Risk Assessment: A Technical Perspective

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Regulatory Jurisdiction: Genetically Engineered Plants

After more than ten years of plant biotechnology research and extensive field evaluation at sites throughout the United States (Gasser and Fraley, 1989), the first of several improved crop products being developed are undergoing regulatory review prior to market introduction. Before these products were submitted for review, the various U.S. regulatory bodies had considerable input and oversight into the research and development undertaken to verify the performance and safety of these products. Initial research in the laboratory was performed under the National Institutes of Health (NIH) Recombinant DNA Guidelines (1976; 1982). Several years of field-testing were carried out under the jurisdiction of the United States Department of Agriculture (USDA), in conjunction with the U.S. Environmental Protection Agency (EPA) for pesticidal plants. Regulatory approvals may be required by all three regulatory agencies (USDA, EPA and FDA [Food and Drug Administration]) prior to marketing these products. Early in this regulatory process, the Office of Science and Technology Policy (OSTP) issued the "Coordinated Framework for Regulation of Biotechnology" (1986) which assigned appropriate regulatory oversight and responsibility for biotechnology to these federal agencies, (see discussion by MacKenzie, p. 47)

USDA regulates the movement and release of genetically engineered plants under the Plant Pest and Quarantine Act since many of these plants are generated using organisms or DNA sequences from organisms that are plant pests. The program is administered by the Animal and Plant Health Inspection Service (APHIS). APHIS will make a determination concerning the plant pest status of varieties derived via biotechnology prior to market introduction. EPA regulates the safety of the plant pesticidal products (e.g., proteins that provide insect or disease resistance) as active ingredients and the protein(s) used in the selection process for generating these plants (e.g., selectable marker proteins) as inert ingredients. FDA oversees the food and feed safety of products derived from these plants.

During the past decade, as progress has been made in the production, testing and development of plant biotechnology products, regulations and safety assessment requirements have developed to assure the safety of these products. There has been significant progress in the past twelve months by each of the three regulatory agencies in providing guidance and requesting public feedback on how these products will be regulated. The USDA published draft guidelines in June of 1992 (APHIS, 1992a) for public comment. The final policy was published in March, 1993 (APHIS, 1993). EPA published their proposed policy on regulating plant pesticides, under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), for public comment and sponsored a Science Advisory Panel discussion on December 18, 1992 to obtain public input on this proposed policy (EPA, 1992). Likewise, FDA published their proposed policy for regulating genetically engineered plants and plant products on May 29,1992 for public comment (FDA, 1992).

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The first of the products produced by plant biotechnology, the Flavr Savr™ tomato by Calgene, Inc. (Sheedy et al., 1988), has been reviewed for plant pest status by the USDA (APHIS, 1992c) and is presently under review for food and feed safety by the FDA (Redenbaugh et al., 1992). A petition to the USDA has also been submitted for virus-resistant squash by the Upjohn Company (APHIS, 1992b). With over 500 field tests that have been performed around the world to date (Huttner et al., 1992; Casper and Landsmann, 1992) many other products are being extensively evaluated for agronomic, environmental and consumer value. The food, feed and environmental safety of each of these will be assessed prior to marketing. One of these, a potato improved to control a specific insect pest without the use of chemical pesticides, serves as a case study for how this safety assessment is being performed to assure product wholesomeness and safety, environmental soundness and to support public confidence in these products.

COLORADO POTATO BEETLE RESISTANT POTATOES

Potato plants that control that crop's most serious insect pest, the Colorado Potato Beetle (CPB), are among the first products that Monsanto has developed and for which we are completing a safety assessment. We have worked for nearly 10 years to develop and evaluate these potato plants, which resist CPB through the production of an insect control protein, found in nature, that selectively controls the beetle without affecting nontarget insects, humans or animals. These plants were produced by inserting and expressing a gene from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) in the potato plant (Perlaket al., 1993) using *Agrobacterium* transformation (Newell et al., 1991). The CPB-resistant plants produce low levels of two new proteins, the *B.t.t.* protein for resistance to CPB and the neomycin phosphotransferase (NPTII) protein produced to enable the selection of cells expressing the *B.t.t.* protein in tissue culture. The *B.t.t.* protein produced in these potato plants is the same as one of the insecticidal proteins contained in several microbial formulations that have been used safely and have been commercially available since 1988 (EPA, 1988).

