

Comments from Environmental Defense on EPA's “Cry9C Food Allergenicity Background Document”

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by

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Introduction

Thank you for the opportunity to present the comments of Environmental Defense on a pesticide petition (9F5050) submitted by AgrEvo USA Company¹ to the U.S. Environmental Protection Agency (EPA). AgrEvo has petitioned EPA to expand the current exemption from the requirement of a tolerance for Cry9C Bt toxin² produced in genetically engineered corn. To date, EPA has limited use of the Cry9C toxin to corn used for animal feed,³ because the biochemical properties of Cry9C toxin indicate that this protein has the potential to cause allergic reactions in humans. AgrEvo would now like to expand use of Cry9C corn to human foods. As explained below, Environmental Defense urges EPA to not approve at this time AgrEvo's petition to expand the use of Cry9C toxin to corn used in human food. AgrEvo has not provided adequate evidence to assure a reasonable certainty of no harm from the use of Cry9C toxin for use in human food. Nevertheless, Environmental Defense does hold open the possibility that the Cry9C could be approved for use in human food if foods containing the toxin were labeled as potentially allergenic and a system of post-market surveillance was implemented.

EPA Allergenicity Assessment Procedure

EPA faces special challenges in evaluating the allergenicity of Cry9C toxin and other pesticidal proteins because there is currently no predictive methodology for testing the allergenicity of most proteins introduced to food via genetic engineering. Such testing is only possible for proteins introduced from commonly allergenic foods, such as nuts. Proteins from commonly allergenic foods can be screened for "antibody-antigen" reactions using blood serum available from individuals with common food allergies. However, for most proteins, including those from non-food sources such as Bt bacteria, no such testing is possible. In other words, most proteins added to foods via genetic engineering cannot be directly tested for allergenicity.

EPA appears to rely primarily on **four criteria** for assessing of the allergenic potential of Bt toxins that crops have been genetically engineered to produce. Choice of these criteria is based on the conclusions of a 1994 Workshop on Food Allergenicity⁴ and an industry-supported paper.⁵ The four criteria are:

1. Amino acid **sequence homology** to known allergens;
2. Resistance to **acid and enzymatic degradation/gastric digestion**;

¹ As the result of a corporate merger, AgrEvo recently became part of the company Aventis.

² A number of companies have genetically engineered corn and other crops to produce in their tissues insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt), with the goal of killing insect pests that feed on these Bt crops.

³ Includes associated residues in meat, poultry milk, or eggs resulting from animals fed with the GM corn and its derivatives.

⁴ In April of 1994, EPA, the Food and Drug Administration, and the Department of Agriculture hosted a "Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops."

⁵ Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants. 1996. *Critical Reviews in Food Science and Nutrition*, 36(s):S165-S1866. CRC Press, Inc.

3. **Heat stability/heat resistance;**
4. **Molecular size.**

Similar criteria were recently advocated in a paper by Kimber and colleagues (1999). In addition to these four criteria, two additional criteria were considered in EPA's assessment of the allergenicity of Cry9C protein for use in animal feed:

1. The **abundance** of the protein, or its **amount** as a percentage of total proteins in representative materials;
2. **Occupational exposure.**

Cry9C Allergenicity Assessment

EPA's four criteria:

As described below, the Cry9C toxin appears a potential allergen according at least two of EPA's four criteria for allergenicity – resistance to acid and enzymatic degradation and heat stability:

1. There is no **homology** of sequences of 8 amino acid stretches of Cry9c with known allergens. It should be noted, however, that EPA has not established the value of this type of assessment as an indicator of allergenicity (MRID No. 44384404). While a positive finding of homology may indicate allergenicity, a negative finding may not be a useful indicator of safety.
2. Cry9c was found to be **resistant to digestion** *in vitro* (MRID No. 44258108) and partially also *in vivo* (MRID No. 44734305);
3. Cry9c was found to be **heat resistant** (90°C): for extended periods the protein appeared to be stable (MRID No.44258108);
4. The **molecular size** of the Cry9C protein (68 kDa) is included in the upper range of the possible values for an allergen (10-70 Kda). Moreover, MRID NO 447343-05 indicates that the protein can be partially degraded to a 55 kDa form, closer to the size of most allergens. EPA's interpretation of this information is unclear, since molecular size is not discussed in the EPA background document (1999).

Additional two criteria:

As discussed above, two additional criteria – protein abundance and occupational exposure -- were introduced for assessment of the allergenicity of Cry9C toxin in animal feed. As detailed below, the utility of protein abundance as an indicator of allergenicity in human food is questionable, especially since a) it is not clear what constitutes low abundance, and b) data is not presented on exposure to Cry9C protein. AgrEvo did not directly assess occupational exposure in its petition to EPA.

The study (MRID 44734304) to determine the **abundance** of Cry9C protein concluded that the protein is present in low amounts. It is unclear, however, what the threshold

might be for a "low amount." In fact, in another context – resistance management – EPA and AgrEvo argue that the Cry9C protein is present in high amounts! According to EPA's Biopesticide Fact Sheet (1999), "the company believes that Cry9C corn expresses a high dose of toxin. Submitted data appears to verify this in all plant tissues except pollen."

In addition, the relevant scientific literature does not to the best of our knowledge show that protein abundance is an indicator of allergenicity. Moreover, if abundance of Cry9C protein is to be used as an indicator, EPA should require exposure assessment for the protein. Exposure assessment is especially important for subpopulations exposed by multiple routes (e.g., have occupational as well as dietary exposures) or who are at particular risk of allergy (e.g., children with a familial history of allergy).

According to EPA, **occupational exposure** to Cry9C protein is negligible, or presents no risk because the Cry9C protein is not toxic to people (Biopesticide Fact Sheet, 1999). However, the basis for this assertion is not clear. No study is presented to substantiate the claim of negligible exposure. To the contrary, scientific evidence clearly shows that occupational exposure to grain dusts can be hazardous.

Occupational exposure to grain dust has been linked to various form of allergy, with some epidemic episodes (Anto et al., 1989). Bakers' asthma is classic example in epidemiology (Baur, 1998), and the role of bacterial enzymes in this ailment has been clearly demonstrated (Pepys, 1992). It is not unreasonable to hypothesize that corn dust could play a similar role as a vector of a new allergen, such as a Bt toxin. Corn dust from animal feed has recently been implicated in respiratory dysfunctions (Park et al., 1998) and in glove-lubricant-powder derived allergy (Crippa et al., 1997). Corn dust's role in acute respiratory inflammation has been demonstrated by testing cytokine expression (Wohlford-Lenane et al., 1999). Thus, corn dust and its potential components, such as Bt toxins, merit close scrutiny as potential causes of occupational allergy.

This conclusion is bolstered by a recent study demonstrating that exposure to Bt extracts leads to specific IgE and IgG formation (Bernstein, 1999). Although this study does not show a link between exposure to Bt and occupationally related respiratory symptoms, it does indicate that exposure to Bt occurs and may lead to skin sensitization and induction of an IgE response – two components of allergic response. In short, since corn dust can clearly convey allergens, and Bt extract is potentially allergenic, there is ample evidence to be concerned about occupational exposure to Cry9C corn.

AgrEvo presents only limited information relevant to occupational exposure -- testimonial letters from employees of Garst Seeds, Slater, IA, who have handled considerable quantities of Bt Cry9C corn seed, tassels, and pollen, and have not experienced adverse reactions they could directly attribute to handling the corn (MRID 44714003). Although these letters may seem reassuring, as an informal survey they have limited scientific value. Moreover, AgrEvo itself has shown that pollen has a lower Cry9c concentration than other plant parts. These testimonial letters may not reflect future experiences of workers exposed to corn dust during shipping, storage, and processing of corn.

