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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Insect Resistance Management Plan Review for Cry51Aa2.834_16 expressed in MON 88702 x MON 15985 x COT102 cotton and target pests *Lygus* spp. and *Frankliniella spp.*
EPA Reg. Nos. 524-AAE
MRID# Unassigned
Decision# 551370
DP Barcode: 457311

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Action Requested

BPPD[1] previously reviewed Monsanto[2] Company's Insect Resistance Management (IRM) plan and supporting data for Section 3 registration of MON 88702 x MON 15985 x COT102 Cotton containing Cry51Aa2.834_16 (abbreviated in this review as Cry51). Therein, Monsanto asked BPPD to consider natural refuge as the primary IRM strategy for MON 88702 cotton containing Cry51 for control of lygus bug and thrips species. In the US EPA (2020) review, several deficiencies were identified by the Agency. Here, BPPD will review the registrant response to those deficiencies and determine the adequacy of the Cry51 IRM plan for Section 3 registration.

[1] The use of BPPD in this review refers to the BPPD IRM team: Kara Welch and Alan Reynolds
[2] Monsanto merged with Bayer CropScience in 2018 but was still doing business as Monsanto at the time of the submission of the Section 3 registration package.

Conclusions and IRM Options

BPPD recognizes there is no ideal IRM plan for a low-to-moderate dose toxin like Cry51 cotton. Thus, per the weight of evidence BPPD recommends a natural refugee paradigm to protect the longevity of the Cry51 trait. This option is also the least burdensome to growers, since it does not involve planting of a structured refuge. Both the structured refuge and limited acreage deployment options (previously discussed by BPPD in its original IRM assessment for Cry51 – see U.S. EPA 2020) may not provide a reliable source of Cry51-susceptible target pests since this (non-Cry51) cotton acreage will still be sprayed with insecticides. Further, a limited acreage deployment scenario is logistically unlikely to be implemented evenly across the landscape. Thus, BPPD concludes that natural refuge will provide some protection for the Cry51 trait although it may not be significantly more durable than other IRM options explored by the Agency or the registrant's modelling.

Additionally, BPPD concurs with the sentinel plot program proposed by Monsanto Company for resistance monitoring of the key target pests of Cry51 cotton. The sentinel plots will be in the mid-south and southeast (for *L. lineolaris*), as well as the Carolinas and the southwest (for the other target species). For the time being, the expected level of control provided by Cry51 cotton is 25-50% of large nymphs, and less control will be considered a possible resistance case. However, it is recommended that Monsanto refine the action threshold for resistance determinations as a term of registration.

BPPD also recommends that the natural refuge strategy for the key target pests of Cry51 cotton be re-evaluated every five years with a data submission similar to Monsanto's (2019) original IRM submission for Cry51 cotton. The information for re-evaluation should contain information relevant to host utilization, cropping pattern analyses, resistance monitoring data, and simulation modelling to reexamine levels of effective refuge in the Cotton Belt states and western region.

BPPD requests annual reports from Monsanto regarding the sentinel plot resistance monitoring program and stewardship activities for potential cases of resistance.

BPPD further recommends that Monsanto submit a technology use guide post-registration outlining the grower stewardship program.

Background

Monsanto Company's MON 88702 x MON 15985 x COT102 cotton (EPA Reg. No. 524-AAE) is a pyramided transgenic cotton that expresses Cry51Aa2.834_16 (MON 88702, hereafter referred to as Cry51), Cry1Ac (MON 15985), Cry2Ab2 (MON 15985), Vip3Aa19 (COT102), and confers dicamba as well as glufosinate herbicide resistance. This registration is the first proposed Section 3 commercial product to contain Cry51, a modified insecticidal crystal protein that provides protection against plant bug species (*Lygus hesperus* and *L. lineolaris*) as well as thrips species (*Frankliniella fusca* and *F. occidentalis*). Cry51 is a receptor-meditated midgut toxin that targets a new spectrum of pest species compared to historically registered Bt PIPs. MON 88702 x MON 15985 x COT102 has been previously registered (EPA Reg. No. 524-657) as a seed increase/breeding product, but with no commercial uses and IRM requirements.

To mitigate the risk of resistance, the EPA has required IRM plans for all commercial Bt crops as part of the terms of registration. The Agency opined on the IRM plan as proposed by Monsanto (2019) in US EPA (2020). Monsanto requested the use of a natural refuge paradigm for IRM in conjunction with the registration of Cry51 cotton. Monsanto proposed to bolster the durability of Cry51 with a stewardship plan alongside natural refuge where farmers will be encouraged to spray conventional insecticides at threshold levels of the target pests for MON 88702.

