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TO: Members of Joint Standing Committee on Agriculture, Forestry and Conservation
FROM: Lebelle Hicks PhD, DABT
RE: Questions from the work session on LD 718 Thursday May 2nd, Survey of the literature

May 10, 2013

During and following the recent work session several questions were raised by members of the Joint Standing Committee on Agriculture Forestry and Conservation. The questions addressed the potential allergenicity of Bt-corn varieties, the question of purified cooking oils as potential sources for proteins which may be allergenic and the source of the herbicide resistance genes.

Corn Allergies

Corn may cause allergic reactions following exposure via skin (corn starch based powders in latex gloves), lungs (farmers and corn processors including bakers) and food. The scope of this review is food allergies from corn (or soybeans) with special emphasis on studies where corn food allergies were evaluated and genetically modified corn and appropriate varieties were compared.

Food allergies, including corn, are commonly brought on by IgE (immunoglobulin E). These reactions require an exposure followed by sensitization reactions. Reactions resulting from exposure to corn in sensitized individuals range from urticarial (hives, wheals and itching) to anaphylaxis (whole body histamine reaction, life threatening). The first report of corn induced anaphylaxis was reported in 1984 (Scibilia *et al.*, 2008).

Current research into corn allergies is attempting to identify the proteins involved in the allergic reaction, followed by determining if the allergenic proteins are amplified in the GMO corn varieties or if the proteins added during the genetic engineering process are allergenic in their own right.

People who have allergic reactions to corn products are identified by clinical tests. These tests include the skin prick test and the double blind placebo control dietary challenge. In test tubes blood from these positive individuals react with proteins obtained from the food causing the allergy and are considered IgE reactive.

Two types of corn preparations are used to determine IgE reactivity, extracts from the corn containing the mix of proteins present and purified proteins known to be present in the GMO corn varieties. The proteins may be purified from the corn extracts using biochemical techniques or they may be formed from DNA specific for the proteins.

If a protein extract or a purified protein binds to the IgE from the sera of sensitized patients, then that protein is recognized as an allergen. The tests which have been performed for the Bt-corn proteins are summarized in Appendix I. Table 1. In no instances were extracts from GMO corn or purified GMO

proteins found to specifically bind to IgE from the sera of either food allergic or specifically corn allergic individuals. *However, not all of the GMO proteins in currently available GMO corn have been tested in these assays.* When the blood was obtained from individuals allergic to corn there was binding to IgE, the patterns of binding for GMO was virtually identical to non-GMO corn (Batista *et al.*, 2005, Takagi *et al.*, 2006, Nakajima *et al.*, 2007, Nakajima *et al.*, 2010).

Contamination of Cooking oils with Proteins

One study was identified where purified cooking oils were evaluated for protein content. Proteins of a size which could be allergenic were identified from two samples of soybean oil, two samples of corn oil, one sample of peanut oil and one sample of sunflower oil. The only protein sample from the oils which contained allergens was peanut oil (Ramazzotti *et al.*, 2008).

Source of the Genes for Herbicide Resistance

The glyphosate (CAS# 1071-83-6) mode of action in plants is the inhibition of the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, an enzyme necessary for the formation of aromatic amino acids (Herbicide Resistance Action Committee (HRAC) and Weed Science Society of America (WSSA) 2013 at wssa.net/wp-content/uploads/HerbicideMOAClassification.pdf). The Roundup Ready gene codes for a glyphosate tolerant enzyme 5-enolpyruvylshikimate-3-phosphatase protein. The source of the Roundup Ready gene is *Agrobacterium* sp Strain CP4. Shorthand for this protein is CP4 EPSPS (Jennings *et al.*, 2013).

The Liberty Link, herbicide resistance gene codes for resistance to glufosinate herbicides. It was obtained from *Streptomyces hygroscopicus* (Thompson *et al.*, 1987, Sutton *et al.*, 2003). This bacterium is the source of multiple antibiotics and the naturally occurring herbicide, bialaphos (Thompson *et al.*, 1987), not registered in the US (NPSIRS 2013). Bialaphos (CAS# 35597-43-4) is a three amino acid peptide and contains the modified glutamic acid residue called phosphinothricin. The ammonium salt of phosphinothricin is also known as glufosinate-ammonium (CAS# 77182-82-2) and marketed as Liberty herbicide. Glufosinate-ammonium inhibits glutamine synthetase in plants and bacteria (Thompson *et al.*, 1987).

