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GM crops unlikely to remain contained, study shows

By Danielle Demetriou

14 October 2003

Genetically modified crops may not remain self-contained, according to a Government study published yesterday which revealed bees are able to carry pollen up to 16 miles away.

Stringent regulations are likely to be enforced on future GM crop trials, after the study discovered that pollen could travel eight times further than previously thought.

A second report found that after GM oilseed rape was grown, it would take 16 years before conventional crops could be grown without fear of breaching the maximum 1 per cent contamination threshold.

The findings, published by the Department for Environment, Food and Rural Affairs, coincided with protests from about 1,000 anti-GM campaigners who marched through central London.

The first study, conducted by the Scottish Crops Research Institute, raises question about previous research in Canada and the UK which claimed the pollen of GM crops could travel only 2.5 miles.

Using "bait plants", it found that contamination could take place as far afield as 16 miles from the original site. While long distance transfer was described as rare, and resulted in a dilution gene flow, the study revealed that bees were the key culprits. They carried the pollen back to the hive then swapping it with other pollen, resulting in the fertilisation of plants.

The second study revealed that unless weeds from GM crops were stringently controlled, there was a risk of contaminating conventional crops for up to 16 years. To ensure crops were not contaminated, weedkiller would have to be sprayed regularly.



Study heightens fears over GM superweeds

By Steve Connor Science Editor

10 October 2003

Cross-pollination between GM plants and their wild relatives is inevitable and could create hybrid superweeds resistant to the most powerful weedkillers, according to the first national study of how genes pass from crops to weeds.

Its findings will raise concerns about the impact of GM crops. Next week the results will be published of farm-scale trials which have studied the impact on the countryside of three types of crop.

The government-funded scientists said the latest findings "contrast" with previous assessments of gene flow between farm crops and weeds. They had suggested that the danger of hybridisation - where two types of plant cross-pollinate to create another, for example a superweed - was limited. Superweeds are considered to be a threat because, in some cases, they might absorb resistance to weedkillers from GM crops engineered to be herbicide-tolerant.

But the results of the research, which involved analysing satellite images of the British countryside and patrolling 180 miles of river banks, reveal that hybridisation is both more widespread and frequent than previously anticipated

Mike Wilkinson of Reading University, who led the study published today in the journal Science, said physical barriers such as isolation distances - buffer zones designed to stop pollen spreading from GM crops into the wild - would have only a limited impact on preventing hybridisation.

"This [study] shows that isolation distances will reduce hybrid numbers but not prevent hybridisation. It depends on what level of hybridisation you deem acceptable but if you want to absolutely prevent hybrids then isolation distances will not do so," Dr Wilkinson said. "Hybridisation is more or less inevitable in the UK context," he added.

The study concentrated on non-GM oilseed rape and assessed how easily it cross-bred with a near-relative in the wild called barge-man's cabbage, also known as wild turnip, which typically grows along river banks. Although the research was based on conventional oilseed rape, Dr Wilkinson said the conclusions applied to any flow of genes that could be expected from the GM varieties of oilseed rape that were undergoing farm-scale trials.

Top 10 genetically modified foods



Corn



Soy



Cottonseed



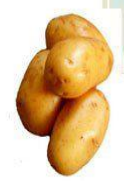
Papaya



Rice



Rapeseed
(Canola)



Potatoes



Tomatoes



Dairy products



Peas

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1999. Stanley W B Ewen, Arpad Pusztai,
THE LANCET

‘Effects of diets containing genetically
modified potatoes’ expressing Galanthus nivalis
lectin on rat small intestine’

Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine

Stanley W B Ewen, Arpad Pusztai

See Commentaries pages 1314, 1315

Diets containing genetically modified (GM) potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

Genetically modified (GM) plant products are becoming increasingly common in the human food-chain, yet in contrast to the general acceptance of the need for the biological testing of novel foods and feedstuffs, few studies have been carried out on the possible effects of GM products on the mammalian gut mucosa. GM potatoes expressing a snowdrop lectin (*Galanthus nivalis* agglutinin [GNA]) under the CaMV35S promoter have been developed to increase insect and nematode resistance.¹ GNA was selected for insertion into potatoes because the initial effect of this mannose-specific lectin on the rat small bowel has been shown to be minimal,² and because its binding to mannose present on the epithelial surface of rat jejunal villi is demonstrable only after feeding for 10 days. We compared the histological indices of the gut of rats fed potato diets containing GM potatoes, non-GM potatoes, or non-GM potatoes supplemented with GNA, to find out whether GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut.