In US EPA (2020), the Agency identified several deficiencies or data needs in the IRM plan and asked the registrant for more information on the following IRM components:

- Exploration of additional modelling scenarios investigating the impact of natural refuge compared to a 20% structured refuge requirement or a limited acreage deployment of Cry51 cotton. BPPD asked Monsanto to include more information on non-random mating in thrips, single versus multigenic resistance, as well as an explanation of various modelling parameters.
- Prior to commercialization, BPPD recommended that Monsanto propose metrics by which resistance can be determined, including a resistance definition, actionable unexpected injury thresholds, and consider developing resistant strains of all four target species for comparison to field collected insects.
- BPPD recommended that Monsanto submit updated host productivity and stochastic resistance modelling analyses every five years to re-evaluate the efficacy of the natural refuge program, if adopted. These submissions will be analogous to the study currently iteratively assessed for Lepidopteran natural refuge products.
- BPPD recommended that Monsanto submit a technology use guide following product registration that outlines that spray program recommended with Cry51 usage.
- BPPD suggested submission of an annual survey conducted by a third party to determine compliance with the IPM program primarily highlighting overspray data for farms that utilize Cry51 cotton as a proxy to monitor for arising resistance concerns if above expected insecticide use is demonstrable.

Herein, BPPD has provided a scientific review of Monsanto Company (2020) response to the deficiency letter requesting additional information on the above. First, BPPD will summarize the contents of the registrant response, then opine on the integrity of the new data.

Summary of Monsanto Company's IRM Submission for MON 88702 x MON 15985 x COT102 Cotton to Support Natural Refuge

MRID Unassigned: Responses to the Non-Target Organism (NTO) and Insect Resistance Management (IRM) data deficiencies of 524-AAE contained in the 75-day deficiency letter received on March 9th, 2020

The primary source document for this review is the 75-day response letter provided by Monsanto and cited above.

MRID 508434-08: Insect Resistance Management Plan for MON 88702 x MON 15985 x COT102 x MON 88701 x MON 88913

The above MRID was reviewed in US EPA (2020) and may be referenced herein.

IRM Considerations and Proposed Plan for MON 88702 x MON 15985 x COT102 Cotton Natural Refuge

Probabilistic Modelling for *L. lineolaris* and Cry51 trait durability

Monsanto (2020) generated a new modelling analysis under a probabilistic, rather than deterministic, approach. Previously, worst case scenario estimates of the durability of Cry51 cotton under the deterministic model were approximately 10 years until Bt resistance for the key target pest, *L. lineolaris* (Monsanto 2019). The probabilistic modeling of the *L. lineolaris* resistance timeline resulted in durability estimates that consistently exceeded 10 years when the impact of over-sprays was included; rarely less than 15 years (see Fig. 1). Here, BPPD will describe the probabilistic model parameters, operation, and results.

Model Inputs

Under the new probabilistic modelling, the parameters identified in Table 1 were varied. Key additions to the new model requested by US EPA (2020) included variable toxin dose and inclusion of natural refuge. In laboratory and greenhouse studies, Cry51 exerts 90% mortality against nymphal stage *L. lineolaris* (US EPA 2020). However, in preliminary field trials, realized dose may be approximately 70% mortality (Monsanto 2020) as reflected in the WSS model parameter. Additionally, a 20% structured refuge was included that varied in the level of grower compliance from 25-75% with a most likely value of 50%.

Due the nature of probabilistic modelling, other parameters were varied as well and are outlined in Table 1. Both the trait: h and RAF parameters are respectively associated with dominance of the resistance allele and resistance allele frequency at the time of commercialization. The variables that address weedy host refuge, corn abundance, and corn productivity are related to the amount of natural refuge present in the environment (see US EPA 2020 for a detailed explanation). The final two parameters, spray survival, were varied on Bt and non-Bt cotton, because the model presumes there are less sprays for *L. lineolaris* control on Cry51-traited cotton and thus such sprays exert less control than on non-Bt cotton.

Table 1. Beta-PERT Model Parameters used to Describe Model Parameter Uncertainty.

