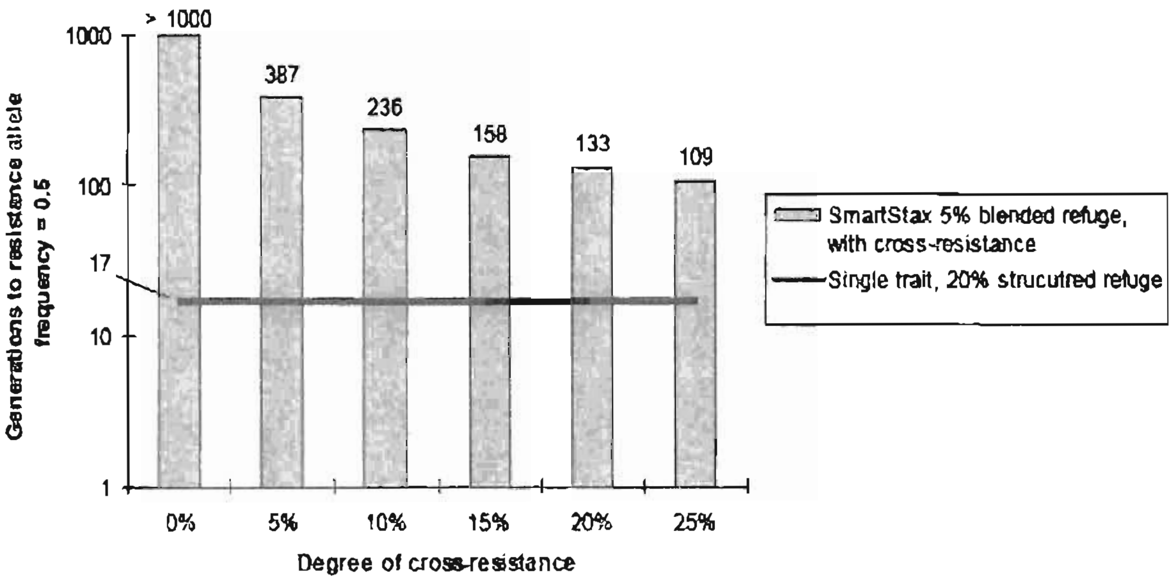


Figure 2. Generations (log scale) to resistance for SSX RIB and a 20% 20% single trait PIP under worst case larval movement/survival assumptions and various degrees of cross-resistance (extracted from Appendix B).

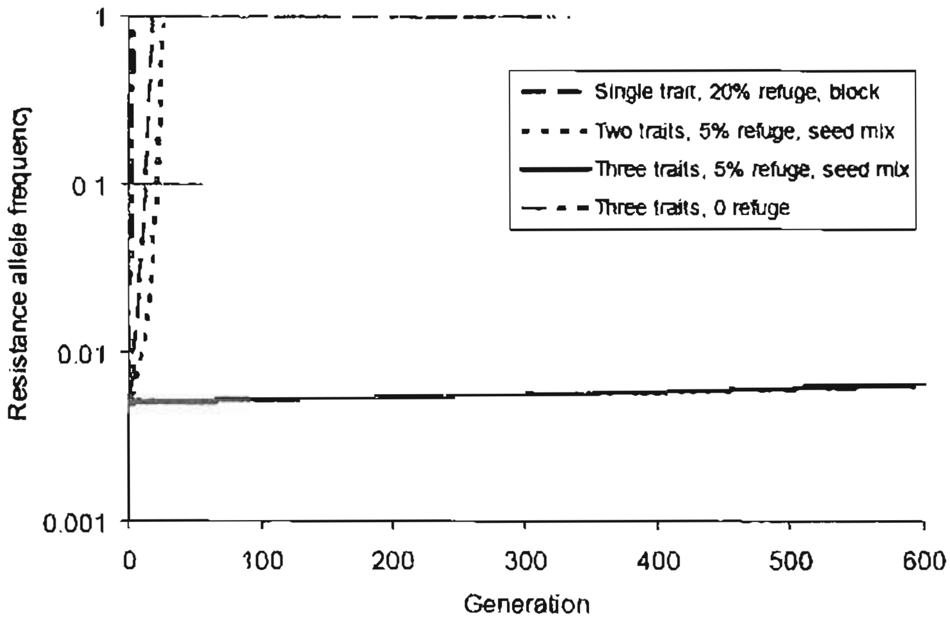


Figure 3. Increase in resistance allele frequency using worst-case larval movement and four different scenarios (extracted from Appendix B)