Appendix I Table1. GMO Corn Extracts and Proteins, Results of Testing for Allergenicity

| Test Sample (registration status in Maine) | Protein(s) | Function | Assay | n | Positive | Negative | Reference |
|---|--------------------------|--|-------------------------------|------------|-----------------|-----------------|-----------------------------|
| Bt11 Corn protein extract (currently registered) | Cry1Ab | Insect resistance lepidoptera ^(a) | Skin Prick Test | 27 | 0 | 27 | Batista <i>et al</i> , 2005 |
| | LL ^(b) | Herbicide resistance | | | | | |
| | PAT ^(c) | Antibiotic resistance marker | | | | | |
| Bt176 Corn-protein extract (never registered) | Cry1Ab | Insect resistance lepidoptera | | 27 | 0 | 27 | |
| | LL | Herbicide resistance | | | | | |
| | PAT | Antibiotic resistance marker | | | | | |
| T25 Corn- Protein Extract (not a pesticide) | LL | Herbicide resistance | | 50 | 0 | 50 | |
| | PAT | Antibiotic resistance marker | | | | | |
| MON810 Corn- protein extract (currently registered) | Cry1Ab | Insect resistance lepidoptera | 50 | 0 | 50 | | |
| Roundup Ready Soy-protein extract (not a pesticide) | CP4 EPSPS ^(c) | Herbicide resistance glyphosate | 27 | 0 | 27 | | |
| Purified proteins (found in GMO corn) | Cry1Ab | Insect resistance lepidoptera | Skin prick test | 77 | 0 | 77 | |
| | | | IgE ^(d) reactivity | 57 | 0 | 57 | |
| | PAT | Antibiotic resistance protein | Skin prick test | 77 | 0 | 57 | |
| | | | IgE reactivity | 57 | 0 | 57 | |
| | CP4 EPSPS | Herbicide resistance | Skin prick test | 27 | 0 | 27 | |
| | | | IgE reactivity | Not tested | | | |

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| Test Sample (registration status in Maine) | Protein(s) | Function | Assay | n | Positive | Negative | Reference |
|--|-------------------|-------------------------------|---------------------------------------|---|-----------------|-----------------|-------------------------------|
| Purified proteins (never registered) | Cry9C | Insect resistance lepidoptera | IgE reactivity | 140 | 0 | 140 | Takagi <i>et al.</i> , 2006 |
| Purified proteins (found in GMO corn registered) | PAT | Antibiotic resistance protein | IgE reactivity | 151 | 0 | 151 | |
| Purified proteins (found in GMO corn registered) | CP4 EPSPS | Herbicide Resistance | IgE reactivity | 132 | 0 | 132 | |
| MON810 Corn- protein extract (currently registered) | Cry1Ab | Insect resistance lepidoptera | Staining patterns on a separation gel | Virtually identical; GMO corn extract and isoline extract | | | Nakajima <i>et al.</i> , 2007 |
| Purified protein (found in corn registered in Maine) | Cry1Ab | Insect resistance lepidoptera | IgE reactivity | 44 | 0 | 44 | |
| MON863 Corn- protein extract (currently registered in Maine) | Cry3Bb1 | Insect resistance rootworms | Staining patterns on a separation gel | Virtually identical; GMO corn extract and isoline extract | | | Nakajima <i>et al.</i> , 2010 |
| Purified protein (found in corn registered in Maine) | Cry3Bb1 | Insect resistance rootworms | IgE reactivity | 55 | 0 | 55 | |

- a) Lepidoptera = caterpillars of corn borers and ear worms
- b) LL = Liberty Link resistance to glufosamine-ammonium
- c) PAT = marker protein for antibiotic resistance, phosphinothricin-N-acetyl transferase
- d) CP4 EPSPS = glyphosate tolerant enzyme 5-enolpyruvylshikimate-3-phosphatase found in Roundup Ready commodities
- e) Immunoglobulin E

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