

Cry34Ab1 protein to pepsin degradation. CSPI contests the interpretation of the results provided by Dow AgroSciences that indicate the Cry34Ab1 degrades under the influence of pepsin. CSPI asserts that EPA cannot make a safety determination in light of these results and international consensus on how to address allergenicity as stated in the Food and Agricultural Organization/World Health Organization (FAO/WHO) expert consultation. Specifically, CSPI claims use of a less sensitive protein detection method, a pH of 1.2 instead of 2.0 for the pepsin buffer solution and a low concentration of the Cry34Ab1 protein in the assays were all utilized to achieve the results. CSPI suggests that all these features combine to artificially skew the results of the pepsin assay to show that Cry 34Ab1 is readily broken down by pepsin. CSPI suggests that the Cry34Ab1 protein is stable to gastric fluid breakdown since it is visible on Coomassie blue stained gels at 7 to 10 minutes of pepsin incubation. CSPI also claims that the initial Dow AgroScience data using a more sensitive Western blot assay clearly show the protein present at the 20 to 30 minute sample and that this endpoint is scientifically agreed upon to indicate resistance to pepsin degradation. CSPI recognizes that the total dietary exposure to the Cry34Ab1 protein likely to occur during an experimental use permit would not be expected to induce an allergic reaction and that there is still considerable scientific controversy around the determination of potential allergenicity of a protein new to the food supply. Finally, CSPI states that a test to determine potential allergenicity is still needed and, in the interim, acceptable standards for performing the currently available tests are provided by the FAO/WHO report on Evaluation of Allergenicity of Genetically Modified Foods (Rome, 2001).

EPA Response

EPA agrees that there is still the need to develop definitive tests to assess potential allergenicity and that the currently employed tests need to follow standardized procedures. EPA also agrees that no single criterion of those currently utilized can alone be an indication of potential allergenicity. However, EPA would suggest that, while the guidance given by the 2001 FAO/WHO report is invaluable, there is still no consensus on how to implement several of the tests suggested in the FAO/WHO guidance nor any direction given as to critical endpoints for the tests suggested. This lack of consensus was confirmed by a CODEX ad hoc

working group on allergenicity which met in Vancouver, Canada in September 2001, to consider the advice of the FAO/WHO expert consultation report from Rome 2001. This CODEX group found that, without development and implementation of the new tests suggested by FAO/WHO expert consultation, the current weight of evidence approach provides essentially the same information for judging allergenicity as that suggested by the 2001 FAO/WHO report. This advice has been incorporated into the latest version of the CODEX food safety assessment for genetically engineered foods. (CODEX, [ftp://ftp.fao.org/codex/alnorm03/Al03_34e.pdf](http://ftp.fao.org/codex/alnorm03/Al03_34e.pdf))

The current criteria used by EPA to judge allergenicity include amino acid sequence similarity analyses, stability to heat, and enzymatic degradation. The Cry34Ab1 protein does not share significant amino acid similarity with known allergens either on a whole sequence level or on the eight amino acid stepwise comparisons, nor does the Cry34Ab1 protein appear to be stable to temperatures above 90 °C. The initial data reported from the company indicated that one of the two proteins, Cry34Ab1, was moderately resistant to the action of pepsin by still being detectable on an SDS-PAGE western blot at 20–30 minutes. EPA questioned the results found in the initial submission on pepsin degradation and requested more information.

In the absence of a definitive endpoint for determining the pepsin resistance of a given protein, the initial results reported by Dow were not conclusive. The 2001 Rome FAO/WHO expert consultation report specifically does not mention a time endpoint for pepsin degradation of a protein other than the protein or a significant sized fragment being present at the final endpoint of 60 minutes. The literature references CSPI itself provided cite a range of values for pepsin stability ranging from 8 minutes to 2 hours and demonstrate a lack of consensus on pepsin resistance. EPA would therefore disagree with CSPI that there is a scientific consensus on visible protein bands in an SDS PAGE assay at 20 to 30 minutes indicating pepsin stability.

Dow AgroScience's second submission presents results that indicate more rapid breakdown than the initial data. Dow AgroScience's approach where enzymatic degradation is expressed as a kinetic rate instead of a definitive substrate disappearance endpoint makes the results less variable since the sensitivity of the detection system does not affect the final result. This is because the pepsin activity can

be expressed as a rate constant, an endpoint that is not dependent on the sensitivity of the detection system, is substrate concentration independent and is the classical method used by protein chemists to determine enzyme activity or in this case substrate disappearance. While this method may not be the final iteration of the pepsin degradation assay, EPA believes that an analysis that lessens assay variability and makes the results independent of the sensitivity of the detection method is an improvement.

EPA finds that the literature references CSPI cited are diametrically opposed in their view of the usefulness of the pepsin degradation assay for prediction of allergenicity (Ref. 1). The Astwood et al. paper shows that, while lowering the pepsin concentration can lead to the appearance of fragments in an otherwise rapidly degraded protein, the pepsin assay is a good predictor of allergenicity (Ref. 1). The Fu et al. paper indicates that both allergens and non-allergens can be stable to pepsin activity so the assay is not predictive (Ref. 2). Both papers emphasize that protein doses, pepsin concentrations, and assay conditions should be equivalent when comparing proteins. Neither paper suggests a definitive timepoint that could be interpreted as indicating protein stability to pepsin. The Fu et al. paper in fact suggests that allergens and non-allergens can both be either resistant to or degraded by pepsin. The final conclusion in the Fu paper is that the pepsin sensitivity assay alone has no predictive value for allergenicity. EPA does not agree with this position but does agree that pepsin stability alone is not a sole criterion to be used for an allergenicity assessment.

EPA agrees with CSPI that the tests used to determine potential allergenicity need standardization and supports efforts in that area. EPA believes that there is sufficient data available, considering all information on the Cry34Ab1 protein, to make a finding that there is a reasonable certainty that no harm will result from the aggregate exposure to the Cry34Ab1 and Cry35 Ab1 proteins as expressed in corn.

The petition requested that 40 CFR part 180 be amended by establishing a temporary exemption from the requirement of a tolerance for residues of the plant-incorporated protectants *Bacillus thuringiensis* Cry34Ab1/ Cry35Ab1 proteins and the genetic material necessary for their production in corn in or on corn. The Mycogen/ Dow AgroSciences and Pioneer Hi-Bred experimental use permits associated with the petition are 68467–EUP–3, 68467–EUP–5, 68467–EUP–T(7), 68467–