<u>Response to SAP findings (epistasis)</u>: The applicants stated that epistasis via 1) a Cry-protease mechanism and 2) altered expression of receptor genes through a regulator gene were unlikely to be of relevance because such mechanisms would provide little or no selective advantage to ECB feeding on the three high-dose Bt proteins in SmartStax. In terms of the third epistatic process (cross-resistance), the applicants stated that there was no or little potential for cross-resistance between the toxins (Cry1F and Cry1A, Cry2A; Cry2A and Cry1A) as previously discussed (Head and Storer 2008). The Cry1F resistant colony exhibited some survival in diet bioassays using Cry1A.105; however, the resistant colony and susceptible ECB exhibited similar mortality on Cry1A.105 at concentration levels relevant to the field. The data indicated that these insects will not survive SmartStax.

The incorporation of some cross-resistance between two toxins would reduce the durability of SmartStax below what was modeled by Monsanto (MRID 479437-01), EPA/ORD (Caprio and Glaser 2010), and the supplemental modeling provided to the Agency by the applicants. The durability of SSX RIB would be intermediate between the two-toxin and three-toxin pyramid shown in Figure 3. The applicants noted that whatever form of cross-resistance would occur between two toxins in SSX, the durability of SSX RIB would always be greater than for a 20% block (single trait) and two-gene pyramid (5% seed mix).

Response to SAP findings (SWCB density effects): Density-dependence in this species arises primarily as a result of competition for overwintering sites in corn stalks and is manifested as cannibalism. This behavior could enhance selection for resistance if resistant insects were able to grow larger and faster than susceptible insects. Cannibalism can also reduce the effective refuge size. The applicants note that no SWCB larvae have been observed to survive on SmartStax in field studies (Head and Storer 2009). The applicants concluded that density dependence would be more important in a structured refuge than seed blend and that incorporating this type of population dynamic would favor seed blends over structured refuges.

Response to SAP findings (dose considerations): The SAP chose lower dose values for the SSX toxins in their model than what were supported by empirical data:

1) Event TC1507 was previously determined to be high-dose against ECB and SWCB. Pereira et al. (2008) showed that Cry1F resistance [in ECB] was functionally recessive on TC1507 plants and confirmed that the event was high-dose.

2) Bioassays conducted in 2009 with ECB showed that Cry1A.105 ($EC_{50}$ range 0.16-0.68 $ng/cm^2$) was approximately 3-fold more active than Cry1F ($EC_{50}$ range 0.68-2.05 $ng/cm^2$), and Cry2Ab ($EC_{50}$ range 1.64-4.71 $ng/cm^2$) was approximately 2-fold less active than Cry1F.

3) Level of expression of Cry1A.105 and Cry2Ab2 in leaf tissue of SSX plants was approximately 2-fold higher than for Cry1F.

4) The expression data showed that Cry1A.105 was present at higher concentration levels than Cry1F, while Cry2Ab2 and Cry1F were present at similar concentrations throughout the growing season. The applicants concluded that since Cry1F was at high-dose in SSX that also Cry1A.105 and Cry2Ab2 had to be at high-dose levels for ECB.

## III. BPPD REVIEW OF MONSANTO/DOW'S SUBMISSION

The applicants agreed with the SAP's conclusion that a 5% SSX RIB would be less durable than a 5% SSX with a structured refuge. However, they noted that when realistic levels of non-compliance were incorporated that this difference in durability was reduced. BPPD concurs with this statement and notes that the SAP did not appear to address compliance in their model.

BPPD concludes that a seed blend expressing (such as SmartStax) three high-efficacy toxins (against mobile lepidoptera pests) with low potential for cross-resistance and low risk for other epistatic effects should generally be more durable than a seed blend expressing two high-efficacy toxins with low or no epistatic effects. For SmartStax, however, the relative difference in durability between a seed blend and a block refuge of equivalent size cannot be determined until Monsanto and Dow address the SAP's modeling recommendations (i.e., explicit larval movement, epistasis, non-uniform oviposition in seed blends, and density-dependent effects) and other concerns described in this review.

