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FNDC Proposed Plan Change 18 & WDC Proposed Plan Change 131 – Genetically Modified Organisms

65 Papers (some with comments) presented as evidence by John Clarke in support of my 5 main points summarised below:

1. We must encourage research so that farming is based on sound science.
2. Unlike the present trend, GMO research must be thorough, not rushed, so that we do not expose our community and environment to needless risks.
3. To maximise safety, open air trials should only ever be carried out after long term safety and effectiveness trials have been completed and independently reviewed by council approved reviewers.
4. Those carrying out the research must be held fully financially accountable for any harm they cause to the community, the environment and livelihoods. To encourage council staff to ensure the financial fitness of those carrying out the research, perhaps add a clause stating the council will reimburse any shortfalls which the research entity fails to meet.
5. I contend that the gravest risk of all would come from allowing field trials to become a discretionary activity. Cutting corners simply because some individuals consider there might be a good opportunity or a net benefit to the community creates grave risks as demonstrated repeatedly in the accompany GMO related research.

The 65 papers are first presented as a summarised list first and then in detail.

Evidence to support the need for long term studies

1. Long term studies, which had not be done previously, showed that 3rd and 4th generation mice fed on corn experienced reduced fertility rates.
4. A 2 year study showed increased aging and reduced metabolism in mice fed GM soya. The comments highlight other studies showing up to 7 fold allergen increase in cooked GM soy.
13. Seralini, well respected and now vindicated of the slurs, calls for more long term studies because of the statistically significant explosion of chronic health effects observed in people consuming GM foods.
15. Bt corn showing toxic effects mostly on kidney and liver, but also in heart, adrenal glands and spleen. New Bt variations and combinations causing differing degrees of toxicity. Conclusion strongly advocates need for long term feeding studies.
20. This study shows GM soy causing changes in uterus and ovaries of rats and possibly being the reason for reproductive problems but requiring further study.
21. A call for long term studies to observe effects of stacked function GM crops (ones designed with multiple pesticides or herbicides) – necessary as tolerance rapidly gained from Bt crops and Roundup) on issues such as multi-generation infertility.

23. Seralini – another well respected, vindicated scientist - reviewed 19 studies which showed that even poorly designed and incorrectly reported results of short GM feed studies (using either GM corn or soy) show organ damage and he calls for longer and broader studies to assess the true harm.

Evidence to support need for thorough research to ensure safety and effectiveness

2. Bt corn has damaging effects on gut and immune system of mice. Italian government and Austrian government studies show that Bt corn harms immune system, deregulates genes and reduces fertility in mice.
5. There are now many studies, including one done by Seralini (who has now been vindicated) showing that Glyphosate kills human placenta cells (possibly explaining high rates of premature births and miscarriages in workers exposed to Roundup) and harms liver cells in rats, causes death of amphibians, frogs, and DNA damage in sea urchins. Roundup, with its added adjuvants, is shown to be more than twice as toxic. Use of Glyphosate and Roundup must be reviewed as approximately 75% of GM crops are Roundup Ready, and it does not break down rapidly as previously claimed by Monsanto.
8. Changes to the testes of mice fed on GM soy.
9. & 10. Transgenic plant DNA is being transferred from plants to pigs and sheep fed on Bt corn and canola, even though GM industry say this cannot happen.
11. Round up residues on GM crops cause cell death. This is particularly disturbing as Roundup is now sprayed on ripening wheat to speed up dessication for harvest.
12. A carefully researched review paper highlights numerous health risks observed with GM foods and urging long term studies to clarify safety and mechanisms at work.
14. Large concentrations of transgenic DNA found in animals and is not destoyed in the digestive process and is finding its way into the soil. The risks posed need urgent study.
17. Spontaneous abortions and malformations caused by Roundup residues.
18. Roundup causing death of non-target, aquatic communities such as tadpoles.
19. Bt toxin from crop residues finding their way into river systems and causing strong immune responses and cognitive impairment in non-target aquatic animals – despite Monsanto denials.
22. Study identifies likely horizontal gene transfer and need for further research to assess.
24. Traces of Bt toxin in mothers and in placenta of 86% of new born babies. How? Implication is that it comes from corn fed meat, can resist cooking, processing and digestion, still survive long term and may well accumulate in the body. The effects are not known and require further study.
25. Several studies showing glyphosate is an endocrine disruptor which may impair male fertility and calling for more studies to assess risk comprehensively.
26. The Bt gene expresses itself differently in different plants. When the Bt gene in tobacco is expressed strongly, it has shown to impair plant growth and development. When it is expressed weakly, the plant growth and development are not impaired. The implication is that the Bt gene, when working as it should, is damaging crops. This needs further study and is an indication of non equivalence with normal tobacco.
27. Bt proteins, especially in GM seed with stacked functions, are toxic to human cells – for safety this needs much further research.
28. Several papers showing that small concentrations of Roundup and Glyphosate are toxic to humans and cause DNA damage – more research needs to be done on the safety of using these in our community and on our food crops.
29. Germany, and 5 other EU countries have banned Monsanto's MON810 Bt corn because it performs poorly while also harming aquatic life.
30. GM soybeans are approximately 13% lower in phytoestrogens than nonGM varieties with wide variability compared to their natural counterparts. New GM crops need to be tested more fully for the ability to deliver what they state.

33. Roundup in very low concentrations reduces biodiversity of food organisms.
34. Glyphosate reduces biodiversity of beneficial gut bacteria in chickens as well as enterococcus bacteria. This may be a factor in the upsurge of clostridia botulinum mediated diseases.
36. New dsRNA GM plants and pesticides do yet not have guidelines for testing and consequently are not being assessed for safety. dsRNA has been shown to survive cooking, processing and digestion and in at least once case, in mice and in humans, has altered the ways that the genes are expressed. This is yet another example of cavalier, unsafe science.
37. Glyphosate and Roundup exposure can lead to permanent modifications to the immune function early in childhood, later leading to the development of allergies, asthma and autoimmune diseases.
38. Strong possibility that Roundup and Glyphosate residues in cattle feed are causing botulinum in cattle and related illnesses in poultry.
39. Various Bt (Cry) toxins caused red blood cells to rupture in mice. The implication is that the Bt Cry toxin could be toxic to humans -the severity depending on level and length of exposure.
40. Glyphosate is toxic to aquatic invertebrates but Roundup is even more harmful.
41. Pigs fed on stacked function maize and soy had more 4x the stomach inflammation and uterine problems of those fed a nonGM diet.
42. In Brazil soybean workers exposed to Glyphosate had genetic damage.
43. Glyphosate found in human urine across europe. Bearing in mind this is the most commone herbicide in the world, and locally, this requires more study to assess safety.
44. A high level of uptake of GM DNA fragments into the human blood stream is associated with inflammation.
45. Glyphosate accumulates in GM Roundup Ready soybeans. GM soybeans have less nutritive value than organic soy and are not substantially equivalent.
46. & 47. Harm to rats fed MON810 GM corn.
35. This study, based on evidence, shows that Bt pollen will kill butterflies and contradicts a previous study by Perry, based on supposition, that it would not.
48. Significant differences between nutritional values of GM and nonGM corn indicate that substantial equivalence does not hold.
49. & 50. Pesticides used with GM crops are much more toxic to human cells than stated by manufacturer.
51. Glyphosate induced DNA damage to liver and blood of mice.
52. Field evolved resistance by western corn rootworm to multiple Bt toxins in GM corn indicate that promises of resistance proved empty and expensive.
53. The combination of glyphosate (with its metal chelating properties) and hard water may be responsible for the epidemic of chronic kidney disease in Sri Lanka's paddy fields, since 1999 when glyphosate use became endemic. Similar glyphosate use and hard water have given rise to very similar outbreaks in India and Central America.
54. Glyphosate residues in animals and humans give cause for concern as chronically ill humans have higher levels of glyphosate in their urine.
55. Glyphosate induce DNA and chromosome damage and are probably carcinogenic.
56. GMO plants accumulate formaldehyde and are thus not substantialy equivalent.
57. GMO feed trials are mostly worthless because of GM contaminated feed.
58. Glyphosate is an endocrine disruptor even below the recommended threshold of use and has neurodevelopmental, reproductive and transgenerational effects.
59. Roundup effect on rats mirrors adrenal insufficiency in humans, a condition whose prevalence has been increasing rapidly in recent years. Adrenal insufficiency manifests as fatigue, anxiety, sweating, anorexia, shaking, nausea, heart palpitations and weight loss.
60. Effects of MON810 GM maize on water fleas harms them and shows that it is not substantially equivalent to normal corn.
61. There is strong evidence that glyphosate is contributing to the upsurge in cancers. The use of glyphosate closely correlates with an equivalent upsurge in cancers and related pathologies.

62. Glyphosate is commonly sold with adjuvants and these mixtures are proving at least twice as toxic as glyphosate and in much smaller concentrations.

Evidence of unprofessional and risky GM industry practices

3. Shonky practise was used to hide kidney and liver damage to mice fed Bt corn.
6. Shonky design claiming to be a long term study but it was only a single generation and aimed to hide pancreas and liver damage to lambs fed Bt corn. The comments by the independent reviewer are enlightening as to the tricks used to get the desired result of “no harm by GM”.
7. Despite identifiable effects attributable to GM soy, it claims no effects. Read the Jeffrey Smith comment after which highlights that the liver is the main detoxifier and the organ most likely to show adverse effects in a short term study, not the heart and kidney.
31. Birth defects and reproductive problems caused by Roundup made to disappear with support of unpublished studies.
32. Substantial equivalence argument shown to be ridiculous after scientists review how differently Bt crops control insects compared to natural crops.
63. The widespread introduction of GMOs in Canada is based more on hype than on a realistic assessment of actual costs and benefits. The only real "benefit|" coming from GMOs is more effective weed management, and even then, only in the early days, before super-weeds (and super-bugs) kick in.
64. GMOs are summarised as 20 years of hype for a worthless technology:
No matter how much money has been thrown at GMOs from government, private investors and corporations, no matter how much PR spin is put on the story, the fact remains that the promises that genetically modified food would revolutionise our world, feed the hungry, boost the yields and therefore the incomes of farmers, cure disease and more recently fight climate change remain spectacularly unfulfilled.

65 Papers in detail

1: GM maize reduces fertility in mice

Citation: Velimirov A, Binter C and Zentek J. (2008)
Biological effects of transgenic maize NK603xMON810 fed in long term reproduction studies in mice.
Report, Forschungsberichte der Sektion IV, Band 3. Institut für Ernährung, and Forschungsinttitut für biologischen Landbau, Vienna, Austria, November 2008.

Abstract

The aim of the study was to examine effects of the stacked GM crop NK603 x MON810 in different models of long term feeding studies. **So far no negative effects of GM corn varieties have been reported in peer-reviewed publications. But the hypothesis, that effects after long term exposure might become evident in multi-generation studies has rarely been investigated.** In this study three designs were used, including a multi-generation study (MGS), a reproductive assessment by continuous breeding (RACB) and a life- term feeding study (LTS), all performed with laboratory mice (strain OF1). The test diets differed only as to the inclusion of 33% NK603 x MON810 corn (GM) versus non- GM corn of a near isogenic line (ISO), both grown under identical conditions in Canada. The MGS also included one group with a non GM corn cultivated in Austria

(A REF). All corn varieties used in the MGS and LTS were harvested in 2005, the transgenic and isogenic corn for the RACB were harvested in Canada in 2007. No Austrian corn was used in this case. In the MGS microscopic and ultrastructural investigations were performed to detect changes at the organ and cell level. Gene expression patterns were compared by micro array expression profiles of the intestine as feed-animal interface and by real time PCR. The results of the MGS showed no statistically significant differences concerning parental body mass. The number of females without litters decreased with time in the GM and ISO group, especially in the 4th generation. In the group fed with A REF corn fewer females were without litters, and accordingly more pups were weaned. The production parameters average litter size and weight as well as number of weaned pups were in favour of the ISO group. These differences were also seen in the RACB design and were statistically significant in the 3rd and 4th litters. In addition, the inter-individual variability was higher in the GM group as compared to the other groups. The LTS showed no statistically significant differences in the survival of 3 groups of mice fed the different maize varieties. In the MGS the continuative investigations revealed differences between the GM and ISO groups. The comparison of organ weights did not indicate directed dietary effects, except for kidneys. The electron histological investigation of the cell nuclei revealed differences as to fibrillar centres, dense fibrillar components and the pore density in hepatocytes. This could point to an effect of the GM crop on metabolic parameters. Immunohistochemistry revealed no systematic differences in CD3, CD20 positive cells and macrophages in gut tissue. The microarrays showed differences between the feeding groups. When the data of both non-GM feeding groups from MGS were combined and compared to the GM feeding group, the discrimination became more evident. Analyses of metabolic pathways indicated, that the groups differed regarding some important pathways, including interleukin signalling pathway, cholesterol biosynthesis and protein metabolism. Summarizing the findings of this study it can be concluded, that multi-generation studies, especially based on the RACB design are well suited to reveal differences between feeds. **The RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions.** The RACB trial with its specific design with the repeated use of the parental generation is a demanding biological factor for the maternal organism. Compared to the findings in the RACB trials it can be assumed that the physiological stress was considerably lower in the MGS trial. **The trial design of using "new" parental generations instead of continuous breeding with the same generation has to be considered as being obviously less demanding. This might have masked the impact of dietary factors on reproductive performance.** However, this part of the experiment is valuable as such because it underlines the need for different experimental designs for the assessment of dietary effects that have an unknown impact on animals. The outcome of this study suggests that future studies on the safety of GM feed and food should include reproduction studies. Physiological and genomic traits and depending on the nature of the genetic modification proteomic and metabolomic methods might be taken into consideration as additional tools to the tests performed in this study.

2: GM Maize Disturbs Immune System of Young and Old Mice

Citation: Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A and Mengheri E. (2008).
Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice.
J Agric Food Chem, 16 November 2008

Abstract

This study evaluated the gut and peripheral immune response to genetically modified (GM) maize in mice in vulnerable conditions. Weaning and old mice were fed a diet containing MON810 or its parental control maize or a pellet diet containing a GM-free maize for 30 and 90 days. The immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of control maize fed mice was similar to that of pellet fed mice. As compared to control maize, MON810 maize induced alterations in the percentage of T and B cells and of CD4+, CD8+, γδT, and αβT subpopulations of weaning and old mice fed for 30 or 90 days, respectively, at the gut and peripheral sites. An increase of serum IL-6, IL-13, IL-12p70, and MIP-1β after MON810 feeding was also found. **These results suggest the importance of the gut and peripheral immune response to GM crop ingestion as well as the age of the consumer in the GMO safety evaluation.**

Comment

by Mae-Wan Ho ISIS Press Release 19/11/08 GM Maize Disturbs Immune System of Young and Old Mice
http://www.i-sis.org.uk/MON810gmMaizeMiceImmuneSystem.php New research add to the weight of damning evidence against the safety of GM food

The Italian government's National Institute of Research on Food and Nutrition has just published a report online in the Journal of Agricultural Food Chemistry documenting significant disturbances in the immune system of young and old mice that have been fed the GM maize MON 810 [1]. This follows hot on the heels of results released by the Austrian government showing that GM Maize Reduces Fertility & Deregulates Genes in Mice (SiS 41) [2]. These revelations confirm a string of previous findings on adverse health impacts of GM food and feed, leave us in little doubt that GM is Dangerous and Futile (SiS 40) [3]. Proponents should stop misleading the public that GM food and feed is safe.