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CPB is the most damaging pest of the $2.3 billion U. S. potato crop (Casagrande, 1987; National Potato Council, 1992) and economically important in the majority of the North American potato production regions. Loss of revenue in Michigan alone was estimated at more than $15 million in a state where potato production is valued at $70 million (Potato Growers of Michigan, 1992; Olkowski et al., 1992). If untreated or poorly managed, the CPB can devastate potato production in some areas (Hare, 1980; Ferro et al., 1983; Shields and Wyman, 1984). Current treatment of CPB primarily involves the use of insecticides that are variably effective due to environmental factors and insect insensitivity, and significantly reduce field populations of beneficial insects which help control other potato pests. These pesticides are also expensive, with costs that can exceed $200 per acre per season (Ferro and Boiteau, 1992).

Field trials conducted in 1991 with CPB-resistant potatoes demonstrated effective control of feeding damage by all stages of the CPB. There were significantly fewer immature larvae, adults and egg masses of CPB on the genetically improved potatoes, compared to the control plants. Without insecticide application, defoliation of the improved potato plants was less than, or equal to, control plants sprayed with insecticides on a regular schedule. In addition, agronomic evaluations consisting of plant vigor, growth habit characteristics and general insect and disease susceptibility, have shown the CPB-resistant potatoes to be equivalent to the parental Russet Burbank potatoes. Field tests were expanded in 1992 with similar results.

These genetically improved potatoes offer several advantages to the grower, the consumer and the environment for controlling this devastating insect pest. The superior CPB control offered by the plants will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop while maintaining comparable yields. Reducing the amount of insecticide applied to potatoes will further aid the implementation of Integrated Pest Management (IPM) practices as beneficial insect populations will be maintained, which can help reduce the other pests of potatoes not directly controlled by the CPB-control protein. The *B.t.t.* protein also has been shown to be safe to nontarget species, including humans (EPA, 1988) and thus provides an environmentally safe means to control CPB. In addition, CPB-resistant potatoes will benefit both large and small growers as no additional labor, planning or machinery is required for adoption. Prior to market introduction, the potato lines will continue to be evaluated for performance and to refine insect management programs. Efforts will focus on confirming CPB control across the potato growing regions and developing production systems that optimize the benefits of these improved potatoes.

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As with other food crops improved through biotechnology, USDA, EPA and FDA exercise joint regulatory oversight for these genetically improved potatoes. All field tests have been carried with the approval of the USDA. In October 1992, an Experimental Use Permit (EUP) was requested from the EPA. The EUP permit, approved in May, 1993, allows for more extensive field evaluation of CPB-resistant potato varieties to be performed on more than 10 acres. Prior to commercialization, a USDA determination of the nonregulated status and an EPA product registration will be obtained under the respective policies described above. Likewise, appropriate consultations with and oversight by FDA will be conducted as described in the FDA policy.

SAFETY ASSESSMENT OF CPB-RESISTANT POTATOES

Monsanto's policy for ensuring the safety of the CPB-resistant potatoes is consistent with the published policies of the three regulatory agencies and relies on two approaches. The first approach is to provide appropriate information to establish that the CPB-resistant potatoes are "substantially equivalent" to the Russet Burbank potatoes from which this variety was derived. The term "substantial equivalence" refers to the concept that the genetically improved potatoes are comparable to the Russet Burbank potatoes in respect to composition, nutritional quality, yield, morphology and in other aspects that could impact the use, value, and the environmental, food and feed safety of this product. The only significant difference that has been observed be-

tween the genetically improved potatoes and the Russet Burbank potatoes is that the CPB-resistant potatoes effectively control the insect pest by expressing two additional proteins (*B.t.t.* and NPTII). No other differences have been observed. The concept of "substantial equivalence" or "substantial similarity" has been used by FDA (1992) and other international organizations (Organization for Economic Co-operation and Development, 1992; International Food Biotechnology Council, 1990) in their recommended approaches for safety assessment. In essence, this is the way new plant varieties and plant products have traditionally been regulated. In addition to establishing the "substantial equivalence" of the CPB-resistant potatoes to the Russet Burbank potatoes, we will also provide data to confirm the environmental, human and animal safety of the two newly expressed *B.t.t.* and NPTII proteins.

SUBSTANTIAL EQUIVALENCE OF CPB-RESISTANT POTATOES TO RUSSET BURBANK POTATOES

Information concerning the source(s) of the genes introduced, the methods used to produce the genetically improved potato plants, the molecular characterization of DNA introduced into these plants, and characterization of the levels of the *B.t.t.* and NPTII proteins serves as a basis for characterizing the CPB-resistant plants. The important nutritional and natural products in potato are being determined for both the genetically improved and Russet Burbank potatoes to show that the composition of the potato tuber has not been altered during the transformation and regeneration processes. Levels of the macronutrients—protein, fat, carbohydrate, dietary fiber and ash—are being determined. The levels of the important vitamins—vitamin C, vitamin B_6, thiamine, niacin, folic acid and riboflavin—are being assessed. Levels of important minerals—calcium, copper, iron, iodine, magnesium, phosphorus, potassium, sodium and zinc—are being evaluated. The only class of important potato natural toxicants, the glycoalkaloids (solanine and chaconine), have been quantified and shown to be comparable in both the genetically improved and Russet Burbank potatoes. Raw potato tubers from both the CPB-resistant and Russet Burbank potatoes were fed, along with the regular rat diet, to rodents in a 28-day study to assess the palatability of these potatoes. No differences in consumption, growth rates, or observations during gross necropsy were observed during these studies. These data have confirmed that the CPB-resistant potatoes are comparable to the Russet Burbank potatoes in all aspects except for the ability to control the CPB pest due to the presence of minor amounts of *B.t.t.* and NPTII proteins.

...use of registered B.t.t. *products offers no significant risks to human health or nontarget organisms.*

SAFETY OF THE *Bt* PROTEIN

The *Bt* gene used to produce CPB-resistant potato plants, designated *cry Ilia,* (Hofte and Whiteley, 1989) was isolated from DNA from *Bacillus thuringienses* subsp. *tenebrionis* (McPherson et al., 1988). The *Bt* gene encodes an insecticidal protein produced by these bacteria during sporulation. The protein is selectively active against coleopteran larvae. Upon ingestion by susceptible species, feeding is inhibited with disruption of the gut epithelium and eventual death of the insect pest (Slaney et al., 1992). The amino acid sequence encoded by the gene inserted into potato plants produces a protein that is identical to that produced by *Bt* found in nature (McPherson et al., 1988).

Based on the available scientific data, EPA and other regulatory agencies worldwide have determined that use of registered *Bt* products offers no significant risks to human health or nontarget organisms (Shields and Wyman, 1984; EPA, 1991). In published reviews and the EPA documents, studies are referenced where large doses (5000 mg per kg) of *Bt* preparations were administered as single or multiple doses to different laboratory animals with no adverse effects. Avian and aquatic organisms have also been fed *Bt* preparations with no adverse effects. The preparations which were administered contained varying amounts of crystalline proteins from *Bt,* either as a mixture with spores or encapsulated in killed *Pseudomonas fluorescent* cells (EPA, 1991). While target insects are susceptible to oral doses of *Bt* proteins (pg per gram of body weight), there was no evidence of any toxic effects observed in nontarget laboratory mammals, including fish or birds given the equivalent of up to 10^6 pg of protein per gram of body weight. No deleterious effects were observed on nontarget insects at doses over 300- to 700—fold that needed to control the target insects (Macintosh et al., 1990). In addition to the predicted lack of receptors for the *Bt* protein, the absence of adverse effects in nontarget animals is further facilitated by the poor solubility and rapid degradation of *Bt* proteins in the acid environment of the digestive system.