BPPD noted that Dow compared the pyramided seed blend strategy solely to the single PIP with a 20% block refuge and showed that (under their modeling construct) the pyramided product was more durable than the single PIP. As stated by the SAP, a comparison between a pyramid and a 20% single trait structured refuge should always show that the pyramid is more durable. The relative comparison, however, is important between single PIP, pyramided PIP (SSX RIB), and the pyramided PIP with a structured refuge. Monsanto included a comparison between the three IRM strategies and reported that a 5% SSX seed blend would be more durable than a single gene product with a 20% refuge and SSX with a 5% structured refuge with 50% grower non-compliance but somewhat less durable than SSX with a 5% structured refuge and 100% compliance (see Figure 1). BPPD concludes that a comparison between the seed blend and a structured refuge with realistic numbers of non-compliance (i.e. 20%-30%, based on surveys conducted by the Agricultural Biotechnology Stewardship Technical Committee) would have improved their analysis. In addition. Monsanto could have used a probability approach to modeling non-compliance with the mean as 20% and worst-case and best-case choice for min and max values. The durability of block refuges should be higher with less non-compliance.

BPPD agrees with the applicants that some of the dose profiles chosen in the SAP's modeling analysis of SSX RIB were lower than what the applicants' empirical data and published literature supported. It is likely that higher dose values would improve overall durability for both blocks and blends.

BPPD's review of the specific concerns raised by the SAP (and Monsanto/Dow's response) is detailed below:

## A. LARVAL MOVEMENT:

*a) Explicit larval movement:*

BPPD concludes that Monsanto and Dow did not directly address the SAP's recommendation to include explicit larval movement into their model. Instead, Dow and Monsanto provided the Agency with supplemental modeling in their response to the SAP report that (as in the previous modeling) used a spatially implicit model to estimate SSX RIB durability. Hence, both applicants have likely overestimated the durability of a SmartStax seed blend as was done in the initial modeling (see BPPD 2010 and SAP 2011).

The SAP demonstrated in their analysis the effect on resistance evolution when larval movement was modeled implicitly and explicitly. In their example they incorporated Monsanto's worst-case assumption for heterozygote survival (5x that of SS-genotypes) and reduction in effective refuge to 1% and demonstrated that as larval movement increased in the spatially explicit model, the durability of the pyramid decreased almost linearly (two genes modeled for simplicity but the argument holds for three gene pyramids as well), while durability remained constant in the spatially implicit model and only decreased when the population density began to decline towards extinction (Figure 4a). At the point where larval movement reached 60% (however, greater values are supported in the literature (Onstad and Gould, 1998)), the estimated durability with explicit larval movement was 50% that of the Monsanto's estimated durability.

A critical point to consider about larval movement is that it can have the effect of increasing the survival of heterozygotes in a seed blend (Figure 4c) beyond what it would be if there was no movement. This is not captured by any parameter in the model but instead is an outcome of modeling larval movement explicitly. Hence, BPPD disagrees with Monsanto's contention that they addressed larval movement by conservatively reducing the effective refuge to 1% and strongly recommends that the applicant incorporate explicit larval movement into their simulation analyses of SmartStax RIB and address this germane point raised by the SAP.