The GM maize and the parental non-GM variety from which it was derived, were grown simultaneously in neighbouring fields in Landriano, Italy, from seeds provided by Seeds Emporda (Girona, Spain). The control maize flour from the non-GM parental strain had a low level of GMO contamination (0.29 percent by PCR test) but only the GM maize had the specific gene coding for the toxin Cry1Ab that acts as a pesticide.

The GM and non-GM maize were also analysed for levels of the fungal aflatoxins B1, B2, G1, G2, fumonisin B1 (FB1), deoxynivalenol (DON), ochratoxin, and zeralenon, that frequently contaminate maize grains. The values were below the maximum allowed in Europe, except for FB1 (1350 and 2450 mg/kg) and DON (1300 and 650 mg/kg) in GM and non-GM maize respectively.

The diets were formulated according to accepted standards and contained 50 percent MON810 or its parental control maize flour. A standard pellet diet containing about 50 percent of commercial non GM maize was also used, which did not contain CrylAb by PCR test.

Weaning mice, 21 days old, were fed with the diets for 30 and 90 days, and the old mice, 18 to 19 months, were fed for 90 days on the test diets; and male Balb/c mice were used in all the experiments.

There were no differences in the mean body weight or in food consumed between the GM-fed and control mice. These are the 'agronomic' characteristics typically measured in feeding tests, and all too often, the only characteristics measured.

The total number of white blood cells in the small intestine, spleen and blood were not different. However, there were significant differences in the percentages of T and B cells, and of CD4+, CD8+, gdT+, and mbT+ subpopulations in both weaning and old mice that were GM-fed for 30 and 90 days respectively compared with controls. These changes appeared in the gut, spleen and blood, and were accompanied by increase in blood cytokines IL-6, IL-13, IL-12p70, and MIP-1b, all involved in allergic and inflammatory responses. These changes were not detected in the mice fed the commercial non-GM pellet diet.

The greatest effects were the weaning mice fed for 30 days on GM maize, whereas those fed for 90 days only had increased B cells. In the old mice, the induced changes were similar to those found for the weaning mice fed for 30 days. These results show that very young and old mice are more susceptible to immunological insults. By the time the mice were 111 days old (90+21), a degree of tolerance had been established, so that the disturbances were reduced.

The immune disturbances are significant also in view of findings from another laboratory [4]; proteomic analysis identified 43 proteins that were up or down regulated in the MON 810 maize seeds compared with the parental strain, among them a 50 kda g-zein, a well-known allergenic protein [5], that was not present in the parental strain.

It is clear that genetic modification is inherently hazardous, as it invariably result in unpredictable and uncontrollable changes in the genome and the epigenome (pattern of gene expression) that impact on safety.

References

1. Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A and Mengheri E. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J Agric food Chem, **http:// pubs.ac.org**, 16 November 2008

2. Ho MW. GM maize reduces fertility and deregulates genes in mice. Science in Society 41 (to appear)

3. Ho MW. GM is dangerous and futile. Science in Society 40 (in press).

4. Zolla L, Rinalducci S, Antonioli P, Righetti PG. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a sresult of genetic modification. J. Proteome Res 2008, 7, 1850-61.

5. Pasini G, Simonato B, Curioni A, Vincenzi S, Cristaudo Q, Santucci B, Peruffo AD, Giannattasio M. IgE-mediated allergy to corn: a 50 kDa protein, belonging to the reduced soluble proteins, is a major allergen. Allergy 2002, 37, 98-106

3: Rats fed with MON810 corn suffered kidney and liver damage

Citation: Kilic, A. and M. T. Akay (2008).
A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation.
Food Chem. Toxicol. 46(3): 1164-1170.

Abstract

For the last ten years, in accordance with the increased use of genetically modified (GM) foods for human and livestocks, a large number of feeding studies have been carried out. However, the evidence is still far from proving whether the long-term consumption of GM foods posses a possible danger for human or animal health. Therefore, this study was designed to evaluate the effects of transgenic corn on the rats that were fed through three generations with either GM corn or its conventional counterpart. Tissue samples of stomach, duodenum, liver and kidney were obtained for histopathological examinations. The average diameter of glomeruli, thickness of renal cortex and glomerular volume were calculated and number of affected animals/number of examined animals for liver and kidney histopathology were determined. Amounts of urea, urea nitrogen, creatinine, uric acid, total protein, albumin and globulin were determined; enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, creatine kinase and amylase were measured in serum samples. No statistically significant differences were found in relative organ weights of rats within groups but there were some minimal histopathological changes in liver and kidney. Changes in creatinine, total protein and globulin levels were also determined in biochemical analysis.

Comment

Preliminary impressions from another worker in this field:

* This paper claims that the feeding programme resulted in no untoward effects on the animals fed with GM maize MON810. However, the quality of the research leaves much to be desired, and it is surprising that it got through the peer review process. The researchers presumably obtained samples of the Bt corn and the non- GM corn from the Turkish Government's own Agriculture Dept. No description is made of the non-GM variety, including its name. Monsanto has in the past, used varieties that have gone through the GM process but not taken up the transgene, as 'controls". No real mention is made of the fact that the GM corn must be MON810 corn and absolutely no mention is made of the fact that it is made by Monsanto. Why not? Researchers have to sign all sorts of agreements with Monsanto to get anywhere near being able to be given samples of GM grain, so we may assume that the researchers expected to find no difference between the GM and non-GM grain and were embarrassed when they did. Specific defects in the research: * **They used fairly old rats, not just post-weaned, so the animals were less likely to show adverse effects. * They used corn at only 20% of the diet. Even Monsanto has used 33%. They have two control groups and many of the analyses are actually between the two control groups. * They have used SE as a measure of variance instead of SD. This tends to reduce the apparent variance and makes the data look tighter than it is. * They have small sample sizes - blood biochemistry by gender is only n=5, so it is hard to find statistical significance. Of course, they found little statistical significance there! * Of real concern to me are the results in table 4. It indicates that there is something very weird happening in one or both of the control groups. The liver weight of group I is 16.4% of the body weight of the rats while the liver weight in Group II (the 20% corn control group) is only 2.7% of the body weight. Either the livers in group I are swollen or the livers in group II have shrunk shockingly. * In table 3, the group I control has less F3 offspring. I didn't think that feeding corn (whether GM or not) increased the number of offspring compared to feeding ordinary rat chow! * They clearly have found problems in the liver and kidneys of animals fed the GM corn (Table 5)** * They have concluded, despite the evidence that feeding the GM corn did cause harm, that it "did not cause severe health concerns on rats". That is a matter of opinion.....? * No mention is made of where funding for the research came from.

Comment from another peer reviewer:

The Turkish paper appears to me to have been remarkably badly conducted. Apart from the almost unreadable English, I'd point out:

1. This information appears to have been drawn entirely from 6 females per treatment, and their subsequent offspring. There is no replication in time - the trial was not repeated with a different starting group of females.

2. We are not told the range in starting weight of the starting group of 18 females. Because large magnitude arithmetic differences in subsequent performance are not found to be statistically different, one may wonder at the possible

heterogeneity in weight of the starting females. This is one of several key omissions. Likewise, F3 rats were sacrified at 3.5 months of age - why this age, and would results have differed from earlier, later, or sequential sampling?

3. The comparability of the 3 diets is quite unclear. We are told nothing about the Standard diet - does it contain any corn at all? This becomes important when comparing performance on the 3 diets and the Group I animals appear to be responding v. differently from II and III, which are more similar. We are given composition of the experimental diets (Table 1) but not the Standard - why?

4. There does not appear to have been any effort to standardize the 3 diets in terms of energy or protein, or to statistically compare the composition of the Bt and non-Bt corn. Table 1 is labelled 'composition of experimental diets', but it appears to have been composition of the corn part of the diet only. If the diets are not themselves standardized then, this introduces a source of ambiguity into interpretation of differences, or lack of differences.

5. Animals received set amounts of feed - not free choice. Is there a reason for this? Does limited feed hinder expression of palatability or other differences among rations?

6. I'm unclear on the mating design. Presumably, the original females were not bred repeatedly, but their offspring were bred to contribute the F2, and then their offspring to make the F3?

7. The number of animals in the F2 and F3 generations appear to differ substantially among trts, particularly in the F2 and F3 generations, and particularly between the Standard and corn-based rations (50% more individuals than on the standard ration). What does it mean that different numbers of individuals made it into the F2 and F3 - depending on the ration - and why were all of them analyzed for body weight etc. rather than just 6 per generation? In Table 4, groups varying in size from 5 to 14 females and from 14 to 22 males were compared statistically, they claim to have tested for homogeneity of variance but this seems implausible, given numerous anomalous results. For example, female liver weights where 6.7 does not differ from 25.1, but both of them differ from 33? Likewise Table 5 where arithmetically large differences are ns. For females in particular, all parameters shown in Table 5 appear to distinguish III from either I or II - but the interpretation ("damages were minor but not critical to animal health") minimizes the meaning of these differences - or gender specific differences. Comparing grossly unequal sample sizes appears to have contributed to the finding of no significant difference.

8. Fig 1 doesn't indicate if it is male or female animals, or which generation - but presumably F3 as this was the only one subjected to examination?

9. Why limit the corn to 20%? Are rats not meant to eat corn? What is the safety margin of 33% corn?

4: GM soy reduces metabolic rate, speeds up ageing process

Citation: Manuela Malatesta, Federica Boraldi, Giulia Annovi, Beatrice Baldelli, Serafina Battistelli, Marco Biggiogera, Daniela Quaglino. (2008)
A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing.
Histochem Cell Biol. 2008 Jul 22; : 18648843 (P,S,G,E,B,D)

Abstract

Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. Although no direct evidence has been reported so far that genetically modified (GM) food may affect health, previous studies on hepatocytes from young female mice fed on GM soybean demonstrated nuclear modifications involving transcription and splicing pathways. In this study, the effects of this diet were studied on liver of old female mice in order to elucidate possible interference with ageing. The morpho-functional characteristics of the liver of 24-month-old mice, fed from weaning on control or GM soybean, were investigated by combining a proteomic approach with ultrastructural, morphometrical and immunoelectron microscopical analyses. Several proteins belonging to hepatocyte metabolism, stress response, calcium signalling and mitochondria were differentially expressed in GM-fed mice, indicating a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. **This study demonstrates that GM soybean intake can influence some liver features during ageing and, although the mechanisms remain unknown, underlines the importance to investigate the long-term consequences of GM-diets** and the potential synergistic effects with ageing, xenobiotics and/or stress conditions.

Comment

GM Soy, allergies and other effects (Jeffrey Smith, cited with full references on Celsias web site)
http://www.celsias.com/article/genetically-modified-foods-unsafe- evidence-that-li/
GM Soy and Allergies - Soy allergies jumped 50% in the U.K. just after GM soy was introduced. If GM soy was the cause, it may be due to several things. The GM protein that makes Roundup Ready Soy resistant to the herbicide does not have a history of safe use in humans and may be an allergen. In fact, sections of its amino acid sequence are identical to known allergens. A portion of the transgene from ingested GM soybeans, along with the promoter that switches it on, transfers into human gut bacteria during ingestion. The fact that the transformed bacteria survives applications of Roundup's active ingredient, glyphosate, suggests that the transgene continues to produce the Roundup Ready protein. If true, then long after people stop eating GM soy they may be constantly exposed to its potentially allergenic protein, which is being created within their gut. (This protein may be made more allergenic due to misfolding, attached molecular chains, or rearrangement of unstable transgenes, but there is insufficient data to support or rule out these possibilities.)
Studies suggest that the GM transformation process may have increased natural allergens in soybeans. The level of one known allergen, trypsin inhibitor, was 27% higher in raw GM soy varieties. More worrisome, it was as much as sevenfold higher in cooked GM soy compared to cooked non-GM soy. Not only is this higher amount potentially harmful, the finding also suggests that the trypsin inhibitor in GM soy might be more heat stable and, therefore, even more allergenic than the natural variety. It is also possible that changes in GM soy DNA may produce new allergens. Although there has never been an exhaustive analysis of the proteins or natural products in GM soy, unpredicted changes in the DNA were discovered. A mutated section of soy DNA was found near the transgene, which may contribute to some unpredicted effects. Moreover, between this scrambled DNA and the transgene is an extra transgene fragment, not discovered until years after soy was on the market. The RNA produced is completely unexpected. It combines material from all three sections: the full- length transgene, the transgene fragment, and the mutated DNA sequence. This RNA is then further processed into four different variations, which might lead to the production of some unknown allergen.
Another study verified that GM soybeans contain an IgE-binding allergenic protein not found in nonGM soy controls, and that one of eight subjects who showed a skin-prick allergic reaction to GM soy had no reaction to nonGM soy. Although the sample size is small, the implication that certain people react only to GM soy is huge. The increased residue of Roundup herbicide in GM soy might contribute to increased allergies. In fact, the symptoms identified in the U.K. soy allergy study are among those related to glyphosate exposure.
The allergy study identified irritable bowel syndrome, digestion problems, chronic fatigue, headaches, lethargy, and skin complaints including acne and eczema. Symptoms of glyphosate exposure include nausea, headaches, lethargy, skin rashes, and burning or itchy skin. It is also possible that glyphosate's breakdown product, AMPA, which accumulates in GM soybeans, might contribute to allergies. Finally, mice fed GM soy had reduced levels of pancreatic enzymes. When protein-digesting enzymes are suppressed, proteins may last longer in the gut, allowing more time for an allergic reaction to take place. Any reduction in protein digestion could therefore promote allergic reactions to a wide range of proteins, not just to the GM soy.
Animal feeding studies with Roundup Ready soy indicated toxic livers, altered sperm cells, significant changes in embryo development, and a fivefold increase in infant mortality, among others.
Other references:
Malatesta, M. et al. Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean. J. of Anat. 201, no. 5 (Nov 2002): 409.
Malatesta, M. et al. Fine structural analyses of pancreatic acinar cell nuclei from mice fed on GM soybean. Eur. J. Histochem. 47 (2003): 385–388.
Malatesta, M. et al. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct. Funct. 27 (2002): 173–180.
Vecchio, L. et al. Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur. J. of Histochem. 48, no. 4 (Oct– Dec 2004):449–454.
Oliveri et al. Temporary depression of transcription in mouse pre- implantion embryos from mice fed on genetically modified soybean. (48th Symposium of the Society for Histochemistry, Lake Maggiore, Italy, Sept 7–10, 2006).
Ermakova, I. Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies. Ecosinform 1 (2006): 4–9.

5: Roundup residues interfere with multiple metabolic pathways

Citation: M Malatesta, F Perdoni, G Santin, S Battistelli, S Muller, M Biggiogera (2008).
Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function.
Toxicol In Vitro. 2008 Sep 18; : 18835430 (P,S,G,E,B,D)

Abstract

Previous studies on mice fed genetically modified (GM) soybean demonstrated modifications of the mitochondrial functions and of the transcription/splicing pathways in hepatocytes. The cause(s) of these alterations could not be conclusively established but, since the GM soybean used is tolerant to glyphosate and was treated with the glyphosate-containing herbicide Roundup(trade mark), the possibility exists that the effects observed may be due to herbicide residues. In order to verify this hypothesis, we treated HTC cells with 1-10mM Roundup and analysed cellular features by flow cytometry, fluorescence and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, as well as most of the cytoplasmic organelles. However, in HTC-treated cells, lysosome density increased and mitochondrial membranes modified indicating a decline in the respiratory activity. Moreover, nuclei underwent morpho-functional modifications suggestive of a decreased transcriptional/splicing activity. Although we cannot exclude that other factors than the presence of the herbicide residues could be responsible for the cellular modifications described in GM-fed mice, the concordance of the effects induced by low concentrations of Roundup on HTC cells suggests that the presence of Roundup residues could be one of the factors interfering with multiple metabolic pathways.

