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# Biopharming: Case Study of Corn-Produced Aprotinin<sup>1</sup>

Aprotinin corn was developed by scientists with ProdiGene, Inc., Pioneer Hi-Bred International, Eli Lilly & Company and PE Applied Biosystems by inserting a modified gene sequence for cow aprotinin into corn (Zhong et al 1999).

## **Medical uses of aprotinin**

Aprotinin is a protease inhibitor – a substance that inhibits the action of protein-degrading enzymes – that has uses in biochemical research, medicine, and potentially in agriculture. It has traditionally been extracted from bovine lung tissue, and is sold by Bayer under the name of Trasylol. It is best known as a clotting agent used to reduce blood loss in heart surgery (Landis et al 2001), and has also been administered for over three decades in the treatment of acute pancreatitis (Belorgey et al 1996, p. 555). Aprotinin’s coagulant activity has led to recommendations that it not be used on normally clotting patients due to the risk of thrombosis (blood clot) (Blomgart et al), though more recently this risk has been discounted (Landis et al 2001). In rare first-use cases, aprotinin has caused life-threatening anaphylactic reactions, a risk that increases significantly (up to 5% of cases) upon re-exposure (Trasylol Label 2000). Since aprotinin is infused intravenously for these medical applications, it probably does not pose the same risks when ingested, inhaled or through skin contact, though studies of these latter routes of exposure appear to be lacking.

## **Has the food supply been contaminated with aprotinin?**

Aprotinin corn has been grown at least since 1998, when it was reportedly cultivated in field trials by farmers under contract with ProdiGene’s partner, Stauffer Seeds, in Hamilton County, Nebraska (Seed and Crops Digest 1998). However, the USDA biotech website does not identify a field trial of aprotinin corn until 2002 in Hawaii (APHIS Permit No. 01-187-01r). This indicates that the identity of the aprotinin gene was kept secret as confidential business information in the listings for the 1998 and any previous or subsequent trials (see Section 6.3.1) until 2002. The only reported biopharm corn field trials conducted by ProdiGene in Nebraska in 1997 and 1998 are listed under APHIS Permit Nos. 97-098-07n and 98-085-42n, on 5 and 4 acres, respectively. While the identity of the gene is kept secret, the gene category is “novel protein” in each case, while the 2002 trial of aprotinin is listed under the “pharmaceutical protein” category. The USDA must have reclassified aprotinin from novel to pharmaceutical protein, otherwise there is no accounting for the independently reported 1998 field trial of aprotinin in Hamilton Country, Nebraska.

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<sup>1</sup> Excerpted from: *Manufacturing Drugs and Chemicals in Crops: Biopharming Poses New Threats to Consumers, Farmers, Food Companies and the Environment*, Appendix 3, by Bill Freese of Friends of the Earth, for Genetically Engineered Food Alert, July 2002. Available at: [www.foe.org/biopharm/](http://www.foe.org/biopharm/). Section references refer to this report.

It is possible that aprotinin corn has been cultivated in strict isolation from normal corn, though the secrecy of the USDA and ProdiGene make it impossible to determine this. If aprotinin corn was grown according to USDA performance standards for “minimization” of gene flow, which recommend isolation distances of either 660 feet or 1320 feet (Section 6.4.5) from other corn, it is possible that food-grade corn has been contaminated. This becomes more likely when we consider that the company is ProdiGene, which has been observed to be negligent in gene containment practices with its avidin corn, and that aprotinin corn is not reported to be even partially male sterile, increasing the likelihood of contamination through cross-pollination.

### **Allergenic potential**

As noted above, aprotinin has been found to cause anaphylaxis, a life-threatening allergic reaction, especially upon repeated intravenous uses. Aprotinin is a fairly stable molecule that resists degradation by enzymes and acids, and also has significant thermal stability (Sigma Aprotinin). Since these are common properties of food allergens, aprotinin should be properly evaluated for possible allergenic effects from ingestion, inhalation and dermal contact – especially in its corn-grown form, which apparently has not been tested for glycosylation (Zhong et al 1999, p. 353).<sup>2</sup> As discussed in Section 4.1.1, plant glycosylation patterns would also heighten allergy concerns.

### **Pancreatic disease from ingestion of protease inhibitors**

Aprotinin presents other, potentially more serious, health concerns as a protease inhibitor. Protease inhibitors are found naturally in legumes and particularly in soybeans, and are known to be toxic to many insects, fungi and animals. Animal feeding studies have shown that these inhibitors depress growth by interfering with the digestive activity of enzymes like trypsin that are secreted by the pancreas. This inhibitory effect on trypsin causes the pancreas to compensate by secreting more trypsin-containing digestive fluids, resulting in abnormal enlargement of the organ’s cells (hypertrophy) and abnormal increase in number of cells (hyperplasia). Prolonged feeding of soybean trypsin inhibitors leads to development of tumorous nodules on the pancreas, which after 60 or more weeks become cancerous (SAP MT 2000, pp. 31-33).

Whether ingestion of protease inhibitors is similarly dangerous to humans is not certain, though there is evidence that aprotinin (Dlugosz et al 1988) and other protease inhibitors (SAP MT 2000, p. 31) do in fact stimulate secretion of trypsin and other digestive enzymes in humans as in animals. “This would indicate that the human pancreas at least responds in a negative fashion to the effects of a protease inhibitor” (Ibid, p. 31). Additional evidence of human impacts is the report of an outbreak of gastrointestinal illness in individuals who had consumed under-processed soy protein extender in tuna fish salad. This outbreak was attributed to the protease inhibitors in the soy protein, which apparently had not been deactivated by the usual heat treatment used in soybean processing (Ibid). This case suggests that a relatively small quantity

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<sup>2</sup> Zhong et al say only that: “There was no evidence to support a protein being glycosylated, nor is it glycosylated in its native form from eggs.” Examination of the paper shows no indication that any specific tests for glycosylation were carried out. The reference to “native” aprotinin from eggs is puzzling, since the aprotinin gene spliced into corn is reverse translated from the bovine protein, and aprotinin derived from bovine tissue would thus seem to be the proper comparator. In addition, bovine lung is by far the most common source of aprotinin for research and medical purposes, and I find no other reference to aprotinin from egg.

of protease inhibitors (that present in a soy protein *additive*) may be sufficient to cause symptoms, at least in certain individuals.