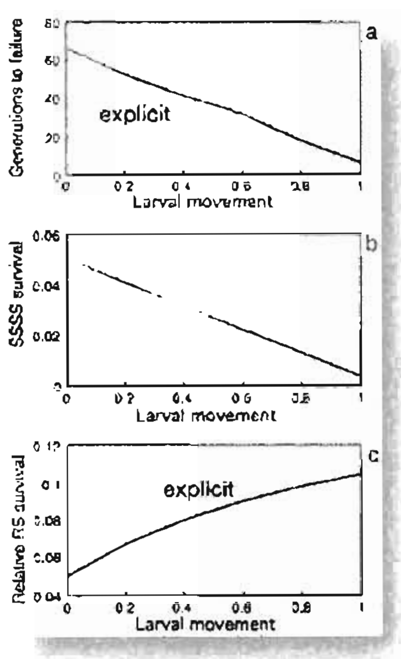


Figure 4. (a) Generations to resistance failure (frequency of both alleles > 0.5), (b) survival of S1S1S2S2 homozygotes, and (c) relative survival of heterozygotes for the cases in which larval movement is explicitly or implicitly modeled. For implicit modeling of larval movement, the survival of S1S1S2S2 susceptibles is decreased (by decreasing the proportion of non-*Bt* plants in the seed mixture, *q*) (panel b), while there is no change in the relative survival of heterozygotes (panel c). In (a), the break in the slope of the line for implicit larval movement (gray line) corresponds to the point above which the insect population starts to decline towards extinction. Baseline parameter values are: proportion of non-*Bt* plants in seed mixture $q = 0.05$; $F = 100$; $s1SS = s2SS = 0.01$; $h1 = h2 = 0.05$; survival is divided equally between pre- and post-larval movement; and initial allele frequencies 0.005. (Fig. A2-8 extracted from SAP report (2011))

BPPD comments regarding Dow's supplemental modeling: It is apparent that a SSX RIB was more durable under the applicants model construct than a two-gene pyramid when larval movement was set at 0.5 and a degree of cross-resistance was incorporated (Figure 2 and Figure 3). Apparently, the single PIP was modeled in a vacuum because no cross-resistance was incorporated for that product. In an environment consisting of a mosaic of toxins, a cross-resistance potential should also be included when estimating the durability of a single PIP; inclusion of cross-resistance would also lower the durability for single gene product.

Dow concluded that their supplemental modeling confirmed the findings of EPA/ORD (Caprio and Glaser 2010) that the addition of a third trait dramatically increased the durability of SSX RIB and that their results also confirmed those of the SAP that a dual gene product could be expected to last about two decades. BPPD does not dispute that a pyramid can be expected to be more durable than a single PIP with a 20% refuge. The important questions to address are what the relative gain and loss in durability would be for a 5% SSX seed blend compared to a single PIP (20% refuge) and a 5% SSX structured refuge. BPPD recommends that Dow include the 5% SSX structured refuge in their relative durability comparison in addition to revising their model structure (i.e. incorporate explicit larval movement).

BPPD has, however, serious concerns with Dow's reported durability for SSX RIB under worst-case movement and dominance assumptions for heterozygotes. The resistance allele frequency after 1000 generations had increased from 0.005 to 0.008. BPPD believes that this durability seems greatly overestimated based on the modeling done by EPA/ORD (Caprio and Glaser 2010) and SAP (2011). BPPD's knowledge of Dow's model structure and assumptions is limited, and it is, therefore, not possible to assess the dynamics in the model: for example, it is unclear to BPPD from Dow's write-up whether a heterozygote individual had to be heterozygous at all three loci to be included in this worst-case movement or whether any degree of heterozygosity allowed for increased movement. BPPD is unable to identify why the worst-case scenario for SSX RIB predicted a durability much above that modeled by, for example EPA/ORD but hypothesizes that it is most likely due to modeling larval movement explicitly. To compare and contrast, the EPA/ORD estimated that resistance could evolve as rapidly as in 158 generations for ECB using a SSX seed blend (but fewer generations to resistance for SWCB) compared to over 1000 generations durability for the 5% block refuge. This model was spatially explicit, incorporated heterozygote movement/survival, and some epistasis. The SAP suggested that EPA increase the heterozygote survival to include more conservative estimates and switch from a frequency-based to a density-dependent model. Such changes would further erode the estimated durability of the SmartStax seed blend in the EPA/ORD model compared to the durability of the block refuge. BPPD concludes that a seed blend with a triple-gene PIP can be expected to increase the durability compared to a single PIP product (20% refuge), but it is unclear whether this increase is as dramatic as Dow demonstrated under their assumptions and modeling construct (more likely to be moderate with respect to the lepidoptera pests targeted).

BPPD specific comments about Monsanto supplemental modeling: Although, dominance was increased in this supplemental analysis, the specific survival levels of heterozygous genotypes were not discussed. According to the SAP, it is the survival of the heterozygotes that determines how fast resistance evolves, not that of the susceptible genotypes, which is what was provided to the Agency in this latest modeling submission (Appendix A). Also, the applicant stated that they ran 1000 simulations for each PERT distribution. The SAP (2011) stated that thousands of simulations needed to be run to adequately sample the min and max values with this sort of probability analysis. Monsanto stated that they were interested in mean results and that, therefore, increasing the number of simulations was not necessary (Head 2011). However, Monsanto reported various risk profiles (number of generations to resistance assuming different levels of risk) for three IRM strategies; therefore, BPPD recommends that Monsanto increase the number of simulations.

