DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

Date	October 28, 1991
From	Edwin J. Matthews, Ph.D. (HFF-156)
Subject	Analysis of the Major Plant Toxicants
To	Toxicology Section of the Biotechnology Working Group
Through	R. Daniel Benz, Ph.D. (HFF-156)

Background

Safety of Whole Food Plants Transformed by Technology Methods

E. An analysis of all major toxins that have been identified to occur naturally in the edible part of the plant that has been transformed, or any of its close relatives (*i.e.* same genus), should be done to show that no change has occurred as compared to the natural parent or relatives. The levels of the toxins should be compared using an appropriate statistical test if the normal range of any of the toxin levels is unknown. If the normal range of any toxin has been established and published, then the edible portion of the transformed plant should be within this range providing that all levels within this range have been shown to be safe.

F. Results from an appropriately designed 28-day feeding study in swine that show that the edible portion of the transformed plant causes no acute toxicity. Endpoints to be examined should include the usual general screen done for 28-day animal studies, These include, but are not limited to effects on: 1) weight gain, 2) organ function, 3) electrolyte levels, 4) metabolism and 5) gastrointestinal tract.

****

Analysis of Major Plant Toxins

A genetically engineered plant may contain an identical profile of expected plant toxicant levels (*i.e.* expected toxicants) as is normally found in a closely related, natural plant. However, genetically modified plants could also contain unexpected high concentrations of plant toxicants. The presence of high levels of toxicants in the bioengineered plant food could occur by two or more mechanisms. For example, normal levels of toxicants could be amplified through enhancement of toxicant gene transcription and translation. This might occur as a result of up-stream or down-stream promotion of gene activities in the modified plant DNA. In addition, plant toxicant genes which were normally inactive could be expressed in the modified plant gene as a result of insertion of the new genetic material (*i.e.* positional mutagenesis). Thus, the task of analysis of all major toxins in genetically engineered plant food includes the assessment of both expected toxicants and unexpected toxicants that could occur in the modified plant food. The unexpected toxicants could be closely related chemicals produced by common metabolic pathways in the same pant genus/species; however, unexpected toxicants could also be uniquely different chemicals that are usually expressed in unrelated plants.

18572

The task of assessing the presence or the absence of expected and unexpected plant toxicants in genetically modified plants and the control plant could be very difficult, because thousands of plant biochemicals have been shown to have toxic effects on animals and microorganisms. While all of these plant toxicants could conceivably be harmful to man by direct ingestion of plant food, or indirectly by ingestion of animal by-products that had consumed plants containing toxicants, the agency's primarily concern is for plant toxicants that could be present in common plant foods. Based upon our current knowledge of plant toxicants which groups all toxicants into one of four categories, we can rank-order the types of toxicants that we would most likely encounter in bioengineered plant foods:

Glycosides > Proteins and Protein By-Products > Alkaloids > Phenolics[1]

Thus, the most common toxicants in plant foods will be glycosides, and least likely toxicants will be phenolics. A more complete discussion of the four types of toxicant chemicals has been provided below.

Analyses for expected and unexpected plant toxicants can be achieved using either chemical/biochemical methods or toxicological bioassays. Chemical/biochemical methods have a high sensitivity for detecting the level of an individual toxicant, but their quantification of toxicant levels require extraction and purification of toxicants from plant cell homogenates. Unfortunately, purification procedures permit the detection of one toxicant while simultaneously destroying or excluding the detection of additional toxicants. Furthermore, current technology has purification procedures for only a fraction of the known plant toxicants.

Alternatively, toxicological bioassays could be used to simultaneously detect both expected and unexpected toxicants. Based upon our current knowledge, if elevated levels of toxicants occurred in genetically modified common plant food, these toxicants should elicit toxic effects in two types of assays, assuming the toxicants were present in the food product at concentrations several-fold higher than normally present in non-stressed, natural plants. First, a portion of the plant toxicants would be expected to be mutagenic in the *Salmonella typhimurium* reverse mutagenesis assay, including: alkaloids, certain glycoside functional groups (isothiocyanates and nitriles) and nitro alkanes. Second, rats and swine would be expected to be sensitive to the toxicological effects of most plant glycoside-, alkaloid-, protein-, and phenolic-toxicants. For some plant foods, the oral short-term study might have to be modified to include a different route of exposure, such as *gavage* for green, leafy foods and food extracts or *i.v.* to overcome the adsorption and solubility problems for saponins. Furthermore, the 28-day study should be optimized to detect hepatotoxicity, toxicity to certain sensitive organs (*i.e.* gastrointestinal tract, pancreas, spleen, and thyroid), anti-nutritive effects (*e.g.* growth retardation), and specific clinical chemistry tests (anemia, electrolytes *etc.*).

Plant Toxicant Addendum

Glycosides

Glycosides are plant toxicants that contain a sugar attached to one of several different functional groups. Important plant food glycoside toxicants include glycosinolates, cyanogenic glycosides, and saponins.

Glycosinolates [n > 100, *e.g.* glucoiberverin]: Toxicant structure includes a sugar linked to an isothiocyanate functional group [R-C(-S-glucose)=NOSO$_3$]. Glycosinolates (EDI = 46 mg) contribute to the flavor of cruciferous vegetables, relishes and condiments, and they are contained in plant foods (*i.e.* brussel sprouts, cabbage, cauliflower, horseradish, mustard, and radishes) and animal feedstuffs (*i.e.* cabbage, kale, rapeseed, swedes, and turnips). Toxic effects include: anti-nutritive (inhibition of growth), impairment of reproduction, blocking of thyroid function, and hepatotoxicity. Glycosinolate-induced toxicity is very common in certain animal feeds (*e.g.* rapeseed).