Model Parameter	Minimum (*a*)	Most Likely (*m*)	Maximum (*b*)	Scale (*k*)
Trait: WSS	0.15	0.3	0.45	4
Trait: h	0	0.10	.4	4
Trait: RAF	0.0025	0.005	0.0075	4
Weedy host refuge, Gen 1-3	0.10	0.20	0.40	2
Corn Abundance, Gen1-2	0.10	0.35	0.70	2
Corn productivity	1	1.5	2	3
Structured refuge Compliance	0.25	0.50	0.75	4
Spray survival, *Bt* cotton	0.33	0.48	0.63	3
Spray survival, non-*Bt* cotton	0.05	0.20	0.35	3

Reproduced from Monsanto (2020)

Model Operation

Probabilistic model runs compared three scenarios for Cry51 cotton use: 1) Cry51 cotton used alone for *L. lineolaris* control; 2) Cry51 cotton supplemented with the insecticidal over-sprays growers will need to use to get full control of *L. lineolaris*; and 3) Cry51 cotton supplemented with insecticidal over-sprays and 20% structured cotton refuge. All three scenarios assumed that all cotton in the landscape was Cry51 cotton (100% product adoption).

Due to the stochastic nature of the model, uncertainty in model parameters using Beta-PERT distributions were specified (see Table 1). In the uncertainty analysis, 5,000 simulations were conducted, for each scenario above (1-3), where model parameter values were randomly sampled. All statistical analyses were conducted using R statistical software.

Note, the model isolated *L. lineolaris* as the key pest for estimating resistance likelihood because this pest had the least durability in terms of years under the worst-case scenario deterministic simulations in the previous Monsanto (2019) submission.

Model Results

The model described above was developed to assess EPA's question of whether natural refuge has equivalent durability to a 20% structured refuge or limited acreage deployment scenario. Monsanto (2020) determined that Cry51 used with insecticide oversprays is nearly equivalent to use with a structured refuge requirement (see Figure 1). Further, Monsanto opined that a limited acreage deployment scenario is functionally like a 20% structured refuge and did not conduct additional modelling.

The new modelling of the *L. lineolaris* Cry51 resistance timeline resulted in median durability estimates for the trait alone of 16 years and 25 years for the trait with the expected use of insecticide oversprays (see Fig. 1). Inclusion of 20% structured refuge with conservative compliance levels had little impact on product durability, typically increasing durability by only 10% for scenarios with the highest resistance risk.

Structured refuge is not notably more protective than natural refuge in this model because of the high insect productivity of corn and weedy hosts (see Monsanto 2019), the reduced impact of refuge due to low levels of likely grower compliance, and the need to spray non-Bt cotton reducing the benefit of this acreage as a source population for refuge purposes.

Limited acreage deployment was considered synonymous with structured refuge by Monsanto (2020) because non-Bt cotton will continue to receive insecticidal oversprays and not be a productive source of *L. lineolaris.* Thus, modelling for this paradigm was not specifically conducted. Additionally, Monsanto (2020) discussed barriers to the feasibility of implementing an acreage cap given that farmer's not beholden to buy seeds from local dealers and thus, an acreage cap may not result in even distribution of Bt cotton and non-Bt refuge.

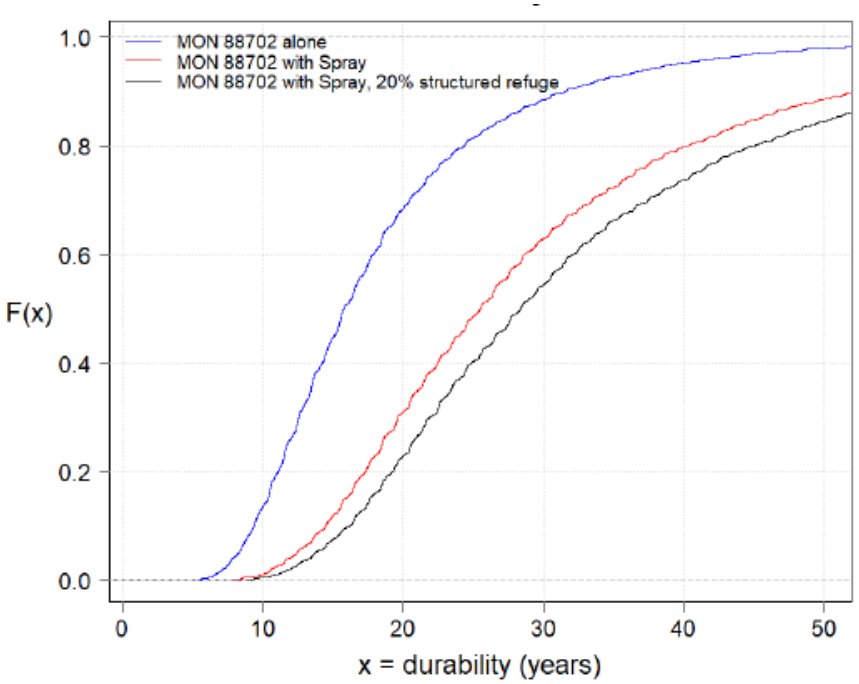

Figure 1. Cry51 cotton durability distribution. Cumulative durability distribution, account for model parameter uncertainty and geographic refuge variability. Reproduced from Monsanto (2020).

Resistance Monitoring Plan for Cry51 Cotton

Monsanto (2019) stated the company will investigate unexpected injury reported by growers, academics, or other stakeholders. However, in the initial submission from Monsanto, no actionable thresholds or resistance monitoring scheme was laid forth by the registrant. Here, Monsanto (2020) proposes to implement unsprayed (for sucking pests) sentinel plots of Cry51 cotton and non-Bt cotton to monitor the abundance of target pest populations in areas of expected high adoption and resistance risk alongside actionable resistance triggers. Regions of interest for placing the sentinel plots will necessarily include the mid-south and southeast, as well as the Carolinas and the southwest, and plots will be run through a combination of Bayer sites and academic cooperators. The expected level of control/reduction (trigger) will vary among the target pests and likely will fall in the range of 25 to 50% reduction based on preliminary data.

Monitoring will be conducted based on activity against late nymph stages of both thrips and *Lygus* spp. Field testing in 2020 may provide enough information to set these triggers and data collection in 2021-2022 will improve these triggers. When the action triggers are exceeded Monsanto will take the below remedial actions.

Remedial Action

In the initial submission Monsanto (2019) proposed that when resistance is ascertained, discussions will be held with the relevant grower/s to understand their current agronomic practices (including choices of insecticides for sucking pest control) and to determine how they can be improved or supplemented. Additional performance monitoring will be carried out around the affected fields to determine the extent of the affected area and key stakeholders (including local extension personnel and consultants) will be contacted to ensure awareness and align on recommended practices.

Additional Deficiencies Identified by EPA and the Monsanto Response

EPA also identified several other deficiencies or points of clarification to be addressed by Monsanto including whether modelling based on single or multigenic resistance was more relevant to the model and oviposition deterrence exerted by Cry51 cotton interfering with non-random mating in thrips.

Single v. multi-locus

EPA identified concerns that due to the low-to-moderate dose nature of Cry51 cotton, resistance may evolve as a multigenic locus rather than a single locus trait. It was unclear to the Agency how this may impact resistance durability or modelling. Monsanto (2020; see therein, ffrench-Constant 2013 and Lande 1983) cited sources that concluded monogenic resistance is a likely resistance pathway for any dose toxin if there is no fitness cost, otherwise, multigenic resistance will prevail unless there is a high dose. Further, Monsanto (2020) conducted modelling with different resistance genetic paradigms and determined that monogenic resistance is a conservative modelling assumption and provides similar durability timelines as compared to multiple resistance scenarios.

Oviposition deterrence in thrips

The Agency noted that random mating was assumed for thrips in the resistance modelling for Cry51 cotton. Random mating is critical for an IRM plan based on refugia because resistant insects must have an equal likelihood of mating with susceptible individuals. However, Cry51 exerts oviposition deterrence against thrips and it was unclear to the Agency how this may affect trait durability.

Monsanto (2020) opined that because of the haplo-diploid mating system of thrips, non-random mating has no impact on durability. In this mating system, haploid males result from asexual reproduction. Thus, heterozygous females will produce fully resistant haploid males (50% of male offspring will be resistant) regardless of who they mate with, so random mating does not have the same effect as it does with diploid mating systems. Furthermore, non-random, directed

movement of mated females after mating has been shown to increase durability (Ives and Andow 2002).

Proposed Terms of Registration

Monsanto (2020) did not formally address in their response memorandum the terms of registration suggested by the EPA regarding: a five-year cyclical re-evaluation of the natural refuge strategy for Cry51 cotton or submission of annual reports regarding the number of overspray applications to Cry51 cotton as an early warning of resistance.

BPPD REVIEW

In EPA (2020), the Agency identified two additional IRM strategies to supplement natural refuge for Cry51 cotton and possibly increase trait durability: a 20% natural refuge or a 70% of total cotton acreage limited deployment cap. Based on the two modelling paradigms conducted by Monsanto (2019, 2020), BPPD concludes that durability of Cry51 in high risk, worst-case scenario areas may be between 10-15 years for *L. lineolaris*. Durability may exceed this threshold in areas where pest pressure is lower.