Comment

This paper follows others examining the effects of GM soy on animals in feeding experiments, and specifically looks into the "indirect" effects of the use of Roundup. Since all RR varieties have to be used with Roundup herbicide, the indirect effects associated with its use as prescribed by the manufacturer MUST be considered by the regulators, as required by law. The EU Directives relating to the planting and use of GM crops are quite clear on this point. The following is one of the "Top 25 Censored Stories for 2007" ---

New Evidence Establishes Dangers of Roundup

Sources:

Third World Resurgence, No. 176, April 2005 Title: “New Evidence of Dangers of Roundup Weedkiller” Author: Chee Yoke Heong http://www.projectcensored.org/top-stories/articles/13-new-evidence- establishes-dangers-of-roundup/

New studies from both sides of the Atlantic reveal that Roundup, the most widely used weedkiller in the world, poses serious human health threats. More than 75 percent of genetically modified (GM) crops are engineered to tolerate the absorption of Roundup—it eliminates all plants that are not GM. Monsanto Inc., the major engineer of GM crops, is also the producer of Roundup. Thus, while Roundup was formulated as a weapon against weeds, it has become a prevalent ingredient in most of our food crops.

Three recent studies show that Roundup, which is used by farmers and home gardeners, is not the safe product we have been led to trust.

A group of scientists led by biochemist Professor Gilles-Eric Seralini from the University of Caen in France found that human placental cells are very sensitive to Roundup at concentrations lower than those currently used in agricultural application.

An epidemiological study of Ontario farming populations showed that exposure to glyphosate, the key ingredient in Roundup, nearly doubled the risk of late miscarriages. **Seralini and his team decided to research the effects of the herbicide on human placenta cells. Their study confirmed the toxicity of glyphosate, as after eighteen hours of exposure at low concentrations, large proportions of human placenta began to die.** Seralini suggests that this may explain the high levels of premature births and miscarriages observed among female farmers using glyphosate.

Seralini’s team further compared the toxic effects of the Roundup formula (the most common commercial formulation of glyphosate and chemical additives) to the isolated active ingredient, glyphosate. They found that the toxic effect increases in the presence of Roundup ‘adjuvants’ or additives. These additives thus have a facilitating role, rendering Roundup twice as toxic as its isolated active ingredient, glyphosate.

Another study, released in April 2005 by the University of Pittsburgh, suggests that Roundup is a danger to other life-forms and non-target organisms. Biologist Rick Relyea found that Roundup is extremely lethal to amphibians. In what is considered one of the most extensive studies on the effects of pesticides on nontarget organisms in a natural setting, Relyea found that

Roundup caused a 70 percent decline in amphibian biodiversity and an 86 percent decline in the total mass of tadpoles. Leopard frog tadpoles and gray tree frog tadpoles were nearly eliminated.

In 2002, a scientific team led by Robert Belle of the National Center for Scientific Research (CNRS) biological station in Roscoff, France showed that Roundup activates one of the key stages of cellular division that can potentially lead to cancer. Belle and his team have been studying the impact of glyphosate formulations on sea urchin cells for several years. The team has recently demonstrated in Toxicological Science (December 2004) that a "control point" for DNA damage was affected by Roundup, while glyphosate alone had no effect. "We have shown that it's a definite risk factor, but we have not evaluated the number of cancers potentially induced, nor the time frame within which they would declare themselves," Belle acknowledges. There is, indeed, direct evidence that glyphosate inhibits an important process called RNA transcription in animals, at a concentration well below the level that is recommended for commercial spray application.

There is also new research that shows that brief exposure to commercial glyphosate causes liver damage in rats, as indicated by the leakage of intracellular liver enzymes. The research indicates that glyphosate and its surfactant in Roundup were found to act in synergy to increase damage to the liver.

UPDATE BY CHEE YOKE HEONG

Roundup Ready weedkiller is one of the most widely used weedkillers in the world for crops and backyard gardens. Roundup, with its active ingredient glyphosate, has long been promoted as safe for humans and the environment while effective in killing weeds. It is therefore significant when recent studies show that Roundup is not as safe as its promoters claim.

This has major consequences as the bulk of commercially planted genetically modified crops are designed to tolerate glyphosate (and especially Roundup), and **independent field data already shows a trend of increasing use of the herbicide**. This goes against industry claims that herbicide use will drop and that these plants will thus be more "environment-friendly." Now it has been found that there are serious health effects, too. My story therefore aimed to highlight these new findings and their implications to health and the environment.

Not surprisingly, Monsanto came out refuting some of the findings of the studies mentioned in the article. What ensued was an open exchange between Dr. Rick Relyea and Monsanto, whereby the former stood his grounds. Otherwise, to my knowledge, no studies have since emerged on Roundup.

For more information look to the following sources: Professor Gilles-Eric, criigen@ibfa.unicaen.fr Biosafety Information Center, http://www.biosafety-info.net Institute of Science in Society, http://www.i-sis.org.uk

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Reference: Sophie Richard, Safa Moslemi, Herbert Sipahutar, Nora Benachour, and Gilles-Eric Seralini (2005) Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase. Environmental Health Perspectives Volume 113, Number 6, June 2005

Abstract Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and **glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems**, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. **We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.**

N. Benachour, H. Sipahutar, S. Moslemi, C. Gasnier, C. Travert and G. E. Seralini (2007). Time- and Dose-Dependent Effects of Roundup on Human Embryonic and Placental Cells. Archives of Environmental Contamination and Toxicology LLC 2007, 10.1007/s00244-006-0154-8 http://www.springerlink.com/content/d13171q7k863l446/fulltext.html

Abstract

Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD50) of Roundup with embryonic cells is 0.3% within 1 h in serum- free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM

glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2–4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1–2%, i.e., with 21–42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short- term basis (1 h), reveal the xenobiotic impacts that are visible 1–2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01% (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature- dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. **Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.**

6: Lambs fed on Bt176 maize had pancreas and liver damage

Citation: Trabalza-Marinucci M, Brandi G, Rondini C, Avellini L, Giammarini C, Costarelli S, Acuti G, Orlandi C, Filippini G, Chiaradia E, Malatesta M, Crotti S, Antonini C, Amagliani G, Manuali E, Mastrogiacomo AR, Moscati L, Haouet MN, Gaiti A, Magnani M (2008).
A three year longitudinal study on the eVects of a diet containing genetically modiWed Bt176 maize on the health status and performance on sheep.
Livestock Sci 113:178–190

Abstract
This study shows that a diet including insect-resistant Bt176 maize, fed to 53 ewes and their progeny for 3 years, did not have adverse effects on their health or performance and that no horizontal gene transfer to ruminal microorganisms or animal tissues was detected. No differences were observed regarding performance, reproductive traits, haematological parameters, antioxidant defences, lymphocyte proliferative capacity, phagocytosis and intracellular killing of macrophages, and ruminal microbial population characteristics between control and genetically modified (GM) maize-fed animals. Immune response to Salmonella abortus ovis vaccination was more efficient in GM maize fed sheep. No modifications of histological features of tissues were found; however, cytochemical analyses of ruminal epithelium by Ki67 staining provided evidence of proliferative activation of basal cells in all GM maize-fed ewes. Preliminary electron microscopy analyses of the liver and pancreas revealed smaller cell nuclei containing increased amounts of heterochromatin and perichromatin granules in GM maize-fed lambs. Meat protein content and water loss by cooking were slightly affected by the dietary treatment. No transgenic DNA was detected in tissues, blood, and ruminal fluid or ruminal bacteria. Longitudinal studies should be included in evaluation of food safety whenever possible and sheep may be a useful animal model for toxicological assessment.

Comment
This Italian feeding trial, in which some sheep were fed the controversial maize line Bt176 over a period of 3 years, appears to have been specifically designed and conducted in order to confirm the "no health risk" hypothesis. In 2002-2005 scientists at the University of Perugia, with funding from the Italian Ministry of Health, conducted a "longitudinal" study involving 106 breeding ewes, of which one group was fed on a diet containing Bt176 maize. The article has crucial information missing, and appears to be systematically biased. The following points emerged in a reading of the text:
1. The preamble states: "There is a lack of long-term studies, performed on a high number of animals over several generations, aimed at evaluating the effects of genetically modified (GM) feeds on livestock species." It is implied that this paper will address this issue, but it does nothing of the sort. Only one generation of ewes was fed on the GM maize, and their lambs were apparently fed no GM at all, in spite of what the Abstract claims. The actual size of the test group is not given anywhere in the paper.
2. The quantity of GM feed fed to the test group of ewes was very small, at 5.6% of their diet, except during lactation, when the proportion increased to 19.4%. Overall, the test group was fed 17 times more hay than GM maize.

3. The researchers found important differences in 4 out of 30 investigated blood parameters (ie. 13.33 percent of parameters). Some items were very close to statistical significance (e.g. ALT, P=0.053; Platelet, P=0.060; WBC, P=0.056) and yet these results were effectively disregarded or dismissed.
4. The histological differences picked up between the control group of ewes and the test (GM) group are said to be 'preliminary' -- we suspect because the scientists on this part of the project were not allowed to perform a detailed or extended study on the histological samples.
5. The PCR cited for Bt176 is not reliable, as it does not give the GM maize used, only the genes in plasmids. This is a common method used by the GM industry in the falsification of results.
6. It is also useful to recall the following: Bt 176 was one of the GM inserts analysed by both French and Belgian scientists. The company Syngenta claimed the transgene is crylAb. Comparison with the public database revealed that the transgene has only 65% homology with the native crylAb, but 94% homology with a synthetic crylAc.
7. Following brief references to "cell nuclear modifications" and "functional modifications" the authors of the paper fail to consider the implications or causes in any detail. In their abstract they say: "No modifications of histological features of tissues were found; however, cytochemical analyses of ruminal epithelium by Ki67 staining provided evidence of proliferative activation of basal cells in all GM maize-fed ewes. Preliminary electron microscopy analyses of the liver and pancreas revealed smaller cell nuclei containing increased amounts of heterochromatin and perichromatin granules in GM maize-fed lambs."
In its investigation of these results GM Free Cymru discovered that the original referees of this paper did express concerns about these physiological changes, and asked for them to be investigated and elucidated, but that the journal accepted the piece in any case for publication. It therefore appears that the journal had no wish to see this essential information in print. One has to wonder why.

7: Rabbits fed on GM soy had changes to heart and kidney

Citation: Tudisco R, Lombardi P, Bovera F, d'Angelo D, Cutrignelli MI, Mastellone V, Terzi V, Avallone L, Infascelli F (2006) Genetically modified soya bean in rabbit feeding: detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis.
Anim Sci 82:193–199
http://journals.cambridge.org/action/ displayAbstract;jsessionid=FC2F24B790EEDE5C085837B07B4590FE.tomcat1?fromPage=online&aid=778124

Abstract
The presence of DNA fragments in tissues from rabbits given genetically modified (GM) soya-bean meal (solvent extracted) was investigated by using the polymerase chain reaction (PCR) approach. Moreover, the possible effects on cell metabolism were evaluated by determination of several specific enzymes in serum, heart, skeletal muscle, liver and kidney. The chloroplast sequence for tRNA Leu by using the Clor1/Clor2 primers designed on chloroplast trnL sequence was clearly detected. On the contrary, two couples of species specific primers for conventional (Le1-5/Le 1-3 which amplifies the soya bean lectin gene) and genetically modified (35S1/35S2 which amplifies the 35S CMV promoter that is present in the genomic structure of GM soya bean) soya bean were not found in all samples. No differences in enzyme levels were detected in serum, but a significant increase of lactic dehydrogenase, mainly concerning the LDH1 isoenzyme was found in particular in kidney and heart but not in the muscle, thus suggesting a potential alteration in the local production of the enzyme. Finally, no significant differences were detected concerning body weight, fresh organ weights and no sexual differences were detected.

Comment
This is yet another paper showing identifiable effects attributable to GM soy in short-term feeding studies. It should therefore be read in conjunction with the papers by Ermakova and Malatesta and others. This is Jeffrey Smith's summary:
Liver and other organ damage
Lab animals fed GM food have showed damage to virtually every system studied. They displayed stunted growth; bleeding stomachs; abnormal and potentially precancerous cell growth in the intestines; impaired blood cell development; misshapen cell structures in the liver, pancreas, and testicles; altered gene expression and cell metabolism; liver and kidney lesions; partially atrophied livers; inflamed kidneys; less developed brains and testicles; enlarged livers, pancreases, and intestines;

reduced digestive enzymes; higher blood sugar; increased death rates; higher offspring mortality; and immune system dysfunction.

The state of the liver — a main detoxifier for the body — is a key indicator of toxins.

* Rats fed potatoes engineered with the GNA lectin (to produce an insecticide) had smaller and partly atrophied livers.8 * Rats fed Monsanto's Mon 863 corn, engineered to produce Bt- toxin, had liver lesions and other indications of toxicity.9 * Rabbits fed GM soy showed altered enzyme production in their livers as well as higher metabolic activity.10 * The livers of rats fed Roundup Ready canola were 12 to 16 percent heavier, possibly due to liver disease or inflammation.11 * Microscopic analysis of the livers of mice fed Roundup Ready soybeans revealed altered gene expression and structural and functional changes.12

Many of these changes reversed after the mice diet was switched to non-GM soy, indicating that GM soy was the culprit.

The findings, according to molecular geneticist Michael Antoniou, Ph.D., "are not random and must reflect some 'insult' on the liver by the GM soy." Antoniou, who does human gene therapy research at King's College London, said that although the long-term consequences of the GM soy diet are not known, it "could lead to liver damage and consequently general toxemia."13

References

8. Arpad Pusztai, "Can science give us the tools for recognizing possible health risks of GM food," Nutrition and Health, 2002, Vol 16 Pp 73-84.

9. John M. Burns, "13-Week Dietary Subchronic Comparison Study with MON 863 Corn in Rats Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Rodent Diet #5002," December 17, 2002**www.monsanto.com/monsanto/content/sci_tech/ prod_safety/fullratstudy.pdf**

10. R. Tudisco, P. Lombardi, F. Bovera, D. d'Angelo, M. I. Cutrignelli, V. Mastellone, V. Terzi, L. Avallone, F. Infascelli, "Genetically Modified Soya Bean in Rabbit Feeding: Detection of DNA Fragments and Evaluation of Metabolic Effects by Enzymatic Analysis," Animal Science 82 (2006): 193-199.

11. Comments to ANZFA about Applications A346, A362 and A363 from the Food Legislation and Regulation Advisory Group (FLRAG) of the Public Health Association of Australia (PHAA) on behalf of the PHAA, "Food produced from glyphosate-tolerant canola line GT73," **www.iher.org.au/**

12. M. Malatesta, C. Caporaloni, S. Gavaudan, M. B. Rocchi, S. Serafini, C. Tiberi, G. Gazzanelli, "Ultrastructural Morphometrical and Immunocytochemical Analyses of Hepatocyte Nuclei from Mice Fed on Genetically Modified Soybean," Cell Struct Funct. 27 (2002): 173-180 13. Jeffrey M. Smith, Genetic Roulette: The Documented Health Risks of Genetically Engineered Foods, Yes! Books, Fairfield, IA USA 2007

[This section on Consumer Health Concerns from genetically modified food crops is derived entirely from the work by Jeffrey M. Smith, director of the Institute for Responsible Technology and author of "Genetic Roulette: The Documented Health Risks of Genetically Engineered Foods" and "Seeds of Deception." **www.responsibletechnology.org**]

8: Testes changes identified in mice fed on GM soy

Citation: Vecchio L, Cisterna B, Malatesta M, Martin TE, Biggiogera M (2004)
Ultrastructural analysis of testes from mice fed on genetically modified soybean.
Eur J Histochem 48:449–453
http://cat.inist.fr/?aModele=afficheN&cpsidt=16413615

Abstract

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.**

Comment

This paper (yet another relating to GM soy) **illustrates the non- specific toxic effects of GM foods that may arise from the highly mutagenic GM transformation process. Although the nature of the "toxins" is unknown (nobody has looked!) and the health consequences (especially in a long-term human context) of the documented effects in this paper is uncertain/unknown, what is clear here (and in many other studies) is that the GM food is causing some metabolic insult to various organs** (the liver, not unexpectedly, is another prime target as other Malatesta studies have shown). This should simply NOT be happening! This is particularly evident in the soya fed mice and which could impact on the fertility of these mice. (Note: Sertoli cells help sperm mature; if there function was impeded them lower counts of normal functioning sperm would be expected).

The authors suggest a role for traces of Roundup in the effects they have identified. The comments below are from Chee Yoke Heong: http://www.projectcensored.org/top-stories/articles/13-new-evidence- establishes-dangers-of-roundup/

Three recent studies show that Roundup, which is used by farmers and home gardeners, is not the safe product we have been led to trust.

A group of scientists led by biochemist Professor Gilles-Eric Seralini from the University of Caen in France found that human placental cells are very sensitive to Roundup at concentrations lower than those currently used in agricultural application.

An epidemiological study of Ontario farming populations showed that exposure to glyphosate, the key ingredient in Roundup, nearly doubled the risk of late miscarriages. osate, as after eighteen hours of exposure at low concentrations, large proportions of human placenta began to die.

9: Transgenicplant DNA found in animal tissues (pigs)

Citation: Mazza R, Soave M, Morlacchini M, Piva G, Marocco A.(2005)
Assessing the transfer of genetically modified DNA from feed to animal tissues.
Transgenic Res. 2005 Oct;14(5):775-84.

NB. This paper relates not to animal harm arising out of the feeding of GM materials, but to the transfer of transgenic DNA from GM plants into animal tissues -- something that the GM industry (and the regulators) have long claimed to be impossible. Fragments of the cry1A(b) genes in the blood, liver, spleen, kidney and muscle of pigs fed with GM maize.

Abstract

In Europe, public and scientific concerns about the environmental and food safety of GM (Genetically Modified) crops overshadow the potential benefits offered by crop biotechnology to improve food quality. One of the concerns regarding the use of GM food in human and animal nutrition is the effect that newly introduced sequences may have on the organism. In this paper, we assess the potential transfer of diet-derived DNA to animal tissues after consumption of GM plants. Blood, spleen, liver, kidney and muscle tissues from piglets fed for 35 days with diets containing either GM (MON810) or a conventional maize were investigated for the presence of plant DNA. Only fragments of specific maize genes (Zein, Sh-2) could be detected with different frequencies in all the examined tissues except muscle. A small fragment of the CrylA(b) transgene was detected in blood, liver, spleen and kidney of the animals raised with the transgenic feed. The intact Cry1A(b) gene or its minimal functional unit were never detected. Statistical analysis of the results showed no difference in recovery of positives for the presence of plant DNA between animals raised with the transgenic feed and animals raised with the conventional feed, indicating that DNA transfer may occur independently from the source and the type of the gene. From the data obtained, we consider it unlikely that the occurrence of genetic transfer associated with GM plants is higher than that from conventional plants.

Comment

"For EFSA to reaffirm its statement that transgenic DNA had not been found in animal tissue when the two studies by Mazza et al. (2005) and Sharma et al. (2006) clearly showed they had, is seriously misleading and ignores the scientific facts. It is unclear why EFSA refuses to state that transgenic fragments have been detected in tissues of farm animals. It is also unclear why the European Commission continues to ask EFSA for scientific advice when the advice it has provided in this case was not scientific but selective and biased."

See Werner Mueller (2008) "EFSA misleads the European Commission and the public over GMOs"**http://www.gmfreecymru.org/pivotal_papers/efsa_misleads.htm**

10: Transgenic plant DNA found in animal tissues (sheep and pigs)

Citation: Sharma R, Damgaard D, Alexander TW, Dugan ME, Aalhus JL, Stanford K, McAllister TA. (2006)
Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal.
J Agric Food Chem. 2006 Mar 8;54(5):1699-709.

NB. This paper relates not to animal harm arising out of the feeding of GM materials, but to the transfer of transgenic DNA from GM plants into animal tissues; something the GM industry (and the regulators) have long claimed to be impossible.
Transgene fragments in the large intestine tissue of sheep and in the cecal tissues of pigs

Abstract
The persistence of plant-derived recombinant DNA in sheep and pigs fed genetically modified (Roundup Ready) canola was assessed by PCR and Southern hybridization analysis of DNA extracted from digesta, gastrointestinal (GI) tract tissues, and visceral organs. Sheep (n = 11) and pigs (n = 36) were fed to slaughter on diets containing 6.5 or 15% Roundup Ready canola. Native plant DNA (high- and low-copy- number gene fragments) and the cp4 epsps transgene that encodes 5- enolpyruvyl shikimate-3-phosphate synthase were tracked in ruminal, abomasal, and large intestinal digesta and in tissue from the esophagus, rumen, abomasum, small and large intestine, liver, and kidney of sheep and in cecal content and tissue from the duodenum, cecum, liver, spleen, and kidney of pigs. High-copy chloroplast- specific DNA (a 520-bp fragment) was detected in all digesta samples, the majority (89−100%) of intestinal tissues, and at least one of each visceral organ sample (frequencies of 3−27%) from sheep and swine. Low-copy rubisco fragments (186- and 540-bp sequences from the small subunit) were present at slightly lower, variable frequencies in digesta (18−82%) and intestinal tissues (9−27% of ovine and 17 −25% of porcine samples) and infrequently in visceral organs (1 of 88 ovine samples; 3 of 216 porcine samples). Each of the five cp4 epsps transgene fragments (179−527 bp) surveyed was present in at least 27% of ovine large intestinal content samples (maximum = 64%) and at least 33% of porcine cecal content samples (maximum = 75%). In sheep, transgene fragments were more common in intestinal digesta than in ruminal or abomasal content. Transgene fragments were detected in 0 (esophagus) to 3 (large intestine) GI tract tissues from the 11 sheep and in 0−10 of the duodenal and cecal tissues collected from 36 pigs. The feed-ingested recombinant DNA was not detected in visceral tissues (liver, kidney) of lambs or in the spleen from pigs. Of note, however, one liver and one kidney sample from the pigs (different animals) were positive for a 278-bp fragment of the transgenic cp4 epsps (denoted F3). Examination of genomic libraries from these tissues yielded no conclusive information regarding integration of the fragment into porcine DNA. This study confirms that feed-ingested DNA fragments (endogenous and transgenic) do survive to the terminal GI tract and that uptake into gut epithelial tissues does occur. A very low frequency of transmittance to visceral tissue was confirmed in pigs, but not in sheep. It is recognized that the low copy number of transgenes in GM feeds is a challenge to their detection in tissues, but there was no evidence to suggest that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material.

Comment
"For EFSA to reaffirm its statement that transgenic DNA had not been found in animal tissue when the two studies by Mazza et al. (2005) and Sharma et al. (2006) clearly showed they had, is seriously misleading and ignores the scientific facts. It is unclear why EFSA refuses to state that transgenic fragments have been detected in tissues of farm animals. It is also unclear why the European Commission continues to ask EFSA for scientific advice when the advice it has provided in this case was not scientific but selective and biased."
See Werner Mueller (2008) "EFSA misleads the European Commission and the public over GMOs"**http://www.gmfreecymru.org/pivotal_papers/efsa_misleads.htm**

11: Roundup residues in GM food cause cell damage and even death

Citation: Benachour, N. and Seralini, G-E. 2008
Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells Chemical Research in Toxicology, DOI: 10.1021/tx800218n
Publication Date (Web): December 23, 2008
http://pubs.acs.org/doi/abs/10.1021/tx800218n

Abstract

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup (R) formulations, from 105 times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from R formulation-treated crops.

Press release:

Press Release CRIIGEN – January 2009

DIFFERENT ROUNDUP FORMULATIONS LEAD TO EMBRYONIC, UMBILICAL CORD AND PLACENTAL CELL DEATH AND ARE POORLY ASSESSED

For the first time, the toxicity mechanisms of four different Roundup formulations were studied in human cells. They act at doses where they are not herbicides anymore. The cells were neonatal cells freshly isolated from the umbilical cord, or less sensitive cell lines specially used to measure pollutant toxicity. The various components of these major herbicides were tested because they are among the most common in the world. Their residues are among the major pollutants, and moreover they are authorized as residues contaminating GM foods and feed at the tested levels. As a matter of fact, Roundup formulations are the most common herbicides used with cultivated GMOs. **Roundup Ready soya, the main GMO imported in Europe for food and feed, contains Roundup residues.** In this research, the formulations were diluted at minimal doses (up to 100 000 times or more) and they programmed cell death in a few hours in a cumulative manner. **We also noted membrane and DNA damages, and found that the formulations inhibit cell respiration. In addition, it was shown that the mixture of the components used as Roundup adjuvants amplified the action of the active principle called glyphosate;** one of its metabolites may be even more toxic. **These effects are greatly underestimated by the legislation,** which does not take these phenomena into account, but instead simply sets arbitrary contaminant thresholds in food or feed. The rules apply to glyphosate whatever its formulation may be, this is wrong. The authorizations for using these Roundup herbicides must now clearly be revised, since their toxic effects depend on, and are multiplied by, other compounds used in the mixtures placed on the market; and glyphosate is only one of them. The detailed blood analyses of each mammal which has received this herbicide during regulatory tests before commercial release must be published immediately, since our research points to undesirable effects which are currently masked or hidden from scientific scrutiny.

This independent work was performed by Nora Benachour and Prof. Gilles-Eric Séralini in the University of Caen in France. It is published in the Scientific American journal Chemical Research in Toxicology. It was supported by CRIIGEN and the Regional Council of Basse Normandie. The support of the Human Earth Foundation and Denis Guichard Foundation is also

acknowledged. Contact in France: Pr Gilles-Eric Séralini, Biochemistry, Institute of Biology, University of Caen, Esplanade de la Paix, 14032 Caen, France. Tel: 33(0) 2-31-56-56-84. Fax: 33(0)2-31-56-53- 20. Corinne Lepage President of CRIIGEN criigen@unicaen.fr "Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic and Placental Cells" by Nora Benachour and Gilles-Eric Séralini. (http://pubs.acs.org/doi/abs/10.1021/tx800218n)
Full text: **http://pubs.acs.org/doi/full/10.1021/tx800218n**

Comment

This new article from CRIIGEN shows that Roundup residues found in GM food and feed can cause cell damage and even death -- even at very low levels. The authors say that their research "..... points to undesirable effects which are currently masked or hidden from scientific scrutiny." This is yet another example of harm associated with GM crops which are currently on the market. In this case the harm is "indirect" -- but it is nonetheless inescapable since all RR crops used for feed and food purposes will contain RR residues at or above the studied level. Please note that this work relates to in vitro studies -- it is now incumbent upon FSA / ACNFP and DEFRA to commission independent in vivo studies which will build on this work and examine just how damaging RR crops and foods are to animals. In the meantime, there must be a moratorium on all sales of food and feed derived from RR varieties which contain these dangerous residues.

12: Health Risks of Genetically Modified Foods***

Citation: ARTEMIS DONA and IOANNIS S. ARVANITOYANNIS, 2009
Health Risks of Genetically Modified Foods
Critical Reviews in Food Science and Nutrition, 49:164–175 (2009)2

Abstract

As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. **Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. The use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which may promote cancer.**

Extract:

ETHICS

The lasting sceptical and/or ambivalent attitude of Europeans towards agro-food biotechnology and the continued controversies about the commercialization of transgenic agro-food products are illustrative of an ongoing legitimacy crisis. One could even interpret the stigma on agro-food biotechnology and its products as testifying to a "robust" societal disapproval: it signals a lack of trust in scientific institutions and expert systems, and voices a social response against the reduction of the complexity of the GMO issue to a solely scientific risk-based problem. Hence, amove from a merely scientific evaluation towards a socially more robust one—that addresses precaution and socioethical issues in a more "sensible"way, whilst making "sense" of the different stances taken in the GMO debate—is still sought after. It will be interesting to see whether new controversies show (triggered, for example, by GMO contaminations or traces of unapproved transgenic events in nontransgenic produces), how these will be communicated and developed in the societal climate, and how they will be interpreted and tackled by, and/or lead to new adjustments in the now running legal system (Devos et al., 2007). The comparison of values relevant to GE crops and foods among EU, Japan, Canada, and the USA is given in Table 2.

CONCLUSIONS

From the review of the toxicity studies concerning GM foods one might see that although toxicity can be assessed, the duration of exposure is too short in order to fully evaluate any potential disruptions in biochemical parameters and to evidence possible signs of pathology within the limited subchronic exposure of animals. Moreover, a larger number of animals should be used in the toxicity tests. The toxicity tests should comply with the guidelines for toxicity testing of drugs. It should be emphasized that since these GM foods are going to be consumed by every human being they should be tested even more thoroughly than drugs and more experiments are required in order to study the possible toxicity and make any

conclusions. **Tests to determine how a GM food affects mutagenesis and carcinogenesis should be conducted as well. Finally, postmarketing surveillance should be part of the overall safety strategy for allergies, especially of high-risk groups such as infants and individuals in "atopic" families.** Evaluation of protein allergenicity in man should also include studies in individuals not only with a history of allergy but with immunodeficiency as well. **The use of recombinant GH in animals, such as cows or the expression of GH in animals such as salmon should be re-examined since it may promote cancer. The results of most of the rather few studies conducted with GM foods indicate that they may cause hepatic, pancreatic, renal, and reproductive effects and may alter hematological, biochemical, and immunologic parameters the significance of which remains unknown. The above results indicate that many GM food have some common toxic effects. Therefore, further studies should be conducted in order to elucidate the mechanism dominating this action. Small amounts of ingested DNA may not be broken down under digestive processes and there is a possibility that this DNA may either enter the bloodstream or be excreted, especially in individuals with abnormal digestion as a result of chronic gastrointestinal disease or with immunodeficiency.** Although intensive scientific effort is currently in progress to thoroughly understand and forecast possible consequences on humans, animals, and the environment, it is anticipated that many years of careful, independent research with animals and clinical trials will be needed in order to accomplish this assessment.

