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Amended Revised Response to EPA's Data Call-In Notice Concerning the Potential for Adverse Effects of Bt Corn on Non-Target Lepidopterans

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PREFACE

The Agricultural Biotechnology Stewardship Technical Committee - Non-Target Organism Subcommittee (the Subcommittee) is submitting a comprehensive response to the Environmental Protection Agency's (EPA or the Agency) December 1999 Data Call-In (DCI) notice for information regarding whether Bt corn pollen poses a significant risk to monarch butterflies. The Subcommittee submitted a revised response to the DCI on March 29, 2001. Subsequent to that submission, the Subcommittee requested the primary authors of four manuscripts that are in preparation for publication to review the Subcommittee's revised response. The Subcommittee utilized data and information from drafts of these manuscripts in addition to information from other sources to prepare the revised response. The purpose of the requested review was to ensure that the Subcommittee's response accurately reflected the results of the research conducted by the authors.

Based on comments received and revisions made by the authors to some of the draft manuscripts, the Subcommittee has made some minor revisions to the March 29, 2001 response to the DCI. These revisions are reflected in the amended revised response that is now being submitted to the EPA. The Executive Summary that follows provides a detailed overview of the Subcommittee's full response to the Agency's DCI.

EXECUTIVE SUMMARY

The Environmental Protection Agency (EPA) in December 1999 issued an extensive Data Call-In (DCI) notice to Bt corn registrants for information regarding whether Bt corn pollen poses a significant risk to the monarch butterfly. The DCI requested information on five areas: the distribution of monarch butterflies, milkweed plants and corn; corn pollen release and distribution in the environment; toxicity of Bt Cry proteins and Bt corn pollen; monarch egg laying and feeding behavior; and monarch population monitoring. The Agricultural Biotechnology Stewardship Technical Committee – Non-Target Organism Subcommittee (formerly the ABSTC Monarch Task Force) is submitting a comprehensive response comprising a thorough summary of the relevant scientific literature and registrant data, as well as data from recent research being prepared for publication. The Subcommittee is composed of representatives from the following producers of Bt corn: Aventis CropScience USA LP (formerly AgrEvo USA Co.), Mycogen Seeds c/o Dow AgroSciences LLC, Monsanto Company, Syngenta Seeds, Inc. (formerly Novartis Seeds, Inc.), and E.I. du Pont de Nemours & Co., Inc.

The information requested by the EPA focuses on two key components of risk assessment: *exposure* and *hazard*. Neither demonstration of exposure nor of hazard is in itself sufficient to establish risk. Any possibility for Bt corn pollen to pose a risk to monarch populations depends jointly on the opportunity for monarch larvae to consume Bt pollen (the potential for environmental exposure), the actual consumption (biological exposure) and the extent of effects of Bt pollen exposure on larval growth and development (toxicity or hazard). Establishment of a reasonable probability of harm (that is, risk), must demonstrate that, under natural environmental conditions, a significant portion of the monarch population consumes hazardous amounts of Bt pollen, and that the effects lead to measurable and significant reductions in the monarch population over and above other significant mortality factors.

Extensive empirical laboratory and field studies on potential hazard and actual exposure indicate that Bt corn pollen poses negligible risk to monarch butterflies. Independent laboratory research studies demonstrate no statistically significant effects on monarch larval survival or development from consumption of high concentrations of Bt pollen from events MON 810 and Bt11, which vastly dominate Bt corn acreage in North America. Furthermore, field experiments confirm that monarch larvae develop normally on milkweed leaves when Bt pollen from these events is naturally deposited during pollen shed, even at high pollen densities. This confirms that, even under maximal exposure conditions, MON 810 and Bt11 pollen poses no measurable hazard. Nevertheless, exposure potential for much of the Corn Belt is further limited by the minor extent of temporal overlap of corn pollen shed (during a relatively brief period each summer) with the presence of sensitive monarch larvae. The absence of hazard to monarchs under conditions of actual exposure, and at much higher levels of exposure in laboratory settings, provides reasonable assurance that monarchs and threatened or endangered species of butterflies are not at risk from planting Bt corn.

After examination of a substantial body of exposure and hazard data, the research shows that monarch larvae develop normally on milkweed plants inside or nearby MON810 and Bt11 cornfields even during the period of peak pollen deposition when exposure of larvae to Bt pollen is maximized.

The following are the key findings concerning the potential for exposure and hazard of Bt corn pollen:

Toxicity Evaluation of Cry Proteins and Bt Corn Pollen

Toxicity of Bt proteins to monarch larvae. Monarchs are members of the same insect order, Lepidoptera, as caterpillar pests targeted for biological control by *Bacillus thuringiensis* (Bt)-based products, including Bt corn and several registered foliar Bt microbial products. The susceptibility of many species of butterfly larvae to lepidopteran-active Bt Cry proteins has been well-documented in the scientific literature. Artificial diet studies with various Cry proteins show predictable, concentration-dependent degrees of mortality and growth inhibition for young monarch larvae in a laboratory setting. The order of sensitivity to the Cry proteins is Cry1Ab > Cry1Ac > Cry9C > Cry1F. Larger monarch larvae are 11 to 30 times less sensitive to Cry1Ab (the Bt protein present in current commercial Bt hybrids) than are young larvae.