Additional information in the current petition:

AgrEvo's petition presents additional information, besides studies concerning the six criteria above. However, much of this additional information is not particularly pertinent to allergenicity and none of it is adequate, even in conjunction with the studies discussed above, to conclude that Cry9C toxin is highly unlikely to be an allergen. For example, in what may be the most pertinent study, AgrEvo assessed the bioavailability of Cry9C toxin in rats' blood, and found Cry9C only in the blood of rats administered relatively high doses of the toxin (MRID 44734305). This finding can be interpreted to suggest that little ingested Cry9C toxin may make it through the human GI tract and into the bloodstream of people, where it may lead to allergic reactions. However, even if this conclusion is ultimately correct, Cry9C toxin may still induce sensitization and elicit allergic reactions in the gut (Dupont and Heyman, 2000).

Just as important, it is at best premature to extrapolate gut permeability data from this single rat study to the entire human population -- including to people with relatively high gut permeability because they are very young, elderly, or suffer GI tract disorders. The scientific literature is clear that gut permeability varies considerably among species (MacKie et al., 1999; Delahunty and Hollander, 1987) and with age and disease-status (Soderholm et al., 1999; Bjarnason, 1995). Thus, even if blood concentration of Cry9C toxin is an important indicator of allergenicity, this study does not appear an adequate to draw a conclusion that ingested Cry9C toxin will not enter the bloodstream of people.

Conclusion:

Environmental Defense urges EPA to reject AgrEvo's petition and to not approve at this time the use of Cry9C toxin in human food. The scientific information provided to EPA by AgrEvo is at best insufficient to assure that exposed populations would have a reasonable uncertainty of no harm from allergic reactions to Cry9C toxin in corn. Two of the four criteria that EPA is using to assess the allergenicity of Bt toxins indicate that the Cry9C toxin has the potential to be an allergen. EPA and AgrEvo have developed additional information concerning the Cry9C toxin beyond the four criteria; however, this supplementary evidence is far from adequate as a basis for a determination that the Cry9c is not, or at least highly unlikely to be, an allergen.

Approval of AgrEvo's petition by EPA would be extremely troubling. Given the paucity of evidence to support a determination that the Cry9C toxin is unlikely to be an allergen, such an approval would suggest that EPA is unlikely to ever make a decision to protect US consumers from potential allergens introduced to the food supply via genetic engineering (unless perhaps an introduced protein was homologous to a known allergen). For EPA to go forward with an approval would be to greatly favor industry profits at the expense of consumer protection. Such an action by EPA would greatly undermine consumer confidence that EPA is capable of meaningful review of genetically engineered plant-pesticides.

At the same time, we recognize that AgrEvo may feel caught in a difficult situation. The company's Cry9C toxin has biochemical properties associated with food allergens, yet there is no clear scientific path to prove or disprove that the toxin is likely to be an

allergen. AgrEvo's situation is in large part a result of inaction by federal agencies. In the six years since the 1994 interagency meeting on food allergy (see footnote 2), federal agencies have done disappointingly little to resolve scientific issues concerning the assessment of allergenicity.

There are at least two constructive approaches – not mutually exclusive -- to the current situation. The first would be to allow AgrEvo to market Cry9C corn for use in human food, with an agreement that the Food and Drug Administration (FDA) would require labeling of food products made from the corn as potentially allergenic. In addition, a rigorous postmarket surveillance system would be established to assess whether the Cry9C toxin causes allergic responses in consumers and in occupational settings. Clinicians who treat allergies and asthma would be alerted to be vigilant for development of new allergies to this novel protein.

A second approach would be for EPA and FDA to use their scientific resources to develop and publish guidance to industry on how to assess the allergenic potential of proteins. Environmental Defense has already urged FDA to develop such guidance. Given the existing uncertainties about assessment of potential allergens, such guidance would be both helpful to AgrEvo and other companies and reassuring to consumers. Without such guidance, there will continue to be no accepted methodology with which to assess the allergenicity of most proteins added to foods via genetic engineering.

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