Comment

Note that this is a review paper, not a primary research paper. Nevertheless, we are including it here because it deserves to be widely read. This is a carefully researched and well-referenced paper which looks at all of the known health risks associated with GM foods. As with most academic publications, the language is cautious, but the authors note a common theme arising from many independent studies of health effects -- the apparently toxic effects of GMOs on internal organs and on the reproductive system. They pick up on the shortcomings of the normal "safety testing" protocols, including the short exposure times experienced by tested animals -- often far too short for GM-related effects to become apparent. They call for testing protocols which are more stringent than drug testing protocols -- on the basis that GM foods are likely (if approved) to be consumed by everybody -- and not just by groups in the population who may be deemed to require "healing." Unusually in a paper of this type, the authors also consider ethical issues, arguing that there is broad societal disapproval of GM crops and foods, based on a lack of trust in scientific institutions and expert systems. The authors argue that in a democracy socio-economic factors should be given much more weight in the regulatory process, since the public will not accept the reduction of the complexity of the GMO issue to a solely scientific risk-based problem.

13: GMO risk assessment procedures are woefully inadequate

Séralini GE, de Vendômois JS, Cellier D, Sultan C, Buiatti M, Gallagher L, Antoniou M, Dronamraju KR.
How Subchronic and Chronic Health Effects can be Neglected for GMOs, Pesticides or Chemicals.
Int J Biol Sci 2009; 5:438-443.
http://www.biolsci.org/v05p0438.htm

Abstract

Chronic health effects are increasing in the world such as cancers, hormonal, reproductive, nervous, or immune diseases, even in young people. During regulatory toxicological sub-chronic tests to prevent these on mammalian health, prior commercialization of chemicals, including pesticides and drugs, or GMOs, some statistically significant findings may be revealed. This discussion is about the need to investigate the relevant criteria to consider those as biologically significant. The sex differences and the non linear dose or time related effects should be considered in contrast to the claims of a Monsanto-supported expert panel about a GMO, the MON 863 Bt maize, but also for pesticides or drugs, in particular to reveal hormone-dependent diseases and first signs of toxicities.

Conclusion

We assume that Séralini et al. [5] methodology can discriminate potential false positive and GM-linked effects, avoiding to some extent false negative ones, in the best way we can do for this discussed and too limited protocol already in use for commercialized GMOs. These GM-linked effects are then considered as signs of toxicity in the 90 days, not proofs of toxicity.

The biological plausibility of a subchronic or chronic side effect of the GM diet, linked to the new toxin in the mammalian regimen, or due to the mutagenesis effect of the genetic modification itself, is thus non negligible. Finally it should be stressed that statistically significant effects of GM diets, or of residues of pesticides that are contained by GMOs, have also been observed in other instances [21-25], but not in all studies [26, 27] enlightening the necessity of a case-by-case approach, and that the real toxicological studies are quite limited up to date for that [28]. All these observations taken together in our opinions do not allow a clear statement of toxic effects, but to suggest them as such, because they are clearly undeniable. Now, to any good researcher similar results would mean that there is much to be improved in the planning of experimental design; and thus to increase their resolution power to obtain unequivocal statements, for instance increasing the duration and/or the number of rats tested. Generally speaking it seems to us unbelievable that a risk assessment carried out only on forty rats of each sex receiving GM rich diets for 90 days (yielding results often at the limits of significance) have not been repeated and prolonged independently. We should overall take into account the fact that the analysed GM product could be fed long-term to people and animals of various ages and sexes, and with various pathologies.
We call for more serious standardized tests such as those used for pesticides or drugs, on at least three mammalian species tested for at least three months employing larger sample sizes, and up to one and two years before commercialization, for GM food or feed specifically modified to contain pesticide residues. We also call for a serious scientific debate about the criteria for testing significant adverse health effects for pesticides or chemicals, but overall for GM food or feed products, such as MON 863

References

1. Daston GP, Cook JC, Kavlock RJ. Uncertainties for endocrine disrupters: our view on progress. Toxicol Sci. 2003;74:245-52
2. Toppari J, Larsen JC, Christiansen P. et al. Male reproductive health and environmental xenoestrogens. Environ Health Perspect. 1996;104:741-803
3. Paris F, Jeandel C, Servant N. et al. Increased serum estrogenic bioactivity in three male newborns with ambiguous genitalia: a potential consequence of prenatal exposure to environmental endocrine disruptors. Environ Res. 2006;100:39-43
4. Belpomme D, Irigaray P, Hardell L. et al. The multitude and diversity of environmental carcinogens. Environ Res. 2007;105:414-29
5. Seralini GE, Cellier D, Spiroux de Vendomois J. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch Environ Contam Toxicol. 2007;52:596-602
6. Dronamraju K.R. Emerging Consequences of Biotechnology. New Jersey, USA: World Scientific Publishing Co. 2008
7. Doull J, Gaylor D, Greim HA. et al. Report of an expert panel on the reanalysis by Séralini et al. (2007) of a 90-day study conducted by Monsanto in support of the safety of a genetically modified corn variety (MON 863). Food Chem Toxicol. 2007;45:2073-2085
8. Hammond B, Lemen J, Dudek R. et al. Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. Food Chem Toxicol. 2006;44:147-160
9. Green S. Toxicology and regulatory process. New York, USA: Taylor and Francis Group. 2006
10. Nielsen E, Østergaard G, Larsen JC. Toxicological Risk Assessment of Chemicals - A Practical Guide. New York, USA: Taylor and Francis Group. 2008
11. Gaylor D, Chen J, Kodell R. Experimental Bioassays for Screening and Low Dose Extrapolation. Risk Analysis. 1985;5:9-16
12. Andersen ME, Barton HA. Biological regulation of receptor-hormone complex concentrations in relation to dose-response assessments for endocrine-active compounds. Toxicol Sci. 1999;48:38-50
13. Bencko V. Human exposure to endocrine disrupters: carcinogenic risk assessment. Folia Histochem Cytobiol. 2001;39(Suppl 2):24-5
14. Melnick R, Lucier G, Wolfe M. et al. Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. Environ Health Perspect. 2002;110:427-31
15. Karanth S, Pope C. Carboxylesterase and A-esterase activities during maturation and aging: relationship to the toxicity of chlorpyrifos and parathion in rats. Toxicol Sci. 2000;58:282-9
16. Howard MD, Mirajkar N, Karanth S. et al. Comparative effects of oral chlorpyrifos exposure on cholinesterase activity and muscarinic receptor binding in neonatal and adult rat heart. Toxicology. 2007;238:157-65
17. Benbrahim-Tallaa L, Siddeek B, Bozec A. et al. Alterations of Sertoli cell activity in the long-term testicular germ cell death process induced by fetal androgen disruption. J Endocrinol. 2008;196:21-31
18. Lu SC, Kuhlenkamp J, Garcia-Ruiz C. et al. Hormone-mediated down- regulation of hepatic glutathione synthesis in the rat. J Clin Invest. 1991;88:206-269

19. Sissung TM, Price DK, Sparreboom A. et al. Pharmacogenetics and regulation of human cytochrome P450 1B1: Implications in hormone- mediated tumor metabolisme and a novel tagert for therapeutic intervention. Mol Cancer Res. 2006;4:135-150
20. Kobliakov V, Popova N, Rossi L. Regulation of the expression of the sex-specific isoforms of cytochrome P-450 in rat liver. Eur J Biochem. 1991;195:585-91
21. Malatesta M, Caporaloni C, Gavaudon S. et al. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. Cell Struct Function. 2002;27:173-180
22. Malatesta M, Biggiogera M, Manuali E. et al. Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean. Eur J Histochem. 2003;47:385-388
23. Vecchio L, Cisterna B, Malatesta M. et al. Ultrastructural analysis of testes from mice fed on genetically modified soybean. Eur J Histochem. 2004;48:449-454
24. Trabalza-Marinucci M, Brandi G, Rondini C. et al. A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livestock Sci. 2008;113:178-190
25. Benachour N, Sipahutar H, Moslemi S. et al. Time- and dose- dependent effects of roundup on human embryonic and placental cells. Arch Environ Contam Toxicol. 2007;53:126-133
26. Brake DG, Evenson DP. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem Toxicol. 2004;42:29-36
27. Brake DG, Thaler R, Evenson DP. Evaluation of Bt (Bacillus thuringiensis) corn on mouse testicular development by dual parameter flow cytometry. J Agric Food Chem. 2004;52:2097-2102
28. Domingo JL. Toxicity studies of genetically modified plants: A review of the published literature. Crit Rev Food Sci Nut. 2007;47:721-733
29. Hotelling H. Analysis of a complex of statistical variables into principal components. J Educ Psychol. 1933;24:417-441

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14: Transgenic DNA does not significantly degrade in the soil food web

Detection of transgenic cp4 epsps genes in the soil food web

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Agron. Sustain. Dev. 29 (2009) 497–501 **www.agronomy-journal.org http://bit.ly/6gauyk**
(Accepted 12 May 2009)

Abstract
The persistence and movement of transgenic DNA in agricultural and natural systems is largely unknown. This movement poses a threat of horizontal gene transfer and possible proliferation of genetically modified DNA into the general environment. To assess the persistence of transgenic DNA in a field of Roundup Ready corn, we quantified the presence of the transgene for glyphosate tolerance within a soil food web. Using quantitative real-time PCR, we identified the cp4 epsps transgene in bulk soil microarthropods, nematodes, macroarthropods and earthworms sampled within the corn cropping system. We found evidence of the transgene at all dates and in all animal groups. Transgenic DNA concentration in animal was significantly higher than that of background soil, suggesting the animals were feeding directly on transgenic plant material. It remains to be tested whether this DNA was still within the plant residues, present as free, extracellular DNA or had already undergone genetic transformation into competent bacterial cells. These results are the first to demonstrate the persistence of transgenic crop DNA residues within a food web.
4. CONCLUSION We found evidence for large concentrations of transgenic DNA in animals from the food web associated with RoundUp Ready corn. This indicates that the transgene does not significantly degrade within the food web. Further, the

guts of these animals may provide opportunity for genetic transformation into native soil bacteria. It remains to be determined how far down the food web the transgene is detectable and whether or not the identified gene is available for transformation. It may be that animals associated with the soil food web provide an excellent starting spot for detecting genetic transformation in the natural environment.

15: GM maize - "clear negative impact" on kidneys, liver, other organs

A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health
Joël Spiroux de Vendômois, François Roullier, Dominique Cellier and Gilles-Eric Séralini
International Journal of Biological Sciences 2009; 5(7):706-726

Abstract
We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose- dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

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5. Conclusions

Patho-physiological profiles are unique for each GM crop/food, underlining the necessity for a case-by-case evaluation of their safety, as is largely admitted and agreed by regulators. It is not possible to make comments concerning any general, similar subchronic toxic effect for all GM foods. However, in the three GM maize varieties that formed the basis of this investigation, new side effects linked to the consumption of these cereals were revealed, which were sex- and often dose-dependent. Effects were mostly concentrated in kidney and liver function, the two major diet detoxification organs, but in detail differed with each GM type. In addition, some effects on heart, adrenal, spleen and blood cells were also frequently noted. As there normally exists sex differences in liver and kidney metabolism, the highly statistically significant disturbances in the function of these organs, seen between male and female rats, cannot be dismissed as biologically insignificant as has been proposed by others [4].

We therefore conclude that our data strongly suggests that these GM maize varieties induce a state of hepatorenal toxicity. This can be due to the new pesticides (herbicide or insecticide) present specifically in each type of GM maize, although unintended metabolic effects due to the mutagenic properties of the GM transformation process cannot be excluded [42]. All three GM maize varieties contain a distinctly different pesticide residue associated with their particular GM event (glyphosate and AMPA in NK 603, modified Cry1Ab in MON 810, modified Cry3Bb1 in MON 863). These substances have never before been an integral part of the human or animal diet and therefore their health consequences for those who consume them, especially over long time periods are currently unknown. Furthermore, any side effect linked to the GM event will be unique in each case as the site of transgene insertion and the spectrum of genome wide mutations will differ between the three modified maize types.

In conclusion, our data presented here strongly recommend that additional long-term (up to 2 years) animal feeding studies be performed in at least three species, preferably also multi-generational, to provide true scientifically valid data on the acute and chronic toxic effects of GM crops, feed and foods. Our analysis highlights that the kidneys and liver as particularly important on which to focus such research as there was a clear negative impact on the function of these organs in rats

consuming GM maize varieties for just 90 days.

16: GM crops do not enhance fitness in stressed conditions

Comment from GM Free Cymru: This is a strange article, straight from the heart of corporate America. Its underlying assumption is that the biotechnology corporations should be allowed to flood the market with GM products, and to control the global food supply if that is what it wants to do, without any reference to things like environmental damage, health and safety issues, and scientific corruption. Rommens is identifying "roadblocks" to commercialization so as to help the GM industry to overcome them -- so he comes from a rather different direction than most of us who might read this note. But at least he's honest -- and quite revealing -- in some respects. The quotes below are interesting -- it's not often that you see these things in black and white from somebody so close to the industry!

He confirms that the process of GM is incredibly expensive and hit-and- miss, and that where GM crops display reasonable "fitness" in the field, in coping with stressed conditions, the fitness generally has little if anything to do with the introduced GM traits. The important characteristics were in the plants already, before they started messing about with them..... The GM miracle? All hype and no substance.....

Review article Barriers and paths to market for genetically engineered crops Caius M. Rommens* J.R. Simplot Company, Plant Sciences, Boise ID 83706, USA Plant Biotechnology Journal (2009) 7, pp. 1–11
http://www3.interscience.wiley.com/journal/123200391/abstract?CRETRY=1&SRETRY=0

Quote: "However, only a few of the many identified genes were tested in the field, and the results from these trials have generally been disappointing, often indicating that indoor effects are not a reliable indicator for what happens outdoors."

"Candidate genes for tolerance against drought, salt and other abiotic stresses also often failed to display field efficacy."

"Despite some promising results for modified nitrogen assimilation, to date, there are no conclusive data on enhanced yield for any transgene."

Summary Each year, billions of dollars are invested in efforts to improve crops through genetic engineering (GE). These activities have resulted in a surge of publications and patents on technologies and genes: a momentum in basic research that, unfortunately, is not sustained throughout the subsequent phases of product development. After more than two decades of intensive research, the market for transgenic crops is still dominated by applications of just a handful of methods and genes. This discrepancy between research and development reflects difficulties in understanding and overcoming seven main barriers-to-entry: (1) trait efficacy in the field, (2) critical product concepts, (3) freedom-to-operate, (4) industry support, (5) identity preservation and stewardship, (6) regulatory approval and (7) retail and consumer acceptance. In this review, I describe the various roadblocks to market for transgenic crops and also discuss methods and approaches on how to overcome these, especially in the United States.