ELISA analysis confirmed that the expression level of GNA in raw GM potatoes was 25.4 µg/g dry matter; this concentration was decreased to 4.9 µg/g after boiling for 1 h. Six rats were randomly allocated to each group, and were fed diets containing either raw or boiled GNA-GM potatoes, parent potatoes (Desiree), or parent-line potatoes supplemented with 25.4 µg/g GNA for 10 days. All potato diets were isocaloric and contained an average of 6% protein. Histological samples (stomach, jejunum, ileum, caecum, and colon) were taken 10 days after the start of feeding. The samples, each 2 cm in length, were opened along the antimesenteric border. The serosal surface was allowed to adhere to card for 3 min and was then fixed in 10% neutral buffered formalin for 18 h at 20°C. Paraffin sections (4 µm) were stained with haematoxylin and eosin, and mucosal thickness (stomach) or crypt length (jejunum, ileum, caecum, and colon) was measured by video image analysis. Intraepithelial lymphocytes are equally distributed in all parts of the small intestine, and are known to increase when non-specific intestinal damage occurs. Thus, to assess potential damage, intraepithelial lymphocytes were counted in eight jejunal villi from each of the six rats fed diets containing GNA-GM potatoes or parent potatoes, both raw and boiled. No such measurements were made for the group fed parent potatoes spiked with GNA because dietary GNA, like other lectins do not induce lymphocyte infiltration. GNA binding to the jejunum and ileum was measured by elution with 0.1 mol/L mannose, followed by ELISA.

	Mean (SD) crypt length (μm) and difference between treatments *						Statistical analysis (p)†			Interaction (p)†	
	Parent	Parent vs parent+GNA (p)	Parent+GNA	Parent+GNA vs GNA-GM (p)	GNA-GM	Parent vs GNA-GM (p)	Effect of GNA	Effect of cooking	Effect of trans-formation	GNA×cook	Trans×cook
Stomach											
Boiled	294 (46)	0.29	347 (42)	0.37	339 (36)	0.02	0.001	0.052	0.868	0.917	0.543
Raw	261 (32)	0.03	312 (32)	0.98	323 (54)	0.07					
p	0.18		0.94		0.35						
Jejunum											
Boiled	75 (19)	0.72	78 (17)	0.97	78 (12)	0.71	0.029	0.171	0.041	0.035	0.037
Raw	57 (8)	0.14	64 (11)	0.01	90 (20)	<0.01					
p	0.06		0.09		0.24						
Ileum											
Boiled	59 (8)	0.20	55 (7)	0.12	63 (13)	0.43	0.221	0.001	0.106	0.209	0.942
Raw	71 (9)	0.24	79 (13)	0.43	87 (25)	0.15					
p	0.02		<0.01		0.06						
Caecum											
Boiled	95 (19)	0.90	98 (21)	0.04	70 (15)	0.05	0.033	0.001	0.566	0.497	0.021
Raw	132 (19)	0.02	104 (17)	0.25	119 (25)	0.35					
p	<0.01		0.55		<0.01						
Colon											
Boiled	148 (15)	0.02	177 (24)	0.02	139 (24)	0.65	0.878	0.002	0.181	0.231	0.001
Raw	192 (34)	0.04	148 (25)	<0.01	215 (34)	0.28					
p	0.02		0.07		<0.01						

Data are the means of six animals calculated from five observations for each. GNA×cook=interaction between GNA and cooking; Trans×cook=interaction between transformation and cooking.

*By Student's *t* test. †By multivariate analysis with Tukey's test.

Table 1: Effect of raw and cooked parent, parent+GNA, and GNA+GM potatoes on histological indices of rat gut

Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean

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In the literature, the reports on the effects of a genetically modified (GM) diet are scanty and heterogeneous; in particular, no direct evidence has so far been reported that GM food may affect human or animal health.

Hepatocytes represent a suitable model for monitoring the effects of a GM diet, the liver potentially being a primary target. In a previous study, we demonstrated that some modifications occur in hepatocyte nuclei of mice fed on GM soybean. In order to elucidate whether such modifications can be reversed, in the present study, 3 months old mice fed on GM soybean since their weaning were submitted to a diet containing wild type soybean, for one month. In parallel, to investigate the influence of GM soybean on adult individuals, mice fed on wild type soybean were changed to a GM diet, for the same time. Using immunoelectron microscopy, we demonstrated that a one-month diet reversion can influence some nuclear features in adult mice, restoring typical characteristics of controls in GM-fed animals, and inducing in control mice modifications similar to those observed in animals fed on GM soybean from weaning. This suggests that the modifications related to GM soybean are potentially reversible, but also that some modifications are inducible in adult organisms in a short time.

Key words: cell nucleus, liver, genetically modified soybean, electron microscopy.

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In the literature, the reports on the effects of a genetically modified (GM) diet are scanty and heterogeneous (e.g., Schubbert *et al.*, 1998; Ewen and Pustzai, 1999; Chiter *et al.*, 2000; Edwards *et al.*, 2000); in particular, no direct evidence has so far been reported that GM food may affect human or animal health, and scarce are the studies on the effects of a diet containing significant amounts of GM plants (Malatesta *et al.*, 2002a,b, 2003; Vecchio *et al.*, 2004). These studies have been obviously focussed on organs and tissues which may be seen as potential targets (either directly or indirectly) of this diet. In this view, the liver is a primary site where the biotransformation of the products of digestion takes place through the degradation and/or detoxification of xenobiotic compounds received from the intestines or from the general circulation; in addition, the liver is involved in the synthesis of many plasmatic protein components and, more generally, in the overall metabolic control of the organism. Hepatocytes may, therefore, represent a suitable model for monitoring—at the cellular level—one of the targets of the diet. In a previous study (Malatesta *et al.*, 2002a), we demonstrated that some modifications occur in hepatocyte nuclei of mice fed on GM soybean: these changes are mainly related to the structural constituents involved in the transcription and splicing processes. In the present investigation, we aimed at elucidating whether such modifications can be reversed: to do this, mice fed on GM soybean from their weaning to the third month of age were submitted to a diet containing wild type soybean, for one month. In parallel, to investigate the influence of GM soybean on adult individuals, mice which had been fed on wild type soybean were administered for one month a GM diet. Morphometrical and immunocytochemical analyses have been carried out on hepatocyte nuclei at electron microscopy,

Ultrastructural analysis of testes from mice fed on genetically modified soybean.

Vecchio L, Cisterna B, Malatesta M, Martin TE, Bigglogera M.

We have considered the possible effects of a diet containing genetically modified (GM) soybean on mouse testis. This organ, in fact, is a well known bioindicator and it has already been utilized, for instance, to monitor pollution by heavy metals. In this preliminary study, we have focussed our attention on Sertoli cells, spermatogonia and spermatocytes by means of immunoelectron microscopy. Our results point out that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II is decreased in 2 and 5 month-old GM-fed mice, and is restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules is higher and the nuclear pore density lower. Moreover, we found enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells. A possible role played by traces of the herbicide to which the soybean is resistant is discussed.

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A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin *Galanthus nivalis* (GNA)

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Abstract

Genetically modified plants expressing insecticidal traits offer a new strategy for crop protection, but at the same time present a challenge in terms of food safety assessment. The present 90-day feeding study was designed to assess the safety of a rice variety expressing the snowdrop *Galanthus nivalis* lectin (GNA lectin), and forms part of a EU-funded project where the objective has been to develop and validate sensitive and specific methods to assess the safety of genetically modified foods. Male and female Wistar rats were given a purified diet containing either 60% genetically modified or parental rice for 90 days. This corresponds to a mean daily GNA lectin intake of approximately 58 and 67 mg/kg body weight for males and females, respectively. Prior to the animal study comprehensive analytical characterization of both rice materials was performed. The chemical analyses showed a number of statistically significant differences, with the majority being within the ranges reported in the literature. In the animal study a range of clinical, biological, immunological, microbiological and pathological parameters were examined. A number of significant differences were seen between groups fed the two diets, but none of them were considered to be adverse. In conclusion, the design of the present animal study did not enable us to conclude on the safety of the GM food. Additional group(s) where the expressed gene products have been spiked to the diet should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin *per se* or to secondary changes in the GM rice. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Genetically modified rice; Safety assessment; Animal study; GNA lectin; SAFOTEST

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. Worldwide it comprises approxi-

mately 23% of all calories consumed; in some countries more than 60% of the dietary calories are derived from this cereal (Khush, 2003).

The ever-increasing demands on yield are responsible for the development of many different high yielding varieties of rice. However, whilst the extensive cultivation of modern high yielding varieties has on the one hand resulted

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Table 14
Absolute and relative organ weights for rats fed on GNA rice diet and control rice diet

	Males		Females	
	GNA rice	Control	GNA rice	Control
<i>Absolute weight</i>				
Body weight	422 ± 33	417 ± 40	244 ± 22	257 ± 16
Adrenals	0.0576 ± 0.007	0.0600 ± 0.013	0.0759 ± 0.015*	0.0666 ± 0.003
Brain	2.02 ± 0.07	2.00 ± 0.09	1.85 ± 0.08	1.86 ± 0.10
Epididymis	1.176 ± 0.10	1.178 ± 0.16	—	—
Heart	1.14 ± 0.11	1.12 ± 0.09	0.805 ± 0.07	0.818 ± 0.08
Kidneys	2.49 ± 0.28	2.32 ± 0.25	1.52 ± 0.16	1.57 ± 0.16
Liver	12.7 ± 1.4	12.7 ± 1.7	7.54 ± 0.88	7.79 ± 0.72
Mesenteric ln.	0.109 ± 0.03	0.108 ± 0.03	0.092 ± 0.03*	0.131 ± 0.04
Ovaries	—	—	0.133 ± 0.03	0.122 ± 0.03
Pancreas	1.279 ± 0.43	1.284 ± 0.36	1.050 ± 0.23	1.047 ± 0.14
Small intestine	8.05 ± 1.03	8.07 ± 1.03	6.14 ± 0.84	5.91 ± 0.59
Spleen	0.775 ± 0.07	0.762 ± 0.10	0.555 ± 0.079	0.552 ± 0.051
Testes	3.91 ± 0.34	3.92 ± 0.34	—	—
Thymus	0.393 ± 0.08	0.385 ± 0.06	0.334 ± 0.078	0.365 ± 0.075
Uterus	—	—	0.500 ± 0.12	0.482 ± 0.14
Length small int.	112.8 ± 7.2	111.5 ± 8.0	100.8 ± 4.4	100.8 ± 3.1
<i>Relative weight</i>				
Adrenals	0.0137 ± 0.002	0.0145 ± 0.003	0.0313 ± 0.006*	0.0261 ± 0.003
Brain	0.481 ± 0.03	0.491 ± 0.04	0.777 ± 0.06	0.727 ± 0.04
Epididymis	0.280 ± 0.02	0.284 ± 0.05	—	—
Heart	0.279 ± 0.02	0.269 ± 0.02	0.331 ± 0.02	0.319 ± 0.03
Kidneys	0.569 ± 0.04	0.557 ± 0.03	0.623 ± 0.04	0.614 ± 0.06
Liver	3.00 ± 0.19	3.04 ± 0.22	3.09 ± 0.26	3.04 ± 0.30
Mesenteric ln.	0.0258 ± 0.006	0.0260 ± 0.009	0.0379 ± 0.011*	0.0509 ± 0.013
Ovaries	—	—	0.0547 ± 0.013	0.0478 ± 0.011
Pancreas	0.324 ± 0.10	0.310 ± 0.09	0.433 ± 0.09	0.410 ± 0.06
Small intestine	1.93 ± 0.28	1.91 ± 0.26	2.32 ± 0.34*	2.30 ± 0.20
Spleen	0.185 ± 0.02	0.183 ± 0.02	0.228 ± 0.027	0.215 ± 0.019
Testis	0.931 ± 0.09	0.945 ± 0.10	—	—
Thymus	0.093 ± 0.02	0.093 ± 0.01	0.137 ± 0.028	0.142 ± 0.024
Uterus	—	—	0.206 ± 0.05	0.189 ± 0.05
Length small int.	0.269 ± 0.018	0.259 ± 0.023	0.416 ± 0.036	0.395 ± 0.026

Relative organ weights expressed as g/100 g body weight. Small intestinal length and relative length is expressed in cm and cm/g body weight. Data is presented as group mean values ± SD.