### **Other potential human health risks of protease inhibitors**

It is interesting to note that scientists still do not understand how protease inhibitors kill insects. Some attribute this effect directly to inhibition of digestive enzymes in the insect gut (SAP MT 2000, p. 31), also the presumed mechanism of gastrointestinal illness in higher animals. But others disagree, proposing different mechanisms.

“The mechanism of action of proteinase inhibitors is not fully understood. Inhibition of enzymes in the alimentary tract of insects is not the main adverse effect. *Depletion of essential amino acids due to over-secretion of digestive enzymes in the presence of inhibitors* is thought to cause most of the toxicity signs observed, but *there are also other targets of toxicity.*” (Kleter et al 2000, section 2.2.3, emphasis added)

Thus, in both insects and mammals, it appears that protease inhibitors: 1) Inhibit digestive enzymes; and 2) Thereby stimulate over-secretion of these same enzymes in a negative feedback loop. In mammals, this leads to pancreatic disease. In insects, it triggers a third effect – depletion of essential amino acids – which some suggest is the chief mechanism of toxicity. Do protease inhibitors have this latter effect in humans as well? If so, depletion of essential amino acids could present the risk of nutritional deficiency. And what of the “other targets of toxicity”? Could they too have human analogues?

### **Aprotinin and other protease inhibitors as plant pesticides**

Because of their insecticidal activity, protease inhibitors like aprotinin are being experimentally spliced into crops to protect against insect attack. ProdiGene is clearly interested in this insecticidal application, as indicated by a passage from its patent for “Commercial production of aprotinin in plants” (Aprotinin Patent 1998):

“Fortuitously, it has been determined that the serine-specific proteinase inhibitor aprotinin has potent insecticidal or larvicidal activity when administered enterically to insects such as European corn borer (ECB) and corn rootworm.”

ProdiGene has shown that aprotinin causes 25% mortality in European corn borer larvae after 7 days of feeding with just 1.0 mg aprotinin per ml of feed. Corn rootworm, the only other insect tested, experienced 60% mortality with 20 mg aprotinin per ml of feed over 7 days (Aprotinin Patent 1998, Tables 3 & 4).

### **Aprotinin shortens the lives of honeybees**

Could aprotinin in plants harm non-target insects? Several feeding experiments have shown that the lives of honeybees are significantly shortened when they consume as little as 3-18 µg aprotinin per day for seven days (Malone et al 2001, p. 64; Burgess et al 1996). The daily dose of 18 µg resulted from feeding honeybees pollen-food containing aprotinin at a concentration of 2.5 mg/g; this concentration was chosen to simulate exposure to transgenic pollen expressing 1% aprotinin (of total protein), approximating a credible field situation (Malone et al 2001, p. 64-5). Unfortunately, ProdiGene does not report the level of aprotinin in the pollen of its corn. Yet its

use of the constitutive ubiquitin promoter suggests that expression in pollen is possible. This possibility becomes more likely when one considers that Cry1F corn, which also contains an ubiquitin promoter, expresses Cry1F in pollen (EPA BRAD 2001b, p. 7). As of 1999, ProdiGene had achieved expression levels of aprotinin in corn kernels averaging about 0.1%, but with some plants expressing up to 0.44%, of total soluble protein; new production lines were to be generated from these higher-expressing plants (Zhong et al 1999, p. 352). The aprotinin content of pollen and anther should be measured and appropriate studies done to determine any possible impacts on a wide range of non-target insects.

### **Stacking aprotinin with other insecticides**

An emerging strategy in the biotechnology industry involves engineering several insecticides into a single crop to achieve broader-spectrum and/or more potent insecticidal activity.

“Combining proteinase inhibitors with lectins or with Cry proteins, either by cross breeding of primary transformants or by multiple gene insertion, is also contemplated in order to enhance insect resistance.” (Kleter et al 2000, 2.2.4).

ProdiGene is clearly interested in this strategy:

“Furthermore, aprotinin and highly similar serine proteinase inhibitors strongly potentiate the insecticidal activity of lectins such as wheat germ agglutinin. It appears that a transgenic plant expressing aprotinin would potentially be more resistant to plant pests such as ECB and corn rootworm” (Aprotinin Patent 1998).

1.0 mg aprotinin per ml of feed combined with 0.2 mg wheat germ agglutinin (WGA) per ml of feed causes 80% mortality in ECB larvae, exhibiting a powerful synergistic effect (Aprotinin Patent 1998, Table 4). Thus, stacking aprotinin and other protease inhibitors with other pesticides could have significant impacts on non-target insects such as honeybees.

### **Conclusion**

Scientific advisors to the EPA recommend that transgenic plants expressing protease inhibitors and/or lectins be subjected to animal feeding studies (SAP MT 2000, p. 33-34). Attempts to discover whether the USDA or FDA had conducted any tests to gauge the potential health risks of aprotinin were unsuccessful. The USDA’s limited response to a Friends of the Earth Freedom of Information Act request contained nothing concerning aprotinin. An FDA scientist said that while the FDA might consult with the USDA on certain biopharm plantings, she was not at liberty to discuss aprotinin or any particular product (personal communication, 2/8/02, Kathryn Stein, formerly of FDA).

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