Monsanto explored the durability for a single toxin and dual-gene and triple-gene pyramid using low, medium, and high efficacy assumptions for SmartStax and incorporated a probability analysis using a PERT-beta distribution for various parameters such as dominance, fitness, and IRAF. For the worst-case analysis, Monsanto reported that the single toxin PIP (25% non-compliance with 20% block refuge) and dual-gene product (with a seed blend option) had a 1% probability that resistance would evolve in less than 10 generations, while the triple gene product (with seed blend) had a durability of 67 generations. BPPD concludes that under Monsanto's model construct and more conservative values for susceptible fitness (0.05) and dominance (0.2), a three gene pyramid using a seed blend was more durable than under the other two scenarios and assumptions modeled (single gene PIP being the least durable, yet not significantly). BPPD concludes that such low efficacy assumptions for a SSX RIB are very conservative worst-case assumptions that are not very likely. BPPD notes that in this new analysis, Monsanto did not consider one of the SAP's main recommendations to model larval movement explicitly. Based on the analysis done by the SAP and mentioned in this review (see Figure 4a), such a change in model structure would have greatly reduced the seed blend durability for a three-gene product compared to the 20% structure refuge for a single toxin and a 5% SSX structured refuge. BPPD is unable to conclude at this point what the relative gain and loss in durability would be from a 5% SSX seed blend (RIB) strategy.

As discussed above, BPPD concludes that Monsanto likely overestimated the durability in their revised seed blend simulations because of implicit modeling of larval movement. The rationale for this conclusion was provided earlier in this section.

*b) Larval movement hypotheses:*

The SAP also recommended that the applicants incorporate different larval movement hypotheses into their model. BPPD found that the applicants did not address this recommendation either, presumably because their field data did not support high larval movement and survival or maybe because of limitations with their current model structure.

BPPD notes, however, that in their preliminary efficacy study (one season, two locations; MRID 479437-01 (Appendix 3)) Monsanto and Dow reported that there was some degree of damage in pure stand SmartStax and MON89034 x TC1507 plots and some SWCB larvae were found (unclear how many), although significantly less than what was observed in 90% and 95% seed blend plots with MON89034 x TC1507. Hence, to add additional conservatism to their analysis, BPPD recommends that Monsanto follow the SAPs advice and incorporate different larval movement hypotheses into their model for SSX RIB.

### B. EPISTASIS

*Cross-resistance:* The applicants addressed epistasis by incorporating various degrees of cross-resistance into their new modeling submission using Dow's deterministic, spatially implicit model. The applicants were able to demonstrate that durability of SSX RIB declined greatly initially and then somewhat slower as the degree of cross-resistance increased (Figure 2). With an assumption of 5% cross-resistance the estimated durability decreased from >> 1000 generations (RAF was 0.008 at 1000 gen) to 387. BPPD notes that this is a drastic drop in durability. Although the applicants appear to argue that the potential for cross-resistance is non-existent or minimal, BPPD concludes that based on the SAP's recommendations, a small degree of cross-resistance should be included in the simulations to create a more conservative model.

*Other forms of epistasis:* The applicants argued that epistasis via a Cry-protease mechanism and altered expression of receptor genes were unlikely to be of relevance because such mechanisms would provide little or no selective advantage to ECB feeding on the three high-dose Bt proteins in SmartStax. BPPD is not convinced of the applicants' argument for not including other forms of epistasis such as, for example, a Cry-protease mechanism. The SAP stated that a Cry-protease could potentially "degrade multiple Cry toxins, reducing or eliminating their toxicity to the insects". The SAP also stated that it is more likely that Cry-protease expression occurs in later instars of ECB and SWCB, which would affect their fitness in a seed mixture when plant-to-plant movement occurred. If such a mechanism confers the ability to tolerate multiple Bt toxins and has genetic heritability, then it should be a "selectable" trait in an environment with significant amounts of Bt corn (SSX). Hence, BPPD recommends that the applicants consider including such a mechanism for older instars of ECB and SWCB in their model.