Extract Conventional variety development programmes are often based on recurrent selection systems in the field. Agronomists score plants for important phenotypes to identify lines that display the highest levels of stress tolerance and yield across multiple sites. In contrast to this selection of plants 'by the environment', artificial and environment controlled laboratory assays are employed to identify genes controlling biotic and abiotic stress tolerance. The apparent success of the latter approach is exemplified by the large number of sequences, articles and patent applications that have been published by molecular biologists during the last two decennia (Vain, 2007). However, **only a few of the many identified genes were tested in the field, and the results from these trials have generally been disappointing**, often indicating that indoor effects are not a reliable indicator for what happens outdoors (Mittler, 2006). For example, overexpression of the Arabidopsis biotic stress tolerance gene Npr1 triggered enhanced tolerance against a broad spectrum of viral, bacterial and fungal pathogens in the laboratory (Cao et al., 1997). But subsequent **field trials demonstrated that this 'systemic acquired resistance' (SAR) was already induced naturally by environmental stresses.** Transgenic plants were hardly distinguishable from untransformed controls, and slightly reduced infection rates were completely off-set by greater susceptibility to insects (Felton and Korth, 2000; Rayapuram and Baldwin, 2007; Walters and Fountaine, 2009). Other once-promising genes in SAR, such as cpr1, cpr5 and cpr6, are now also known to trigger negative pleiotropic effects in the field (Heidel et al., 2004; Century ª 2009 J. R.

Simplot Company et al., 2008). Similarly, plants carrying the Rpm1 gene, which provides resistance against Pseudomonas syringae pv pisi, exhibited a lower shoot biomass, fewer siliques, and an average decrease in seed production of 9% relative to control lines (Tian et al., 2003). Candidate genes for tolerance against drought, salt and other abiotic stresses also often failed to display field efficacy (Yamaguchi and Blumwald, 2005; Mittler, 2006). And the difficulties in studying stress tolerance are dwarfed by attempts to assess the efficacy of candidate yield genes. Despite some promising results for modified nitrogen assimilation, to date, there are no conclusive data on enhanced yield for any transgene (Ameziane et al., 2000; Jing et al., 2004; Hirel et al., 2007). The small group of effective input traits developed through GE consists mainly of insecticidal protein genes from Bacillus thuringiensis. Discovery of these genes was facilitated by the fact that strains of this soil bacterium have been used to control insect pests since the 1920s (Lemaux, 2008). Molecular biologists have also succeeded in controlling certain RNA viruses, especially potyviruses, through targeted RNA silencing (Prins et al., 2008). A more-recently developed strategy provided enhanced stress tolerance through expression of the bacterial RNA chaperone-encoding gene cspB (Castiglioni et al., 2008). Gene efficacy was demonstrated at both the vegetative and reproductive stages of various plant species. More testing is required, however, to confirm there is no fitness cost to the incorporated stress tolerance.

17: Glyphosate residues in food can cause vertebrate malformations and spontaneous abortions

Note: This is a hugely important paper which shows for the first time the precise mechanism involved in the malformations and spontaneous abortions being reported in communities where pregnant women (for example, in Argentina and Paraguay) are exposed to excessive doses of Roundup associated with the spraying of RR soy. Other papers (including those from Prof Seralini and his group) have also highlighted the toxic effects of glyphosate-based herbicides. The findings of the Carrasco group have caused so much concern in the GM soy industry that Prof Carrasco has been the target of yet another campaign of vilification, involving intimidation and hired thugs, into which Amnesty International has now been drawn. Monsanto is clearly implicated.

See also:**HIRED THUGS SEEK TO SILENCE CARRASCO** and **Glyphosate whistle-blower comes under sustained attack**

Pusztai 1999 - Carrasco 2009

Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling

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ReceiVed May 20, 2010 Chem. Res. Toxicol. XXXX, xxx, 000

ABSTRACT

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. Xenopus laeVis embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in Xenopus embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of otx2 expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate

embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.

18: Highly Lethal Effects of Roundup on Amphibians

We include this paper (from 2005) in our collection because it now assumes great importance in view of the findings of the group led by Prof Andres Carrasco in Argentina. It is also significant in that it attracted a vigorous response from the Monsanto hired guns, who were intent in demonstrating that a highly toxic chemical is not really toxic at all to non-target organisms. That of course is biological nonsense, and Prof Relyea gave these people pretty short shrift in his response posted under our "Further Reading" heading.

Ecological Applications, 15(2), 2005, pp. 618–627 q 2005 by the Ecological Society of America

THE IMPACT OF INSECTICIDES AND HERBICIDES ON THE BIODIVERSITY AND PRODUCTIVITY OF AQUATIC COMMUNITIES

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Abstract.

Pesticides constitute a major anthropogenic addition to natural communities. In aquatic communities, a great majority of pesticide impacts are determined from singlespecies experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents an assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals. Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions.

19: Contamination of streams by BT crop detritus

It will, of course, be denied by the GM industry that this contamination has any biological impact. Monsanto et al have been saying that for years, and pretending that BT toxins only affect "target" organisms -- which is of course biological nonsense. The insecticide in GM crops (Bt) causes a strong immune response in any insect that can survive its toxicity. Any immune response in any insect causes cognitive impairment of that insect. We await further work on this -- to see what the actual measurable effects are in aquatic ecosystems.

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Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape

by Jennifer L. Tanka, Emma J. Rosi-Marshall,Todd V. Royer, Matt R. Whiles, Natalie A. Griffiths, Therese C. Frauendorf, and David J. Treering

Abstract

Widespread planting of maize throughout the agricultural Midwest may result in detritus entering adjacent stream ecosystems, and 63% of the 2009 US maize crop was genetically modified to express insecticidal Cry proteins derived from Bacillus thuringiensis. Six months after harvest, we conducted a synoptic survey of 217 stream sites in Indiana to determine the extent of maize detritus and presence of Cry1Ab protein in the stream network. We found that 86% of stream sites

contained maize leaves, cobs, husks, and/or stalks in the active stream channel. We also detected Cry1Ab protein in stream-channel maize at 13% of sites and in the water column at 23% of sites. We found that 82% of stream sites were adjacent to maize fields, and Geographical Information Systems analyses indicated that 100% of sites containing Cry1Ab-positive detritus in the active stream channel had maize planted within 500 m during the previous crop year. Maize detritus likely enters streams throughout the Corn Belt; using US Department of Agriculture land cover data, we estimate that 91% of the 256,446 km of streams/rivers in Iowa, Illinois, and Indiana are located within 500 m of a maize field. Maize detritus is common in low-gradient stream channels in northwestern Indiana, and Cry1Ab proteins persist in maize leaves and can be measured in the water column even 6 mo after harvest. Hence, maize detritus, and associated Cry1Ab proteins, are widely distributed and persistent in the headwater streams of a Corn Belt landscape.

20: GM Soy and reproductive effects in rats

Note from GM - Free Cymru: This article needs careful examination -- it looks as if the intention of the authors was to find NO difference in the effects of consuming GM soy and organic soy. Stanley Ewen has looked at this paper, however, and Jeffrey Smith reports his reaction as follows:

"Female rats fed GM soy for 15 months showed significant changes in their uterus and reproductive cycle, compared to rats fed organic soy or those raised without soy. Published in The Anatomical Record in 2009, this finding adds to the mounting body of evidence suggesting that GM foods contribute to reproductive disorders.

Unlike women whose menstrual cycle starts automatically at puberty, female rats need to be "inspired." Their (estrous) cycle conveniently kicks in only after being introduced to male rats. Since no males were present in this study, the females fed organic soy or no soy were appropriately untriggered (diestrus). For some odd reason, however, those fed GM soy appeared to have their ovulation cycle in full gear.

Although the researchers did not perform a check on the estrous cycle directly, their microscopic analysis of ovaries and uterus tissue showed that the hormone-induced changes (i.e. early ovulation and formation of corpus luteum) were well underway. In addition, the lining of the uterus (endometriim) had more cells than normal and the glands were dilated. In simpler terms, according to senior UK pathologist Stanley Ewen, something in the GM soy diet was "wrecking the ovary and endometrium" of the rats."

The Impact of Dietary Organic and Transgenic Soy on the Reproductive System of Female Adult Rat
Flávia Bittencourt Brasil, LavíNia Leal Soares, Tatiane Silva Faria, Gilson Telles Boaventura, Francisco José Barcellos Sampaio, Cristiane Fonte Ramos,
The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology Volume 292, Issue 4, pages 587-594, April 2009 **http://onlinelibrary.wiley.com/doi/10.1002/ar.20878/abstract**

Abstract

The goal of this article was to compare the effects of a prolonged use of organic and transgenic soy on the lipid profile and ovary and uterus morphology. Wistar rats were fed three different diets from weaning until sacrifice at 15 months of age. The three diets were: casein-based diet control group (CG), organic soy-based diet group (OSG), or transgenic soy-based diet group (GMSG). There were no differences in food consumption or in the diet isoflavone components among the groups. Compared with the CG diet, both the OSG and GMSG diets were associated with significant reductions in body weight, serum triglycerides, and cholesterol ($P < 0.05$) (CG = 406 ± 23.1; 104.3 ± 13.2; 119.9 ± 7.3 GMSG = 368 ± 17.6; 60.3 ± 4.6; 83.3 ± 5.7 OSG = 389 ± 23.5; 72.3 ± 12.5; 95.5 ± 8.0, respectively). The volume density of endometrial glandular epithelium was greater in the GMSG group (29.5 ± 7.17, $P < 0.001$) when compared with the CG (18.5 ± 7.4) and OSG (20.3 ± 10.6) groups. The length density of endometrial glandular epithelium was shorter in both GMSG (567.6 ± 41.1) and OSG (514.8 ± 144.5) diets compared with the CG ($P < 0.05$) diet. GMSG also resulted in reduced follicle number and increased corpus luteum number compared to the OSG or CG diets ($P < 0.05$). **In summary, both GMSG and OSG diets resulted in decreased body weight and lower serum triglyceride and cholesterol levels, and alterations in uterine and ovarian morphology were also observed. The prolonged use of soy-based diets and their relation to reproductive health warrants further investigation.**

21: Debate on GMOs Health Risks after Findings in Regulatory Tests

by Joël Spiroux de Vendômois, Dominique Cellier, Christian Vélot, Emilie Clair, Robin Mesnage, and Gilles-Eric Séralini

International Journal of Biological Sciences 2010; 6(6):590-598 (Communication)

Abstract

We summarize the major points of international debate on health risk studies for the main commercialized edible GMOs. These GMOs are soy, maize and oilseed rape designed to contain new pesticide residues since they have been modified to be herbicide-tolerant (mostly to Roundup) or to produce mutated Bt toxins. The debated alimentary chronic risks may come from unpredictable insertional mutagenesis effects, metabolic effects, or from the new pesticide residues. The most detailed regulatory tests on the GMOs are three-month long feeding trials of laboratory rats, which are biochemically assessed. The tests are not compulsory, and are not independently conducted. The test data and the corresponding results are kept in secret by the companies. Our previous analyses of regulatory raw data at these levels, taking the representative examples of three GM maize NK 603, MON 810, and MON 863 led us to conclude that hepatorenal toxicities were possible, and that longer testing was necessary. Our study was criticized by the company developing the GMOs in question and the regulatory bodies, mainly on the divergent biological interpretations of statistically significant biochemical and physiological effects. We present the scientific reasons for the crucially different biological interpretations and also highlight the shortcomings in the experimental protocols designed by the company. The debate implies an enormous responsibility towards public health and is essential due to nonexistent traceability or epidemiological studies in the GMO-producing countries.

Extract: Conclusions and perspectives

Controversy on biological interpretations is a usual way of advancement in science. It would however have been beneficial for the acceptance of biotechnologies by the public at large, to close this scientific debate by longer, more detailed, and transparent toxicological tests on GMOs, and in particular twenty years ago when the most widely grown GMOs were still experimental.

We wish to reassert that our work does not claim to demonstrate the chronic toxicity of the GMOs in question, especially since it is based on the data originating from insufficient tests that were accepted by regulatory authorities and Monsanto et al., a fact for which we are not in any way responsible. For the regulatory authorities, as well as Monsanto et al, these tests prove chronic innocuousness for mammalian and human public health. And they claim it is not essential to demonstrate the GMOs innocuousness. This again raises the same issues and consequences. We have revealed the inefficiency both of these tests and of their statistical analysis and biological interpretations, for the various reasons detailed above.

However, some of the in vivo 90-day tests are not performed any longer today to get worldwide commercial authorizations, especially for GMO with "stacked events" (i.e., producing one or several insecticides and tolerating one or two herbicides), and this is even more seriously inadequate since the so-called "cocktail effects" are not taken into consideration.

The same controversy took place (February 2010) in India, in relation to the authorization process for a transgenic eggplant that produces a new Bt insecticide. This authorization was based on three-month tests on three mammals and other animals for shorter times, which presented significant biological effects after this GM consumption [10, 25]. The same arguments were used in the debate in India. But in this case, the government decided to take the time to study chronic health effects, following our expertise, and therefore to implement a moratorium [26].

In the present case, we wish to underline that the commercial GMOs in question contain pesticide residues, some of which have been demonstrated as human cellular endocrine disruptors at levels around 1000 times below their presence in some GM feed [27]. Such Roundup residues are present in more than 80% of edible cultivated GMOs. This does not exclude other possible effects.

As a conclusion, we call for the promotion of transparent, independent and reproducible health studies for new commercial products, the dissemination of which implies consequences on a large scale. Lifetime studies for laboratory animals consuming GMOs must be performed, by contrast to what is done today, like the two-year long tests on rats for some pesticides or some drugs. Such tests could be associated to transgenerational, reproductive or endocrine research studies. And moreover, shortcomings in experimental designs may raise major questions on other chemical authorizations.

22: Another route for horizontal gene transfer from GM plants

This is a highly technical, understated but nonetheless important paper which again shows that horizonal gene transfer from GM crops into other living organisms actually happens. Beneath the abstract we reproduce a note from Ignatio Chapela.

Investigating Agrobacterium-Mediated Transformation of Verticillium albo-atrum on Plant Surfaces

Claire J. Knight, Andy M. Bailey, Gary D. Foster* PLoS ONE 5(10): e13684. doi:10.1371/journal.pone.0013684
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0013684

Abstract

Agrobacterium tumefaciens has long been known to transform plant tissue in nature as part of its infection process. This natural mechanism has been utilised over the last few decades in laboratories world wide to genetically manipulate many species of plants. More recently this technology has been successfully applied to non-plant organisms in the laboratory, including fungi, where the plant wound hormone acetosyringone, an inducer of transformation, is supplied exogenously. In the natural environment it is possible that Agrobacterium and fungi may encounter each other at plant wound sites, where acetosyringone would be present, raising the possibility of natural gene transfer from bacterium to fungus.

Methodology/Principal Findings

We investigate this hypothesis through the development of experiments designed to replicate such a situation at a plant wound site. A. tumefaciens harbouring the plasmid pCAMDsRed was co-cultivated with the common plant pathogenic fungus Verticillium albo-atrum on a range of wounded plant tissues. Fungal transformants were obtained from co- cultivation on a range of plant tissue types, demonstrating that plant tissue provides sufficient vir gene inducers to allow A. tumefaciens to transform fungi in planta.

Conclusions/Significance

This work raises interesting questions about whether A. tumefaciens may be able to transform organisms other than plants in nature, or indeed should be considered during GM risk assessments, with further investigations required to determine whether this phenomenon has already occurred in nature.

Comment by Ignacio Chapela, Berkeley/Tromsø

The careful and understated presentation, beginning with the title, belies research results that I think should be considered a major landmark in the growing evidence demonstrating how little we know about the ecological consequences of transgenesis, in particular the potential for horizontal gene transfer in real field situations. It also shows a definite and probably very important source of concern, the real possibility that DNA vectored into plants could move out, with full reproductive capacity, via a microbial route into the genomic environment far and beyond the immediate space and phylogeny of the host plant. Any environmental evaluation of field releases should now be required to seriously consider this possibility.