Event-specific Cry protein expression in pollen. Cry1Ab protein is at trace levels (<90 ng/g dry weight) in corn hybrids derived from events MON 810 and Bt11, which represent the vast majority of the Bt corn acreage in the US. Event 176 has a comparatively high level of Bt protein in pollen because a pollen-specific promoter elevates expression, but hybrids from this event comprise less than 2% of US corn acres and this acreage will continue to decline with time.

Bt pollen effects in the laboratory. Under highly conservative, “no-choice” laboratory conditions, no statistically significant effects on monarch larval survival or development were observed following consumption of high concentrations of Bt pollen from events MON 810 and Bt11. These studies exposed the youngest, most sensitive larval stages to a wide range of pollen concentrations, including extremely high densities that exceeded those observed within cornfields. Because even these extreme pollen densities have shown no significant effect on larval weight, a very sensitive indicator of hazard, it is highly unlikely that longer-term exposures of monarch larvae will result in effects on survival or fitness at environmentally relevant pollen levels. Moreover, sensitivity to Cry proteins decreases markedly in later larval stages, therefore longer-term exposures would not likely yield a measurable effect. Event 176 pollen does have a significant adverse effect on monarch larvae in laboratory assays. However, a variety of factors will tend to mitigate the potential for such effects to occur under field conditions.

Study artifacts. Laboratory studies using collected corn pollen samples are subject to experimental artifacts and misinterpretation. Pollen sample preparation and handling can lead to the presence of anther fragments from the plant tassel; these fragments are believed to result from fractured anthers. In the case of MON 810 and Bt11 pollen, anthers contain considerably higher Cry protein concentrations than the pollen itself. Fractured corn anthers do not appear as a natural feature on milkweed under field conditions, and intact anthers may be too large to be consumed by young larvae. Therefore, the results of assays using manually collected and processed pollen samples rather than natural field deposits of pollen must be interpreted with caution.

Bt pollen effects in the field. Field studies confirm that young monarch larvae develop normally on milkweed leaves when Bt11 pollen is naturally deposited during pollen shed at densities greater than the 96th percentile of measured in-field pollen concentrations. Event 176 pollen has more than 50 times the Cry1Ab level found in MON 810 and Bt11 pollen and, for this event, mortality and growth effects on monarch larvae are observed at Bt pollen densities typical of cornfields and cornfield edges. Event 176 corn, however, represents a very minor portion of corn acres (<2% in 2000), and acreage planted with Event 176 will continue to decline. Thus, the potential for risk to the monarch population from Event 176 pollen is small when the limited acreage is considered in conjunction with factors serving to minimize exposure of monarch larvae to Bt corn pollen (described elsewhere in this summary). These factors include the degree of temporal overlap of sensitive larvae and Bt corn pollen and the relatively low corn pollen densities beyond a few meters from cornfields that limit exposure concerns to the cornfield and the near cornfield edge.

Distribution Of Monarch Butterflies, Milkweed Plants And Corn

Bt corn as a percentage of monarch habitat. The overlap of monarch distribution, host milkweed plants and corn production defines an area of potential Bt pollen exposure that is restricted to less than one-fifth of the summer breeding range for the population of monarchs east of the Rocky Mountains. Additionally, monarch production varies across habitats relative to other habitats. For some habitats, there is a greater potential for exposure of a proportion of the monarch population where cornfields contribute a larger proportion of the overall monarch population. Limited temporal overlap of monarch larvae occurrence with pollen shed within the heart of the Corn Belt where corn production intensity is the greatest further restricts the monarch habitat where exposure may occur.

Monarch host plant and density in cornfields. Monarch butterflies passing through the Corn Belt lay eggs on milkweed species found in that area, which can include milkweed within and around cornfields. Therefore, larvae are potentially exposed to corn pollen to the limited extent to which they co-occur with pollen shed. Even when actively managed for weed control, milkweed populations are reduced but not eliminated. While common milkweed, the most prevalent of the milkweed species, is frequently found in corn and soybean fields, average frequency and patch size are generally much greater in non-crop areas. Therefore, the potential presence of monarch larvae on milkweed within cornfields is very much determined by land use patterns and weed control measures. The importance of cornfields for monarch production varies considerably in different geographic regions. In some areas, where cornfields represent a substantial portion of the land use, cornfields appear to represent significant sites for monarch production, whereas in areas with more heterogeneous land use patterns, cornfields appear to represent only a minor component of monarch production.

Corn Pollen Release and Distribution in the Environment

Spatial-temporal overlap in pollen shed and monarch larval occurrence. Modeled spatial-temporal overlap of corn pollen shed with various life stages of northward migrating monarch populations show, in general, little or no overlap between early instars and the corn pollination period at the southern and central portions of the Corn Belt, with increasing overlap as latitude increases. These modeling conclusions are generally supported by empirical data showing temporal overlap is more consequential in northerly portions of the US Corn Belt. This overlap represents a minor potential for exposure, on a population-wide basis, considering the proportion of the landscape planted to Bt corn and that, for a given corn hybrid, pollination occurs over a much more restricted period than suggested by regional ranges in pollen shed duration.

