DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Memorandum

Date: January 31, 1992

From: Samuel I. Shibko (HFF-152) S. I. Shibko 1/31/92
Director, Division of Toxicological Review and Evaluation

Subject: Revision of Toxicology Section of the *Statement of Policy: Foods Derived from Genetically Modified Plants*

To: James H. Maryanski (HFF-300)
Biotechnology Coordinator, CFSAN

Background Information

The Toxicology Section of the DRAFT document entitled "*Statement of Policy: Foods Derived from Genetically Modified Foods*" was revised on 12/4/91, and these revisions were discussed by the Toxicology Section of the Biotechnology Working Group on 12/10/91. At this meeting it was decided that a number of footnotes would be inserted in the Toxicology Section of the document to provide supplemental information on our "scientific concerns". The attached memorandum summarizes our recommended changes.

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(3) Toxicology[1]

At this time it is unlikely that molecular and compositional analysis can reasonably detect or predict all possible changes in toxicant levels or the development of new toxic metabolites as a result of genetic modifications introduced by the new methods of biotechnology. FDA believes that, until sufficient data and experience with the new techniques of gene transfer have accumulated, the possibility of unexpected, accidental changes in genetically engineered plants justifies a limited traditional toxicological study with the edible part of the plant. This study would provide a basis for assuring the absence of any new highly toxic materials that are not present in the parental plant variety, and would establish the wholesomeness of the food for subsequent limited studies in humans. Additional assurance of safety would be provided by *in vitro* genotoxicity and digestion studies with the food or appropriate extract.

(a) Short-Term Feeding (Wholesomeness) Study

The feeding studies should follow currently available *Redbook* guidelines[2] for short-term studies, with food from a corresponding parental plant variety used as the control. Only one level of genetically modified product would have to be administered, with the ratio of genetically modified (and control) product to normal diet to be determined based on considerations of palatability and adequacy of nutrient

[1] This section is now called Section I.1 Unknown Toxicants and Anti-Nutrients

[2] Redbook (1982). In, Chapter III, Section B of Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition.

content. Because of the bulk occupied by food as a test material, a major problem exists in adequately exaggerating the dose fed to the experimental animals in order to maximize the sensitivity of the study for detecting potentially toxic ingredients. The use of freeze-dried food preparations could be helpful in this regard by eliminating much of the weight and volume (trapped or cellular water) of the test material. Volatiles removed during freeze drying could be trapped and the composition compared to volatiles obtained from freeze-drying food from the corresponding parental plant variety. Changes in composition of volatiles from genetically engineered plant varieties would need to be carefully evaluated to determine toxicological implications.

(b) Genotoxicity Test

Changes in levels of known toxicants[3] associated with particular plants as well as unknown toxicants originating from pleiotropic effects can be monitored in some cases by *in vitro* genotoxicity tests. As an example, the *Salmonella typhimurium* reverse mutation assay has been used to screen exotic plants for the presence of anti-mutagenic substances. Testing complex biological mixtures such as foods in the mutation assay must overcome the problem of interfering substances that cause a false positive reaction, such as histidine and its metabolic precursors.[4] In many cases, these false positives can be significantly reduced by utilizing solvent extracts of the food in the mutagenesis assay.[5] The *Redbook* describes FDA recommendations for conduct of mutagenicity studies. If food from genetically engineered plant varieties

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[3] The Agency's primary concern is for know plant toxicants that could be present in common plant foods (*i.e.* glycosides, proteins and protein by-products, alkaloids, and phenolics {refer to Cheeke, P.R. (1989) Toxicants of Plant Origin. Volumes I-IV. CRC Press}.

[4] Testing of complex mixtures of chemicals from parental and novel plant foods must overcome two problems: 1) the mixture must be devoid of histidine which would interfere with detection of *Salmonella typhimurium* histidine revertants and 2) the mixture must have a low concentration of simple carbon sources (*i.e.* sugars, amino acids *etc.*) that could be rapidly converted to histidine.

[5] The Agency recommends that the edible portion of the novel plant food (and the parental plant food as the control) be tested using a suitable extraction procedure that includes 1) preparation of a homogenous mixture of the food and 2) extraction of the food using appropriate non-polar and polar organic solvents [*e.g.* extraction with cyclohexane and dimethyl sulfoxide (DMSO)]. Many mutagenic plant toxicants (*i.e.* alkaloids and phenolics) could be extracted from the homogenate using a non-polar solvent (*e.g.* cyclohexane) and concentrated for testing. Prior to testing for mutagenic activity, the concentrated extract can be solubilized using a using a non-toxic, polar organic solvent (*e.g.* DMSO). Using this method most of the simple carbon sources in the food will remain in the aqueous phase, and thereby not interfere with the *Salmonella* mutation assay.

Nonetheless, the Agency is aware of two technical problems which could occur during the testing of extracts of plant food. First, it is possible that some non-mutagenic substances may be extracted by a non-polar organic solvent. These substances could act as simple carbon sources and cause false positive responses; thus, control experiments will have to be performed on the food extract to determine the highest concentrations of the extract that would not support the growth of *his⁻ Salmonella typhimurium* tester strains. Second, the combination of non-polar and polar organic solvents would not be expected to extract some plant toxicants --*e.g.* glycoside toxicants; thus, a portion of the plant homogenate may have to be separately treated. In the case of glucoside plant toxicants, the sugar portion of the glycoside may have to separated from the toxic functional groups of the glycoside to permit isolation and concentration of the toxic portion of the glycoside. The agency will consider alternative methods for testing plant foods for expected and unexpected mutagenic toxicants; however, we suggest that any protocols be reviewed prior to submission of petitions.

contains significantly higher levels of mutagenic components than the corresponding parental variety, this may suggest the need for additional toxicological studies.

(c) *In Vitro* Digestion Study.

We cannot assume that ~~all~~ gene products, particularly those encoded by genes from non-food sources, will be digestible. For example, there is evidence that certain types of proteins (*e.g.*, plant lectins and protein allergens) are resistant to digestion and can be absorbed in biologically active form. A properly designed *in vitro* digestion study can help to provide assurance that enzymes and other products of inserted genes are digested at rates comparable to other dietary proteins. If the genetically engineered protein appears to be resist ant to digestion, this could increase concern about immunologic or allergenic potential and may necessitate additional toxicological studies.

cc HFF-152 (Hattan)
HFF-156 (Benz, Matthews, Pribyl)
HFF-158 (Johnson)

E. Matthews: Biotech: wp50: 1/21/92: Revised (D.Benz) 1/22/92: Revised (S.Shibko) 1/23/92: