

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

NEW PUBLICATIONS RELEVANT TO THE SAFETY OF GM FOODS

Issue

Members are invited to consider three recently published papers comparing the effects of feeding GM crops to rodents on various ultrastructural, histological, immunological and biochemical parameters, and to provide advice on what conclusions may be drawn from this work.

Background

1. Recently, three new GM feeding studies have been published in peer reviewed journals:
 - (i) A long term (24 month) proteomic and ultrastructural study on the effects of GM soya on liver ageing in rats¹
 - (ii) A three generation study on the effects of GM Bt corn on various biochemical and histopathological parameters in rats²
 - (iii) A study of the gut and peripheral immune response to GM maize MON810 in mice³

(i) GM Soya

2. This article is the latest in a series from this Italian research group reporting on mainly ultrastructural changes in tissues of mice fed GM soya. The Committee previously looked at several studies from this group in June 2006 (ACNFP78/7). A similar diet was used in the present study.
3. This particular study looks at liver as a suitable model for monitoring dietary effects due to its central role in the control of metabolism. Morphological and proteomic parameters were measured in older (24 months old) mice to investigate the possibility of an effect of GM soya on ageing.
4. One group of 10 female swiss mice were fed on a laboratory diet containing 14% glyphosate resistant GM soya that contained the bacterial CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene. A second group were fed the

¹ Malatesta et al.: 2008, *Histochem Cell Biol*, **130**, pp967-977 (**Annex A**).

² Kilic and Akay: 2008, *Food and Chemical Toxicology*, **46**, pp1164-1170 (**Annex B**).

³ Finamore et al.: 2008, *J Agric Food Chem*. DOI: 10.1021/jf802059w (**Annex C**).

same diet containing commercial non-GM soya. Both groups were fed from weaning to 24 months of age and the livers then processed for morphometric and immunoelectron microscopy and proteome analysis.

5. Proteome analysis: The proteome of the liver tissue in the two groups was compared by 2-dimensional gel electrophoresis in two independent assays with samples from all animals run in triplicate. Protein abundance was quantified on analytical gels loaded with 60µg protein using an automated system with statistical software. Once significant differences had been verified, preparative gels were run using 1mg of protein and bands of interest excised for peptide sequence analysis by mass spectrometry.
6. A total of 49 gel spots were found to be differentially expressed in GM fed mice, with 39 showing increased expression and 10 decreased. Of these it was possible to obtain sufficient peptide sequence to get matches with protein database entries for 20 gel spots (table 1). The database entries are variously associated with hepatocyte metabolism, stress response, calcium signalling and mitochondria and the authors interpret these differences as indicating a more marked expression of senescence markers in GM fed mice compared to controls.
7. Morphometry and immunoelectron microscopy: There were no differences in the localisation of the enzyme polymerase II and the RNA splicing factor SC-35 in GM and non-GM fed mice. However, some differences in certain morphological features of the nucleus and mitochondria are described, including cell and nuclear area and nuclear pore frequency (table 2); which the authors claim indicate a reduced metabolic rate in the liver of GM fed mice.

(ii) GM Bt corn

1. This study was carried out over 3 generations of rats and compared 3 diets. Group I rats were fed the 'standard' rat diet and groups II and III were fed the 'standard' rat diet supplemented with either 20% 'Bt corn' or its 'reference', respectively. The Bt corn and its reference were supplied by the Turkish Ministry of Agriculture and Rural Affairs, but apart from the statement that the reference was of the 'same genetic and breeding background but lack of the Bt gene', no further information on the origin of this material is given in the paper. A comparison of the composition of the Bt and the non-Bt (reference) corn is given in table 1.
9. In the study rats were fed the 3 diets through 3 generations and at 3.5 months the F3 rats were sacrificed and tissue and blood samples taken for endpoint analysis. Tissue samples from the stomach (corpus), small intestine (duodenum), liver and kidney were processed for histopathology and serum samples analysed for levels of urea, urea nitrogen, creatinine, uric acid, total protein, albumin and globulin.

Levels of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatine kinase (CK) and amylase were also determined.

10. Minimal histopathological changes were observed in the liver and kidney (tables 5 and 6; figure 2). Statistically significant differences ($p < 0.05$) were observed for creatinine, with levels increased in group II females, but decreased in group III males, while globulin and total protein levels were statistically different from controls in group II, but not group III (tables 7 and 8).