Pollen deposition and retention on milkweed leaves. The amount of corn pollen deposited on milkweed leaves on a local scale depends on the proximity of milkweed plants to corn, milkweed architecture and morphology, pollen movement away from the field, and environmental factors that can dislodge pollen from leaf surfaces after initial deposition. Empirical measurements inside over 100 individual cornfields show average pollen densities at pollen shed range from tens of grains to several hundred grains per cm^2. Overall, the average pollen density inside cornfields is about 120 grains per cm^2 and falls by half at the immediate field edge. The exposure distribution shows that over 96% of pollen measurements inside cornfields are less than 500 grains per cm^2. Corn pollen is of large size and rapidly settles by gravitational deposition. Thus, off-field pollen deposition on milkweeds is sharply lower along the cornfield edge compared to in-field measurements, decreasing from about 35 grains per cm^2 at one meter to 14 grains per cm^2 at two meters and less than 10 grains per cm^2 at four to five meters. All of these various measures of pollen density fall well below the high levels of MON 810 or Bt11 pollen shown to have no measurable effects on monarch survival or fitness.

Factors affecting pollen deposition and retention. Milkweed plants will intercept less than half of the pollen to which they are exposed. Milkweed leaf characteristics, environmental conditions, and sampling time all impact the deposition and subsequent retention of pollen. Top leaves, where the majority of monarch eggs are deposited and where small larvae tend to feed, retain significantly less pollen than middle and lower leaves due to rainfall, wind, and the tendency for the upper-most leaves to orient more vertically than horizontally.

Monarch Egg Laying and Feeding Behavior

Oviposition behavior. Monarch preference for laying eggs on milkweed in corn versus non-corn habitats varies regionally and temporally. In several areas of the Corn Belt, monarchs show no preference for habitat type (milkweeds in cornfields versus non-cornfields) when selecting milkweeds for egg laying, but in some areas of the Corn Belt monarchs tend to lay more eggs on milkweeds in cornfields, particularly later in the summer. This may reflect differences in cultural practices, because monarchs appear to favor new, succulent milkweed leaves for egg laying.

Monarch larval feeding behavior. Feeding damage from young larvae tends to be located close to where eggs have been deposited. This suggests that, since monarch eggs tend to occur mainly on the underside of milkweed leaves, larvae will begin eating tissue with few or no pollen grains before moving to the upper leaf surface where pollen density may be higher. This, in conjunction with the extensive spatial-temporal variation in pollen concentrations on the milkweed plant itself, indicates a low likelihood that monarch larvae will be continually exposed to Bt corn pollen during development.

Feeding behavior and pollen density. Monarch larvae respond to the presence of corn pollen when feeding on milkweed leaves in a manner that depends upon the density of pollen grains. Monarch larvae feeding on milkweed leaves are not normally expected to avoid cornfields or pollen-covered leaves in choosing where to feed. However, feeding avoidance has been observed when pollen densities correspond to hundreds of grains per cm^2. This is true for Bt corn pollen and non-Bt corn pollen. The amounts consumed, however, are not enough to cause any adverse effects, with the exception of localized effects of Event 176 pollen.

Comparative Risks

Environmental and biological factors limiting monarch populations. Numerous environmental and biological factors may negatively affect monarch populations, reducing their numbers in both southern over-wintering sites in Mexico and northern breeding grounds in the U.S. and Canada. Predominant contributors are threats to over-wintering habitat, survival and fitness, predation, weather, physiological stress and, to a small degree, human activities, including use of broad-spectrum insecticides in crop production. Monarch populations appear to exhibit wide year-to-year variability and resiliency in recovering from highly variable and substantial mortality and stress factors. Given the negligible to non-existent hazard to monarch populations posed by Bt corn pollen, it is not possible to discern an adverse Bt corn impact.

*Relative risks of insect control practices in field corn***.** Consideration of alternatives to Bt corn for control of European corn borer (ECB) is of direct relevance to assessing risks to monarchs. High rates of grower adoption of Bt corn are not surprising given the advantages that Bt corn offers in efficacy, worker safety, and ease-of-use over conventional insecticides for ECB control. Moreover, the same features that have lead to widespread adoption illustrate that Bt corn has likely reduced the overall impact of agricultural practices on the monarch and other non-target insects.

Conclusions

Overall, consideration of hazard and exposure factors indicates that Bt corn pollen represents a low to negligible risk to monarchs and other non-target lepidopterans. Any hazard associated with MON 810 and Bt11 pollen is very low to non-existent, based on both laboratory experiments and field studies. Additionally, a number of factors reduce exposure to Bt corn pollen, including pollen that displays hazard potential, so that any risk associated with Bt corn pollen to non-target lepidopteran populations is very low.