* Statistically significant different from control group ($p < 0.05$).

† Statistically significant different from control group ($p < 0.01$).

(+14% and +20%, respectively). Furthermore, this group had a significantly reduced absolute (−30%) and relative (−26%) weight of the mesenteric lymph node compared with the female control group. No macroscopic or histological findings were observed.

4. Discussion

Even though the two rice varieties were grown under almost identical environmental conditions, chemical analyses revealed a number of statistically significant differences between transgenic and parental rice. Differences were detected for proximates (starch, fiber, sugars, protein, and ash), amino acids, minerals (copper, iron, manganese, phosphorus, and potassium) and vitamins (B_1 , B_6 , pantothenic acid, folic acid). Minor, but statistically significant differences were also observed for distributions of fatty acids and steryl ferulates. Additional field trials would be necessary to determine whether the differences detected

are due to the genetic modification or due to biological variability in the field.

The compositional data for transgenic rice were within the ranges reported in the literature except for protein, amino acids, ash and potassium. One has to keep in mind that existing food composition databases do not necessarily reflect the complete natural variation (Burlingame, 2004). In the present case, for example, protein contents exceed literature data for both the transgenic and the parental line. To assess the overall relevance of statistically significant differences in the light of natural variability within species, more comprehensive databases for the different plant species are necessary, which include samples with different genetic and/or environmental backgrounds. Recently, the International Life Science Institute released a comprehensive crop composition database that provides information on the natural variability in compositions of maize, soybean and cotton (Ridley et al., 2004). The intended extension of the database to other crops including rice will assist



Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

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ABSTRACT

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

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1. Introduction

There is an ongoing international debate as to the necessary length of mammalian toxicity studies in relation to the consumption of genetically modified (GM) plants including regular metabolic analyses (Séralini et al., 2011). Currently, no regulatory authority requests mandatory chronic animal feeding studies to be performed for edible GMOs and formulated pesticides. However, several studies consisting of 90 day rat feeding trials have been conducted by the biotech industry. These investigations mostly concern GM soy and maize that are rendered either herbi-

cide tolerant (to Roundup (R) in 80% of cases), or engineered to produce a modified *Bt* toxin insecticide, or both. As a result these GM crops contain new pesticide residues for which new maximal residual levels (MRL) have been established in some countries.

If the petitioners conclude in general that there is no major change in genetically modified organism (GMO) subchronic toxicity studies (Domingo and Giné Bordonaba, 2011; Hammond et al., 2004, 2006a,b), significant disturbances have been found and may be interpreted differently (Séralini et al., 2009; Spiroux de Vendômois et al., 2010). Detailed analyses have revealed alterations in kidney and liver functions that may be the signs of early chronic diet intoxication, possibly explained at least in part by pesticide residues in the GM feed (Séralini et al., 2007; Spiroux de Vendômois et al., 2009). Indeed, it has been demonstrated that R concentrations in the range of 10^3 times below the MRL induced endocrine disturbances in human cells (Gasnier et al., 2009) and toxic effects thereafter (Benachour and Seralini, 2009), including *in vivo* (Romano et al., 2012). After several months of consumption of an R-tolerant soy, the liver and pancreas of mice were affected, as highlighted by disturbances in sub-nuclear structure (Malatesta et al., 2008a, 2002a,b). Furthermore, this toxic effect was reproduced by the application of R herbicide directly to hepatocytes in culture (Malatesta et al., 2008b).

Abbreviations: GM, genetically modified; R, Roundup; MRL, maximal residual levels; GMO, genetically modified organism; OECD, Organization for Economic Co-operation and Development; GT, glutamyl-transferase; PCA, principal component analysis; PLS, partial least-squares; OPLS, orthogonal partial least-squares; NIPALS, Nonlinear Iterative Partial Least Squares; OPLS-DA, Orthogonal Partial Least Squares Discriminant Analysis; G, glycogen; L, lipid droplet; N, nucleus; R, rough endoplasmic reticulum (on microscopy pictures only); U, urinary; UEx, excreted in urine during 24 h; APPT, Activated Partial Thromboplastin Time; MCV, Mean Corpuscular Volume; PT, Prothrombin Time; RBC, Red Blood Cells; ALT, alanine aminotransferase; MCHC, Mean Corpuscular Hemoglobin Concentration; A/G, Albumin/Globulin ratio; WBC, White Blood Cells; AST, aspartate aminotransferase.