### C. NON-UNIFORM OVIPOSITION

The applicants did not address the SAP's recommendation regarding non-uniform oviposition in seed blends. The Panel stated that: 1) in seed blends of SSX, refuge plants might incur root damage from CRW (and other tissue damage from ECB), which could stunt their growth and allow protected SSX plant to effectively crowd out refuge plants. First generation females would then be more likely to oviposit onto SSX plants in seed blends than non-Bt plants; and 2) in a seed blend environment, second generation females could discriminate between damaged (non-Bt) and non-damaged (Bt protected) plants and could, therefore, be more likely to oviposit onto Bt plants than they would otherwise. Hence, BPPD recommends that the applicants incorporate a degree of non-uniform ovipositing behavior by both first and second generation females favoring Bt plants. This would reduce the seed blend durability due to a reduction in effective refuge compared to a structured refuge of comparable non-Bt proportion.

### D. DENSITY-DEPENDENCE FOR SWCB

BPPD disagrees with Monsanto and Dow's justification (lack of movement onto and SWCB larval presence on SSX) for not addressing density-dependence in their model. As stated in section 2.1 above, some SWCB larvae were found in pure stand SSX and MON 89034 x TC1507 plots and seed blend plots. Hence there is evidence for larval establishment on and movement onto SSX plants. BPPD recommends that the applicants incorporate density-dependence into their simulation models as was recommended by the SAP (2011)

## E. IRM EMPHASIS ON SPECIES AT GREATEST RISK OF EVOLVING RESISTANCE

The SAP concluded that IRM strategies should be designed around the pest that shows the greatest potential rate of resistance evolution. The Panel suspected that this might be SWCB for SmartStax. Should the applicants conduct new modeling incorporating BPPD's recommendations as outlined in this review, a separate analysis should be provided for ECB and SWCB (as was done in the original submission -- discussed in BPPD 2010).

## OVERALL CONCLUSIONS

- Based on a review of the Science Advisory Panel (SAP) report (SAP 2011) and revised modeling submitted by Monsanto/Dow, BPPD concludes that a 5% seed blend for SmartStax corn will likely be less durable (perhaps significantly so) than a comparable (5%) block refuge for the product. BPPD notes, however, that a SmartStax 5% seed blend should be more durable than a 20% block refuge for a single toxin Bt corn product or a comparable (5%) seed blend for a two toxin pyramid. Larval movement, potential survival (and selection) of heterozygote genotypes, and loss of refuge effectiveness during the growing season are the primary factors that are likely to reduce durability in seed blends.
- BPPD has major reservations regarding the modeling approaches taken by Monsanto and Dow (in separate models) to evaluate the SmartStax seed blend. Monsanto and Dow addressed larval movement implicitly (as opposed to explicitly as recommended by the SAP) and did not incorporate other important recommendations made by the SAP (i.e., epistatic resistance mechanisms, density-dependent effects). As detailed in the SAP report, this approach is likely to result in overestimates of durability. For this reason, BPPD is unable to quantify the relative differences in durability estimates between refuge options (i.e., 5% SmartStax seed blend, 5% SmartStax block refuge, and 20% single toxin block refuge).
- Despite the modeling uncertainties described above, BPPD believes that, in general, a three toxin product such as SmartStax should have greater durability than Bt corn products with two or fewer toxins and comparable refuge deployment. In other words, a three toxin seed blend can be expected to be more durable than a two toxin product with the same blend percentage. Similarly, a block refuge for a three toxin product should be more durable than the same block refuge for a two toxin product. This conclusion assumes that the three toxins have high efficacy and low cross-resistance potential. BPPD cautions, however, that the relative gain and loss in durability with multi-toxin pyramids should be evaluated on a case-by-case basis with product-specific data.
- BPPD agrees with Monsanto/Dow that the SAP's modeling analysis 1) did not include three toxins for lepidoptera (two toxins were modeled for simplicity), 2) incorporated low dose scenarios for the toxins in SmartStax, and 3) neglected to consider effects of non-compliance on block refuge. The first two factors likely resulted in lower durability

estimates for both block and blended refuge; the third probably led to an overestimate of durability for block refuges. Monsanto and Dow's revised modeling addressed these three components.

- Block refuges and seed mixes present different potential risks and benefits for resistance management. A summary of these factors is described below:

*Block Refuges*

- **Pros:**
  - Greater durability than other refuge approaches (including seed mixes, strip refuges, and natural refuge) in simulation models;
  - Allows for high production of susceptible insects;
  - Refuges can be managed to preserve yield.