(iii) GM Maize MON810

11. This study examined the intestinal and peripheral immune response of mice to GM maize MON810. Weaning (21 days old) and older mice (18-19 months old) were used as they are described as being more susceptible to immunological insult due to a less efficient immune response.

12. The diets used in the study were formulated according to AIN-93G standard diets,⁴ and contained 50% MON810 or its parental control maize flour. A standard pellet diet, containing about 50% commercial non-transgenic maize, was also used. Weaning mice were fed for 30 and 90 days, with older mice fed for 90 days. The mice had free access to food and water and food intake and bodyweight were recorded every other day. No statistically significant differences were found in the food intakes or bodyweights of the mice used in the study.

13. At the end of the experimental periods (30 and 90 days) lymphocytes were isolated from the small intestine, spleen and blood of experimental animals and analysed to determine:

- The percentage of T and B cells and T cell subsets that can be referred to as the immunophenotype of lymphocyte populations (Fluorescence Activated Cell Sorting (FACS) analysis using a flow cytometer).
- The proliferative response of splenic lymphocytes in the presence of a polyclonal mitogen (ConA) or the purified transgenic protein, cry1Ab (³H-thymidine incorporation).
- The levels of serum cytokines (FACS analysis).

Lymphocyte populations

14. The immunophenotype of all lymphocyte populations fed the control maize was similar to that of pellet fed maize (data not shown), but several changes were

⁴ Reeves et al.: 1993, J Nutr. 123, pp1939-1951. <http://jn.nutrition.org/cgi/reprint/123/11/1939>

evident in mice fed MON810, depending on the tissue localisation and the age of the mice.

2. Weaning mice: in this group fed MON810 for 30 days the total number of T-cells increased in the intestine, whereas the total number of B-cells decreased in the intestine, but increased in the spleen and blood (Fig. 2). In addition, there were a number of changes to various T-cell subpopulations as indicated in Table 1 below (Figure 3).

Table 1: Changes in T-cell subpopulations in weaning mice fed MON810 for 30 days

T-cell subpopulation	Tissue localisation		
	Intestine	Spleen	Blood
CD4 ⁺	↓	↓	-
CD8 ⁺	-	↑	↑
TCRγδ	↑	↑	↑
TCRαβ	↓	-	↓

↑ = Increased ↓ = Decreased

16. Interestingly, in the group of weaning mice fed MON810 for 90 days the only statistically significant differences found were increased levels of B-cells in the intestine and blood.
3. Old mice: In this group of mice fed 90 days the percentage of B-cells was lower in the intestine and blood. The changes to various T-cell subpopulations in this group are indicated in Table 2 below (Figure 4).

Table 2: Changes in T-cell subpopulations in old mice fed MON810 for 90 days

T-cell subpopulation	Tissue localisation		
	Intestine	Spleen	Blood
CD4 ⁺	↓	-	↑
CD8 ⁺	-	-	↓
TCRγδ	↑	-	-
TCRαβ	-	-	-

↑ = Increased ↓ = Decreased

Proliferative response

4. No statistically significant differences were found in the proliferative response to ConA or Cry1Ab in any group of animals (Fig.1). Proliferation was low in the presence of Cry1Ab, suggesting the protein has low immunogenicity.

Levels of serum cytokines

5. Differences in cytokine levels are summarised in Table 3 below (Table 3).

Table 3: Changes in cytokine levels in weaning (W) and old (O) mice fed MON810

Group	Cytokine										
	IL4	IL5	IL6	IL10	IL13	IL12p	IL21	TNF α	IFN γ	MIP1 β	MC
W 30	-	-	↑	-	↑	↑	-	-	-	↑	-
W 90	-	-	-	-	-	-	-	-	-	↑	-
O 90	-	-	-	-	-	-	-	-	-	-	-

↑ = Increased ↓ = Decreased

20. The authors state that the significance of the data and whether the changes identified represent significant immune dysfunction remains to be established.

Committee action sought

21. The Committee is asked to consider these papers and to provide advice on what conclusions may be drawn from this work.

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Annexes attached:

Annex 1: Malatesta et al.: 2008, Histochem Cell Biol, 130, pp967-977

Annex 2: Kilic and Akay: 2008, Food and Chemical Toxicology, 46, pp1164-1170

Annex 3: Finamore et al.: 2008, J Agric Food Chem. DOI: 10.1021/jf802059w

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