The research for this paper is carefully conceived and conducted, using various sources of confirmatory evidence. The frequency of "spontaneous" transformations out of the bacterium and into the fungus (2 out of 17, 1 out of 15, 10 out of 31 and 14 out of 42 trials in various repetitions) is exceedingly high. Although the paper demonstrates the transfer "only" from whole bacterial cells onto fungal spores (or hyphae), a precautionary approach should dictate that the possibility be also considered that transfers could occur through back-transformation, since much of the Agrobacterium wherewithal necessary to accomplish it is present in the transgenic plant. It is also known that whole Agrobacterium can "hide" through the process of regeneration of plants out of callus in the transgenesis process, providing accessible cells for the transformation, and of course encounters of Agrobacterium and different fungi (and other organisms?) at a plant-wound site must be considered common in the field.

Finally, the authors minimize the possible importance of their findings (perhaps appropriately so, especially to avoid a firestorm over their heads) by suggesting that there should be no biological consequences to the transformation unless the transferred DNA provides some measurable advantage to the carrier fungus. I disagree: We know that there are (a) many examples of apparently "silent" DNA that nevertheless has very important consequences, and (b) many functions of transgenic DNA that may not be predicted by the designs of the people doing the original transformations. DNA does not

necessarily need to give an advantage to the carrier; all it needs to do is survive and reproduce. It is unwarranted arrogance to suggest that we know what its functions may be or indeed may become downwind, downtime and down across the phylogenetic landscape.

23: GM Safety tests --- too short, but still revealing organ damage

The paper reproduced below is of great importance, since it demonstrates that even with the use of 90-day feeding studies (which are far too short to reveal significant chronic effects on animal physiology) cell damage to the vital organs of animals fed on GM materials is apparent. The results of 19 feeding studies are reviewed. The recorded damage is generally dismissed by EFSA and other regulators as being "within the range of statistical variation" or as "statistically significant but not biologically significant" ---- and Seralini and his colleagues are highly critical of this complacency. They say that the damage to organs (particularly kidneys and liver) is real, and should be a cause of great concern to EFSA -- and they recommend a transformation of the safety assessment protocols, including lifetime and multi-generational animal studies.

Genetically modified crops safety assessments: present limits and possible improvements

Gilles-Eric Seralini, Robin Mesnage, Emilie Clair, Steeve Gress, Joël S de Vendômois and Dominique Cellier
Environmental Sciences Europe 2011, 23:10 doi:10.1186/2190-4715-23-10http://www.enveurope.com/content/23/1/10/#ins1

Abstract

Purpose

We reviewed 19 studies of mammals fed with commercialized genetically modified soybean and maize which represent, per trait and plant, more than 80% of all environmental genetically modified organisms (GMOs) cultivated on a large scale, after they were modified to tolerate or produce a pesticide. We have also obtained the raw data of 90-day-long rat tests following court actions or official requests. The data obtained include biochemical blood and urine parameters of mammals eating GMOs with numerous organ weights and histopathology findings.

Methods

We have thoroughly reviewed these tests from a statistical and a biological point of view. Some of these tests used controversial protocols which are discussed and statistically significant results that were considered as not being biologically meaningful by regulatory authorities, thus raising the question of their interpretations.

Results

Several convergent data appear to indicate liver and kidney problems as end points of GMO diet effects in the above-mentioned experiments. This was confirmed by our meta-analysis of all the in vivo studies published, which revealed that the kidneys were particularly affected, concentrating 43.5% of all disrupted parameters in males, whereas the liver was more specifically disrupted in females (30.8% of all disrupted parameters).

Conclusions

The 90-day-long tests are insufficient to evaluate chronic toxicity, and the signs highlighted in the kidneys and livers could be the onset of chronic diseases. However, no minimal length for the tests is yet obligatory for any of the GMOs cultivated on a large scale, and this is socially unacceptable in terms of consumer health protection. We are suggesting that the studies should be improved and prolonged, as well as being made compulsory, and that the sexual hormones should be assessed too, and moreover, reproductive and multigenerational studies ought to be conducted too.

Background, aim, and scope

Recently, an ongoing debate on international regulation has been taking place on the capacity to predict and avoid adverse effects on health and the environment for new products and novel food/feed (GMOs, chemicals, pesticides, nanoparticles, etc.). The health risk assessments are often, but not always, based on the study of blood analyses of mammals eating these products in subchronic tests, and more rarely in chronic tests. In particular, in the case of GMOs, the number and nature of parameters assessed, the length of the necessary tests, the statistics used and their interpretations are the subject of

controversies, especially in the application of Organization of Economic Cooperation and Development (OECD) norms. Confusion is perceived even in regulatory agencies, as in the European Food Safety Authority (EFSA) GMO panel working group and its guidelines. Doubt has arisen on the role and necessity of animal feeding trials in safety and nutritional assessments of GM plants and derived food and feed [1]. Based on the literature data, EFSA first admitted (p. S33) that for other tests than GMOs: "For 70% (57 of 81) of the studies evaluated, all toxicological findings in the 2-year tests were seen in or predicted by the 3-month subchronic tests". Moreover, they also indicated (p. S60) that "to detect effects on reproduction or development [...] testing of the whole food and feed beyond a 90-day rodent feeding study may be needed." We fully agree with these assumptions. This is why we think that in order to protect large populations from unintended effects of novel food or feed, imported or cultivated crops on a large scale, chronic 2-year and reproductive and developmental tests are crucial. However, they have never been requested by EFSA for commercial edible crops. We therefore wish to underline that in contrast with the statements of EFSA, all commercialized GMOs have indeed been released without such tests being carried out, and as it was the case recently with maize stacked events without 90-day in vivo mammalian tests being conducted. GM stacked events have the cumulated characteristics of first generation of GMOs (herbicide tolerance and insecticide production), which are mostly obtained by hybridization. For instance, Smarstax maize contains two genes for herbicide tolerance and six genes for insecticide production. In fact, this contradictory possibility was already highlighted in the same review by EFSA (p. S60), when substantial equivalence studies and other analyses were performed: "animal feeding trials with rodents [...] adds little if anything [...], and is not recommended." This is why, in this work we will analyze and review deficiencies in GMO safety assessments, not only performed by biotech companies, but also by regulatory agencies.

We will focus on the results of available 90-day feeding trials (or more) with commercialized GMOs, in the light of modern scientific knowledge. We also suggest here an alternative to conventional feeding trials, to understand the biological significance of statistical differences. This approach will make it possible to avoid both false negative and false positive results in order to improve safety assessments of agricultural GMOs before their commercialization for cultivation and food/feed use and imports.

Overview of the safety studies of GMOs performed on mammals

Our experience in scientific committees for the assessment of environmental and health risks of GMOs and in biological, biostatistical research, and medicine, as well as in the research relative to side effects [2-6] allowed us to review and criticize mammalian feeding trials with GMOs and make new proposals. Mammalian feeding trials have been usually but not always performed for regulatory purposes in order to obtain authorizations or commercialization for GM plant-derived foods or feed. They may have been published in the scientific literature afterwards; however, without public access to the raw data.

We have obtained, following court actions or official requests, the raw data of several 28- or 90-day-long safety tests carried out on rats. The thing we did was to thoroughly review the longest tests from both a biostatistical and a biological point of view. Such studies often analyze the biochemical blood and urine parameters of mammals eating GMOs, together with numerous organ weights and histopathology. We have focused our review on commercialized GMOs which have been cultivated in significant amounts throughout the world since 1994 (Table 1).

We observe and emphasize that all the events in Table 1 correspond to soybean and maize which constitute 83% of the commercialized GMOs, whilst other GMOs not displayed in the table, but still commercialized, are canola or cotton. However, they are not usually directly consumed [7]. Only Sakamoto's and Malatesta's studies have been more than 90 days long (104 weeks and 240 days with blood analyses in Japanese for the first one). Moreover, such tests are not obligatory yet for all GMOs. No detailed blood analysis is available for Malatesta's study, as it mostly includes histochemistry at the ultrastructural level; moreover, the latter tests have not been used to obtain the commercial release by the firm. However, this work has been performed by researchers independent from the GMO industry; it is an important element to take into account for an objective interpretation of the facts, as pointed out in the case of the risk assessments conducted by regulatory agencies with Bisphenol A.

For instance in the latter case, it was observed that none of the industry-funded studies showed adverse effects of Bisphenol A, whereas 90% of government-funded studies showed hazards at various levels and various doses [8]. However, regulatory agencies still continue to refer only to industry-funded studies because they are supposed to follow OECD norms, even if such standards are not always appropriate for the detection of environmental hazards [9]. In this paper, Myers et al. showed that hundreds of laboratory animals and cell culture studies were rejected by regulatory authorities because they did not follow the Good Laboratory Practices (GLP). The Food and Drug Administration and EFSA have based their final decision on two industry-funded studies, claiming that they were superior to the others because they followed GLP. Yet, GLP are based on ancient paradigms. They have serious conceptual and methodological flaws, and do not take into account the latest knowledge in environmental sciences. For example, in the case of Bisphenol A assessment, the animal models used are known

to be insensitive to estrogen (CD-1 mouse). Also, assays and protocols in some OECD guidelines are out of date and insensitive. It is obvious that new product assessments should be based on adapted studies using state-of-the-art experiments. The significant gap between scientific knowledge and regulations should be filled also in the case of GMOs [9]. Therefore, some tests presented here show controversial results or statistically significant results that were not considered as biologically significant by EFSA, raising the question of their interpretation.

Table 1. Review of the longest chronic or subchronic toxicity studies in mammals fed with commercialized GM soybean and maize representing more than 80% of edible GMOs (2010).

First of all, the data indicating no biological significance of statistical effects in comparison to controls have been published mostly by companies from 2004 onwards, and at least 10 years after these GMOs were first commercialized round the world. This is a matter of grave concern. Moreover, only three events were tested for more than 90-days in feeding experiments or on more than one generation. This method was not performed by industries which conducted 90-day tests (with blood and organ analyses), but it was in some cases only. However, a 90-day period is considered as insufficient to evaluate chronic toxicity [1,5]. All these commercialized cultivated GMOs have been modified to contain pesticides, either through herbicide tolerance or by producing insecticides, or both, and could therefore be considered as "pesticide plants." Almost all GMOs only encode these two traits despite claims of numerous other traits. For instance, Roundup ready crops have been modified in order to become insensitive to glyphosate. This chemical together with adjuvants in formulations constitutes a potent herbicide. It has been used for many years as a weed killer by blocking aromatic amino acid synthesis by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Most Roundup ready plants have been modified thanks to the insertion of a mutated EPSPS gene coding for a mutated enzyme, which is not inhibited by glyphosate. Therefore, GM plants exposed to glyphosate-based herbicides such as Roundup do not specifically degrade glyphosate. They can even accumulate Roundup residues throughout their life, even if they excrete most of such residues. Glyphosate and its main metabolite AMPA (with its own toxicity) are found in GMOs on a regular and regulatory basis [10,11]. Therefore, such residues are absorbed by people eating most GM plants (as around 80% of these plants are Roundup tolerant). On the other hand, about 20% of the other GMOs do synthesize new insecticide proteins through the insertion of mutated genes derived from Bacillus thuringiensis (Bt). Usually, pesticides are tested over a period of 2 years on a mammal, and this quite often highlights side effects. Additionally, unintended effects of the genetic modification itself cannot be excluded, as direct or indirect consequences of insertional mutagenesis, creating possible unintended metabolic effects. For instance, in the MON810 maize, the insertion of the transgene in the ubiquitine ligase gene caused a complex recombination event, leading to the synthesis of new RNA products encoding unknown proteins [12]. Thus, genetic modifications can induce global changes in the genomic, transcriptomic, proteomic, or metabolomic profiles of the host. The frequency of such events in comparison to classical hybridization is by nature unpredictable. In addition, in a plant producing a Cry1Ab- modified toxin, a metabolomic study [13] revealed that the transgene introduced indirectly 50% changes in osmolytes and branched amino acids.

Review of statistical effects after GMO consumption

Some GMOs (Roundup tolerant and MON863) affect the body weight increase at least in one sex [2,14]. It is a parameter considered as a very good predictor of side effects in various organs. Several convergent factors appear to indicate liver and kidney problems as end points of GMO diet effects in these experiments [2,5,15,16]. This was confirmed by our meta-analysis of all in vivo studies published on this particular topic (Table 2). The kidneys are particularly affected, concentrating 42% of all parameters disrupted in males. However, other organs may be affected too, such as the heart and spleen, or blood cells [5].

Table 2. Meta-analysis of statistical differences with appropriate controls in feeding trials

Liver parameters

For one of the longest independent tests performed, a GM herbicide- tolerant soybean available on the market was used to feed mice. It caused the development of irregular hepatocyte nuclei, more nuclear pores, numerous small fibrillar centers, and abundant dense fibrillar components, indicating increased metabolic rates [17]. It was hypothesized that the herbicide residues could be responsible for that because this particular GM plant can absorb the chemicals to which it was rendered tolerant. Such chemicals may be involved in the above- mentioned pathological features. This became even clearer when Roundup residues provoked similar features in rat hepatic cells directly in vitro [18]. The reversibility observed in some instances for these parameters in vivo [19] might be explained by the heterogeneity of the herbicide residues in the feed [20]. Anyway, these are specific parameters of ultrastructural dysfunction, and the relevance is clear. The liver is reacting. The Roundup residues have been also shown to be toxic for human placental, embryonic, and umbilical cord cells [21-23]. This was also the case for hepatic human cell lines in a comparable manner, inducing nuclei and membrane changes, apoptosis and

necrosis [24].

The other major GMO trait has to do with the mutated (mBt) insecticidal peptidic toxins produced by transgenes in plants. In this case, some studies with maize confirmed histopathological changes in the liver and the kidneys of rats after GM feed consumption. Such changes consist in congestion, cell nucleus border changes, and severe granular degeneration in the liver [16]. Similarly, in the MON810 studies, a significantly lower albumin/globulin ratio indicated a change in hepatic metabolism of 33% of GM-fed male rats (according to EFSA opinion on MON810 and [5]). Taken together, the results indicate potential adverse effects in hepatic metabolism. The insecticide produced by MON810 could also induce liver reactions, like many other pesticides. Of course, the mCry1Ab and other mBt (mutated Bt toxins derived from native Bacillus thuringiensis toxins) in GMOs are proteic toxins; however, these are modified at the level of their amino acid sequence by biotechnologies and introduced by artificial vectors, thus these could be considered as xenobiotics (i.e., a molecule foreign to life). The liver together with the kidneys are the major reactive organs in case of food chronic intoxication.

Kidney parameters

In the NK603 study, statistically significant strong urine ionic disturbances and kidney markers could be explained by renal leakage [5], which is well correlated with the effects of glyphosate-based herbicides (like Roundup) observed on embryonic kidney cells [23]. This does not exclude metabolic effects indirectly due to insertional mutagenesis linked to the plant transformation. Roundup adjuvants even stabilize glyphosate and allow its penetration into cells, which in turn inhibit estrogen synthesis as a side effect, cytochrome P450 aromatase inhibition [21]. This phenomenon changes the androgen/ estrogen ratio and may at least, in part, explain differential impacts in both sexes.