- **Cons:**
  - Random mating may be less likely than with seed mixes or strip refuges if adult movement is limited (though not the case for mobile lepidoptera);
  - Compliance must be monitored (i.e., with a compliance assurance plan);
  - Non-compliance can result in no refuge deployment or inadequate refuge distance from Bt field to assure random mating (more important for high-dose PIPs such as in SmartStax), which can increase the risk of resistance;
  - Refuge may need to be treated with insecticides (potential economic and environmental costs);
  - There have been reports of a lack of available refuge seed in some areas;
  - Planting refuges can incur inconveniences and expenses for growers.

*Seed Blends*

- **Pros:**
  - Non-compliance is not an issue -- all seed bags are assumed to have the same amount of refuge seed (± standard error);
  - A compliance monitoring program should not be necessary (cost/resource savings);
  - No separate refuge management and insecticide use are needed;
  - Ease of use for growers.

- **Cons:**
  - Lower durability (perhaps substantially) than block refuges in simulation modeling;
  - Potentially lower "effective" refuge due to damage to non-Bt plants and/or Bt pollination within the growing season, reduced larval movement from Bt onto non-Bt plants, and within-plant density-dependent mortality;
  - Possible yield loss due to lodging of refuge plants within the Bt field, particularly with higher (>10%) blend percentages;

- Difficulty detecting "unexpected pest damage" (a key component of resistance monitoring);
- Increased risk of resistance for pests with greater adult dispersal and larval plant-to-plant movement (driven by heterozygous genotypes).

**RECOMMENDATIONS FOR ADDITIONAL INFORMATION:**

To improve BPPD's ability to assess the risks of resistance for a SmartStax seed blend, BPPD recommends that Monsanto and Dow address the following topics and uncertainties:

- Revised modeling incorporating the structural elements recommended by the SAP (i.e., explicit larval movement, switch from a frequency-based model to once including density-dependent larval mortality, epistatic mechanisms for resistance in target pests) with separate analyses for SWCB and ECB. Non-uniform oviposition should be modeled for both ECB and SWCB, especially (but not only) for the second generation of adults which will more likely lay eggs on Bt rather than on damaged (or crowded out) non-Bt refuge plants in seed blends.
- Biological research on adult movement (related to mating and movement from refuges), larval movement, larval feeding (i.e., selective feeding within corn ears or on pollen), survival of heterozygote genotypes on SmartStax (markers may need to be determined for heterozygotes), and the potential for epistatic mechanisms of resistance (particularly with older instars).

## IV. REFERENCES

BPPD. 2010. EPA PRELIMINARY RISK ASSESSMENT of Monsanto's and Dow's 5% seed mixture request for a Section (3) full commercial registration of SmartStax corn. Memo from J.C. Martinez to M. Mendelsohn on November 8.

Caprio M and J Glaser. 2010. Risk Assessments of the evolution of resistance in European Corn Borer and Southwestern Corn Borer to SmartStax corn. Letter report to EPA-OPP/BPPD dated October 29, 2010.

EPA Scientific Advisory Panel (SAP). 2011. Transmittal Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held December 8-9, 2010 to Address Scientific Issues Associated with Insect Resistance Management for SmartStax™ Refuge-in-the-Bag, a Plant-Incorporated Protectant (PIP) Corn Seed Blend. Memorandum from Dr. Sharlene Matten (DFO) to Dr. Steven Bradbury (Director of OPP) on March 3, 2011.

Head GP and NP Storer. 2011. Dow AgroSciences' and Monsanto's Response to Uncertainties Raised by the SmartStax RIB Scientific Advisory Panel, Dec 8-9, 2010. Registrants' unpublished report submitted to the Agency on March 18, 2011.

GP Head. 2011. Comment made by representative of Monsanto during a meeting with BPPD on Tuesday, March 15.

Head GP and NP Storer. 2009. Five percent seed mix refuge as an insect resistance management option for MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Registrants' unpublished report submitted to the Agency on December 14, MRID 479437-01.

Head GP and NP Storer. 2008. Insect resistance management plan for MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Registrant unpublished report submitted to the Agency, MRID 474449-11

Onstad DW and F Gould. 1998. Modeling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 91: 585-593.