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Relevance of the scientific context

Criticisms

- No scientific context
- OECD guidelines not respected
- Protocol not adapted to tumor findings
- GLP violation because of amendments

Answers

This study addresses biological interpretations of early signs of toxicity in biochemistry after 90-day feeding trials (Spiroux de Vendomois et al., 2010)

No guidelines exist for GMO animal studies. Protocol based and adapted from OECD 408 and 452

This is not a carcinogenesis study, but a long term full toxicological study

Research protocols not adapted to GLP agreement because of amendments. The experiment was conducted under a GLP environment and conditions

Criticisms

- History of flaw by the authors which are not toxicologists. Previous studies of the group rejected
- Lack of signs in 90 days
- Not the first long term study

Answers

More than 26 international scientific peer reviewed papers by the team with the lead author on the topic in the last 5 years, and 11 in toxicological journals on the same period only in PubMed. One author, Malatesta, has also published on GMO/ pesticide health risks. None of the papers was considered as flawed by the scientific community. Regulatory agencies or Monsanto are not scientific peer reviewed journal systems

Statistical differences in biochemical parameters of liver and kidney function recognized by both industry and agencies

First chronic investigation with NK603 GM maize; others of two years in farm animals are not over the entire lifespan; the most detailed study for all agricultural GMOs and a formulated pesticide

Originality and limits of the experimental design

Criticisms

- Choice of the rat strain (sensitivity to mammary tumors and nephropathies in males)
- Number of rats per group

Answers

Necessity to have sensitive strains, recommended by the US National Toxicology Program (King-Herbert et al. 2010). Rats and mice have been preferred experimental models because of their susceptibility to tumor induction (OECD guidelines) Relevant comparisons to controls in this work

OECD 408 (90-day toxicity study) 10 animals per group OECD 452 (Chronic toxicity study) 20 animals per group but at least 10 animals per group are studied for hematological and clinical biochemical function

Focus on statistics

Criticisms

- Not enough statistical power
 - No Kaplan Meier's curves
 - Variability expected by chance
 - Only raw data in Figs. 1–3 and Table 2
- No means and standard deviations in Table 3

Answers

Statistics do not tell the truth, but may help in understanding results. The biological interpretations and the crossing of methodologies are the key. Enough and high statistical power for OPLS-DA, and this is why raw data only were presented in Figs. 1 and 2 and Table 2; no statistical power of Kaplan Meier's analyses for a conclusion demonstrating effects or no effects.

OPLS-DA is not a method to compare mean differences which were presented for understanding of biochemical measurements with highly discriminant parameters in bold. In accordance to guidelines and usual practices

Criticisms

- No incidence / severity Lack of histopathology data
- Endocrine disruption not sufficiently supported
- Wilm's tumors are only of genetic origin
- Feeding state explains glycogen in electron microscopy
- Pictures of control rat not shown

Answers

Taken into account as indicated in the legend of Table 2 which consists in a summary of the most relevant data

Convergent body of evidence stemming from mammary tumors, pituitary dysfunctions, histopathology and sex hormone biochemistry

Promotion by pesticide exposure is plausible and as evidenced by gestational exposure described in the literature

No difference in feed consumption; experience in the domain by M. Malatesta

Rats representative of each group shown, controls do not present tumors in majority during the experiment, pictures non necessary

Ethical issues and deontology

Criticisms

- Maize illicitly grown
- Animal welfare problems, a veterinarian would not authorize such tumor development
- Conflicts of interests
- Role of funders

Answers

Not at all; grown and imported with appropriate authorizations

The work follows GLP conditions. All rats followed by veterinarians on the site, applying the rules of the ethical committee and guidelines

No conflict for us. Conflicts of interests for companies testing their own products

See acknowledgments, funders identified. No interference in study or results; confidential up to the embargo