To confirm the safety of the *Bt* protein expressed in CPB-resistant potatoes, we have obtained gram quantities of purified *Bt* protein by expressing this protein in microbial systems (*E. coli).* Limited expression of this protein prohibited the isolation of large quantities of this protein from the potato tubers or potato plant directly. Minor amounts of this protein, purified from the potato tuber and from microbes, were shown to be chemically and functionally equivalent. A series of commonly used analytical assays were used for this equivalence assessment. An acute gavage study was conducted in mice to confirm the safety of the *Bt* protein. A dose, following EPA guidelines, was used that was equivalent to over 2.5 million-fold safety factor based on the average consumption of potato and the level of the *Bt*

protein present in the tuber. No adverse effects were observed in terms of food consumption, weight gain, mortality or gross necropsy observations. Purified protein was also used in an *in vitro* digestion experiment which demonstrated that the *Bt* protein has an extremely short half-life (less than 20 seconds) under simulated gastric conditions (The United States Pharmacopeia, 1990). These studies confirm the safety of the *Bt* protein to humans and animals.

The specificity of the *Bt* protein to CPB was confirmed by host-range studies using the purified *Bt* protein. Five nontarget, beneficial insects (including honey bees, lacewings, ladybird beetles and a parasitic wasp) were also shown to be unaffected by doses of the purified *Bt* protein that are greater than 100 times the amount required to affect the CPB-target insect. These studies confirm the specificity of the *Bt* protein and the safety of this protein to nontarget insects. We are also performing studies to confirm the rapid degradation of the *Bt* protein in the soil after potato tubers are harvested.

SAFETY OF THE NPTII PROTEIN

The NPTII protein, and hence the gene encoding this protein, was used as a selectable marker to enable the identification of potato cells that contained the *B.t.t.* gene. The description and safety assessment of the NPTII protein has been discussed in detail in the FDA submission for an advisory opinion by Calgene (1990) and by recent articles by Flavell et al. (1992) and Nap et al. (1992). In addition to this information, we have performed similar equivalence acute gavage and digestive fate studies as described for the *Bt* protein above, with similar results. No adverse effects were observed in the acute gavage study with greater than a 5 million-fold safety compared to projected consumption, and the half-life of the NPTII protein in the simulated digestive fate study was also less than 20 seconds, confirming the mammalian safety of this protein.

SUMMARY

A variety of plant biotechnology products have been developed and extensively tested under field conditions. Appropriate regulatory oversight has evolved, and continues to evolve, through the various stages of the development of this technology. All three regulatory agencies in the U.S. issued either draft or final policies during 1992 that outline their policies on regulating genetically engineered plants. Two plant products are currently being reviewed under these policies and several more are expected to follow closely behind. One of these is the CPB-resistant potatoes that is described in this report. We have described the approaches that we are using to assess the food, feed and environmental safety of this product as an example. The

philosophy is based on the concept of "substantial equivalence." The new potato variety, derived using plant biotechnology, is established to be comparable to traditionally bred potatoes by comparing nutritional quality, level of important natural products, and agronomic and environmental performance. In addition, a direct safety assessment of the newly expressed proteins *(Bt* and NPTII, for the CPB-resistant potato) confirmed the safety of these components. These safety assessments have confirmed the food, feed and environmental safety of the CPB-resistant potatoes. Similar assessments are being performed for other plant varieties derived using plant biotechnology to assess the safety of these products.

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