BPPD does not fully concur with the model parameters selected by Monsanto (2020) for their revised modeling analysis. For example, the structured refuge compliance values ranged from 25% to 75% with a most likely percent compliance of 50% (see Table 1). While historically, refuge compliance has been lower in Southern agriculture states, compliance is not a static parameter. A strong compliance program, including on-farm assessments, could be developed to bolster grower acceptance and integration of this IRM strategy. This avenue was not explored by Monsanto (2020) and a model with full refuge compliance was not presented to the Agency.

BPPD also does not agree that non-Bt cotton refuge area should be de-valued as a source of pest insects because it will be sprayed. Historically, when EPA required a cotton refuge in the 1990's, non-Bt refuge area had a sprayable and non-sprayable size option. Were EPA to mandate non-Bt cotton refuge, the refuge size would be varied depending on whether refuge would be sprayed or unsprayed. This scenario was not explored by Monsanto (2020).

While there are no ideal IRM options for a low-to-moderate dose toxin, BPPD concludes that based on the weight of evidence, the pathway that provides a reasonable measure of product protection and is most likely to be adopted by cotton growers is the natural refuge strategy. Monsanto (2020) notes that there are similar technical concerns with structured refuge and limited acreage deployment scenarios including that those areas will receive additional insecticide applications limiting their value as refuge. Further, a limited acreage deployment will not distribute non-Bt cotton uniformly across the landscape due to logistical concerns with implementation. Natural refuge provides some durability for Cry51 cotton with less complexity for growers since it aligns with the current Lepidopteran pest refuge strategy. BPPD proposes that Monsanto submit a re-evaluation of the natural refuge strategy every five years to justify the durability of the system for the target pests of Cry51 cotton.

Resistance monitoring program and remedial action

BPPD concurs that a sentinel plot program for resistance monitoring, alongside following up on grower damage reports, with an actionable threshold of a reduction in large target pest nymphs by 25-50% is acceptable. BPPD proposes that Monsanto identify a specific action threshold as a term of registration in the subsequent years after Cry51 commercialization which will then be formally reviewed by EPA as a term of registration. BPPD recommends defining suspected resistance as less than 50% control of large nymphs and confirmed resistance as less than 25% control of large nymphs. At the suspected resistance trigger, BPPD recommends that follow the plan laid forth by the company: discussions held with the relevant grower/s to understand their current agronomic practices (including choices of insecticides for sucking pest control) and to determine how they can be improved or supplemented, additional performance in the affected area, and inform key stakeholders to ensure awareness and align on recommended practices. BPPD also recommends the company to inform the Agency of suspected resistance cases. Following confirmed resistance, BPPD recommends that Monsanto consult with the EPA to determine a best course of action for IRM.

Reporting requirements

Monsanto has not proposed to submit annual reports to EPA. For other Bt PIPs and target pests, EPA currently requires annual reports including resistance monitoring, compliance, and stewardship plans. BPPD recommends requiring an annual report to be submitted by Monsanto per the terms of registration detailing the results of the sentinel plot monitoring and any stewardship activities in response to suspected or confirmed resistance.

Further, as summarized in US EPA (2020), the BPPD IRM team recommends that Monsanto conduct a five-year cyclical re-evaluation of the natural refuge strategy for Cry51 cotton for thrips and *Lygus* spp. This report should be similar to Monsanto (2020), including cropping pattern analyses and host use productivity to examine levels of effective refuge. Furthermore, Monsanto is advised to develop complex, spatial models to identify resistance 'hotspots' (i.e., localized areas with a high risk of resistance at a county-level).

References

Monsanto Company. 2019. Data deficiency response letter dated September 24, 2019. MRID Unassigned.

Monsanto Company. 2019. Insect Resistance Management Plan for MON 88702 x MON15985 x MON 88701 x MON 88913. MRID 50843408

Monsanto Company. 2020. Responses to the Non-Target Organism (NTO) and Insect Resistance Management (IRM) data deficiencies of 524-AAE contained in the 75-day deficiency letter received on March 9th, 2020. MRID Unassigned.

United States Environmental Protection Agency. 2020. Insect Resistance Management Plan Review for Cry51Aa2.834_16 expressed in MON 88702 x MON 15985 x COT102 x MON

88701 cotton and target pests *Lygus* spp. and *Frankliniella spp.* Memorandum from K. Welch to M. Weiner, through A. Reynolds. Dated March 4, 2020.