Kidney dysfunctions are observed with mBt maize producing mutated insecticides such as in MON863. For instance, we quote the initial EFSA report: "Individual kidney weights of male rats fed with the 33% MON863 diet were statistically significantly lower compared to those of animals on control diets", "small increases in the incidences of focal inflammation and tubular regenerative changes in the kidneys of 33% MON863 males." This was confirmed by the company tests [25] and another counter analysis revealed disrupted biochemical markers typical of kidney filtration or function problems [2]. The first effects were not always but sometimes greater than the ones with non- isogenic maize (called reference lines), which contain different salts, lipids, or sugars. Moreover, both results described are different between males and females; this is quite usual in liver or kidney pesticide reactions. These facts do not exclude that such effects can be considered as treatment-related. Other studies also confirmed effects on kidneys. Tubular degeneration and not statistically significant enlargement in parietal layer of Bowman's capsules were also observed with GM maize fed rats [16].

Last but not least, a total of around 9% of parameters were disrupted in a meta-analysis (Table 2). This is twice as much as what could be obtained by chance only (generally considered as 5%). Surprisingly, 43.5% of significant different parameters were concentrated in male kidneys for all commercialized GMOs, even if only around 25% of the total parameters measured were kidney-related. If the differences had been distributed by chance in the organs, not significantly more than 25% differences would have been found in the kidney. Even if our own counter analysis is removed from the calculation, showing numerous kidney dysfunctions [2], around 32% of disturbances are still noticed in kidneys.

Discussion

Need for chronic tests and other tests

Chronic toxicity tests (both with males and females) and reproductive tests with pregnant females and then with the developing progeny over several generations (none of these steps exist at present) are called as a whole the Toxotest approach (or Risk management test, see "Details on the new suggested Toxotest approach"). This could address the long-term physiological or pathological relevance of the previous observations. The physiological interpretations of 90-day-based effects are otherwise somewhat limited. These studies should be complementary to the present regulations or the Safotest and the sentinel test suggested by EFSA [1]. The Toxotest could provide evidence of carcinogenic, developmental, hormonal, neural, and reproductive potential dysfunctions, as it does for pesticides or drugs. Additionally, it is obvious that the 90-day-long trials on mature animals performed today cannot scientifically replace the sensitivity of developmental tests on neonates. A good example is the gene imprinting by drugs that will be revealed only at maturity; this is an important subject of current research, and many findings have been reported for some chemicals such as bisphenol A [26,27]. Even transgenerational effects occur after epigenetic imprinting by a pesticide [28]. These effects cannot be detected by classical 90-day feeding trials and will be visible after many decades by epidemiology in humans if any, as illustrated in the case of diethylstilbestrol, which induced female genital cancers among other problems in the second generation [29]. The F3 multigenerational study for a GMO (Table 1) was too rarely performed. This is why, because of the number of parameters disrupted in adult mammals

within 90 days, the new experiments should be systematically performed to protect the health of billions of people that could consume directly or indirectly these transformed products.

The acute toxicity approach (less than a month of investigations on rodents with high doses) may give effects which are more proportional to the dose, as it might correspond to a rapid poisoning of the animals, generally with force-fed experiments. However, for many pesticide studies in the scientific literature, some long-term side effects of pesticides at environmental doses are described, which are not apparent in short-term experiments [30]. Classical toxicology is quite often based on the concept of revealing linear dose-responses as defined by Paracelsus, which generally fails to evidence U or J curves observed after hormonal sex-specific disruptions. Moreover, the effects of mixtures are also neglected in long-term studies, when supposed active principles of pesticides are not assessed with their adjuvants, which also are present as residues in GMOs. Such pesticides may have the capacity to disrupt the "cell web", i.e., to interfere with a signaling pathway, and this could be unspecific. For instance Roundup is known to disrupt the EPSPS in plants, but is also known to interact with the mammalian ubiquist reductase [21] common and essential to cytochromes P450, a wide class of detoxification enzymes. The so-called Roundup active principle, glyphosate, acts in combination with adjuvants to increase glyphosate-mediated toxicity[21,31], and this may apply to other environmental pollutants [22]. Moreover, all new metabolites in edible Roundup ready GMOs, as acetyl-glyphosate for the new GAT GMOs, have not been assessed for their chronic toxicity [11], and we consider this as a major oversight in the present regulations.

Therefore, as xenobiotic effects are complex, the determination of their toxic effects cannot be determined using a single method, but rather converging pieces of evidence. In GMO risk assessment, the protocols must be optimized to detect side effects, in particular for herbicide-treated GM plants. These cannot be reduced to GM assessment on one side and herbicide residues with any diet on the other side, but unfortunately this has been the case, and this approach has been promoted up to now by regulatory authorities.

In fact, it is impossible, within only 13 weeks, to conclude about the kind of pathology that could be induced by pesticide GMOs and whether it is a major pathology or a minor one. It is therefore necessary to prolong the tests, as suggested by EFSA, since at least one third of chronic effects visible with chemicals are usually new in comparison to the ones highlighted in subchronic studies [1]. The so-called Toxotests, which are supposed to include the studies of chronic pathologies in particular, should be performed on three mammalian species, with at least one non-rodent, similar to the type of rodents used for pesticides and drugs. However, the chronic feeding tests for GMOs cannot be based on the no observed adverse effect level, nor on the lowest observed adverse effect level approach, as in classical toxicology. There are several reasons for that. There is not only one chemical, but also several unknown metabolites and components, in Roundup tolerant varieties for instance, and therefore toxicity is enhanced thanks to the fact that they are mixed together. There is also no possibility of increasing the doses of GMOs in an equilibrated diet over an acceptable level. The diets should be rather representative of an equilibrated diet with GMOs like it could be the case in a real population in America. To prolong 90-day subchronic tests with three normal doses of GM in the diet (11%, 22%, 33% for instance) is the solution.

Sex- or dose-specific pathological effects are common

When there is a low or environmental dose impregnation of the feed (with a pesticide GM plant for instance), the chronic effects could be more differentiated according to the sex, the physiological status, the age, or the number of intakes over such and such a period of time in the case of a drug. These parameters (chronic intake, age of exposure, etc.) are more decisive for pathologies like cancers, than the actual quantity of toxin ingested in one intake. This is in part because the liver, kidney, and other cytochrome P450-rich organs are concerned for long-term metabolism and detoxification, and this phenomenon is hormone dependent. It is also due to the process of carcinogenesis or hormone-sensitive programming of cells [32]. The liver for instance is a sex differentiated organ as far as its enzymatic equipment is concerned [4]. An effect in subchronic or chronic tests cannot be disregarded on the rationale that it is not linear to the dose (or dose-related) or not comparable in genders. This would not be scientifically acceptable. However, this reasoning was adopted both by companies and EFSA for several GMOs, as underlined by Doull et al. [33]. Indeed, most xenobiotics or pollutants may have non-linear effects, and/or may have sex- and age-specific impacts.

One of the pivotal requirements for regulators nowadays, in order to interpret a significant difference as biologically relevant, is to observe a linear dose-response. This allows them to deduce a causality. However, this dose-response cannot be studied with only two points, which is nonetheless the case for all major commercial GMOs today, which are given in the diet in 11% and 33% concentrations only, in subchronic tests. This is true overall if no preliminary data has been obtained to choose the given doses, which is the case in regulatory files. As we have already emphasized, most of pathological and endocrine effects in environmental health are not directly proportional to the dose, and they have a differential threshold of sensitivity in both sexes [34]. This is, for instance, the case with carcinogenesis and endocrine disruption.

Improving the knowledge on impacts of modified Bt toxins

One of the interpretations of the side effects observed (Tables 1 and 2) would be that the insecticide toxins in maize lines may have more pleiotropic or specific actions than originally supposed. The toxins could generate particular metabolites, either in the GM plant or in the animals fed with it. The Bt toxins in GMOs are new and modified, truncated, or chimerical in order to change their activities/ solubility in comparison to wild Bt. For instance, there is at least a 40% difference between the toxin in Bt176 and its wild counterpart [10]. None of the modified Bt toxins have been authorized separately for food or feed, neither has the wild Bt, and neither have they been tested by themselves on animal or human health to date. Even if some studies were performed, the receptors have not been cloned and the signaling pathways have not been identified as yet, nor required for authorizations, and the metabolism of these proteins in mammals are unknown [35]. Thus, the argument about "safe use history" of the wild Bt protein (not designed for direct consumption, in contrast to several GMOs) cannot, on a sound scientific basis, be used for direct authorizations of the above-cited GM corns, overall without in vivo chronic toxicity tests (or Toxotest approach), as it is requested for a pesticide. Some improvements may even be included with regard to pesticide legislation, since these human modified toxins considered as xenobiotics are continuously produced by the plants devoted to consumption.

The proteins usually compared (modified Bt toxins and wild ones) are not identical, and the tests on human cells of Bt proteins are not performed nor are they requested by authorities. Their stability has been assessed in vitro, and GM insecticide toxins are never fully digested in vivo [36]. If some consumers suffer from stomach problems or ulcers, the new toxins will possibly act differently; the digestion in children could be affected too; however, these GMOs could be eaten anywhere and all proteins are never fully decomposed in amino acids by the digestive tract.

Details on the new suggested Toxotest approach

The suggested Toxotest would basically include an extension of the existing 90-day tests, but with at least three doses plus controls (0%, 11%, 22%, 33% GMOs for instance; today the equilibrated diets tested contain 0%, 11%, and 33% GMOs in the best regulatory tests). The purpose would be to characterize scientifically the dose-response approach. The latter cannot be taken seriously with only two GM doses. The final goal is the best health protection for the population without really possible clinical trials, in our case for practical and ethical reasons. There is also no epidemiological follow-up for lack of traceability and labeling in GM-producing American countries. In addition, the fact that the Toxotest includes the best possible toxicological approach will also be in favor of the biotechnology economy and the European Community because it is more expensive to address an issue concerning a whole population afterwards, rather than to work with laboratory animals beforehand; it is also more ethical to work on rats and other mammalian experiments, in order to get the relevant information, rather than to give pesticide plants directly to humans on a long-term basis.

As previously underlined, the health effects such as those suggested in Table 2 (if any, are revealed by adapted studies, such as Safotests or Toxotests), could only be due to two possibilities:

Firstly, the side effects may be directly or indirectly due to a pesticide residue and/or its metabolites. The direct effect is about the pesticide effect on the consumer, and the indirect one is about a metabolism disruption that it has provoked within the plant first. This could not be visible by a detailed compositional analysis, such as the one performed to be assessed by a substantial equivalence study. This concept is not a well-defined one (how many cultivations of crops, over how many years, under which climate, and to measure what precise parameters).

Secondly, the pathological signs may be due to the genetic transformation itself, its method provoking either insertional mutagenesis or a new metabolism by genetic interference. This is the reason why separating intended effects (the direct genetic trait consequence itself) from unintended effects (linked to biotechnology, e.g., insertional mutagenesis), such as spiking the control diet with the purified toxin in the Toxotest approach, is clearly inadequate. It could work in the case of a direct action of the toxin in mammals, but conversely one could not conclude, between an insertional mutagenesis and a specific metabolic action in the plant due to the toxin. However, this is more a research question about the mode of genesis of an effect on health, and new research avenues could be, for instance, to compare the GM diet with or without herbicide treatment in long- term tests with the isogenic control diet including herbicide residues added. This is only necessary for the understanding of the potential signs of toxicity and not for a conclusion of the Safotest or the Toxotest, which would rather suggest, if positive, excluding immediately the corresponding GMO from food and feed.

Improvement of statistical analysis

A serious experimental design is based on a proper choice of the groups, with only one question studied per experiment if possible, and balanced sample sizes. In several authorized GMOs, the sample sizes appear inadequate in 90 days: ten animals per group for the measurement of biochemical parameters out of 20, as performed by the major stakeholders, and accepted by EFSA for MON863, MON810, or NK603 for instance. This is too limited a size to ensure that parametric statistical methods

used by the company are reliable. Moreover, an important discrepancy between GMO-treated rats (40 measured out of 80) and the total number of animals (400) renders more difficult the evidencing of relevant effects, and confusion factors are brought in at the same time with six different reference diets in addition to the two normal control groups as performed in three commercialized GMOs at least [5,6]. This introduces new uncontrolled sources of variability about the effects of the diets and new unnecessary questions not relevant to the GMO safety. The representation of a standard diet with multiple sources could have been studied with only one control group of the same size than the GMO group, eating a mix of six different regular non-GM diets.

Several questions have been raised by companies and authorities as well as comments on statistically significant effects that would supposedly not be biologically meaningful. A subjective part is introduced at this level because it is necessary to take into account the context and the general and detailed knowledge of toxicology and endocrine disruption, as EFSA underlines. This might be highly expert dependent. This is why, to avoid or prevent any misunderstanding, we suggest, in addition to a new statistical approach based on classical methods, to analyze the 90-day tests, even with control and reference diets called the "SSC method" (according to the initials of the authors in [2]).

Briefly, following the necessity to model and analyze the growth curves, multivariate data analysis and data mining of all parameters can be used to correlate, cluster, and select meaningful variables. This kind of approach is not performed at all today. Thereafter, the detailed comparison between GM-treated and control groups, fed with the near isogenic line (because the real isogenic line does not often exists anymore), will necessarily be followed by the study of specific diet effects, when there are non-substantially equivalent diets for reference groups. For that purpose, the controls will be first compared using multivariate inference with reference groups, and thereafter, similarly GMO-treated groups with reference groups. The significant differences linked to the GMO and/or the composition of the diet will be classified according to organ and function. The results will appear more clearly than with the simple statistics accepted today by the authorities (that is, comparison of the highest GM dose group with the mean value of all six control groups), and will reveal in addition new information, as it can be demonstrated.

As recommended by EFSA, an appropriate and relevant statistical analysis is crucial. It should follow the following series of steps, allowing the use of several methods depending on the questions raised:

- Obtaining and modeling the growth curves and feed consumption, assessed by non-linear regression, validation, and statistical comparisons in order to test if the curves are significantly different, thus taking into account individual variability. This necessitates the use of time series analysis, selection models, and non-parametric tests, Akaike Information Criteria and related methods. Water consumption should also be an important factor to follow-up and therefore better understand kidney and urine data.

- The study of dose-response predictions using non-linear regression should be the goal, but the only two doses generally used in these tests do not make it possible to evidence linearity as we indicated. Moreover, in the cases where there are not dose-related trends or relationships using the two doses mentioned, the absence of linear dose-response curves cannot be a reason to neglect the effects. For instance, as previously cited, U or J curves may be characteristic of endocrine effects [37], and spiky irregular curves may be detected in carcinogenesis.

- Simultaneous analysis of all observed variables: multivariate data analysis, principal component analysis, correlations analysis, factorial analysis and clustering

- Multivariate comparisons of the different variables: hypothesis testing, multiple ways ANOVA, MANOVA, and others to determinate if the groups differ relative to the different questions: specific GMO effect or diet effect per se. To evidence a detail, when comparing two mean values, SEM should be calculated to determine confidence intervals; however, SD have been used up to now by the company for MON863 and NK603 files for instance.

Apart from empirical curves in some instances, ANOVA and univariate hypothesis testing only the GMO effect, none of the other statistical approaches is currently used nor requested by the authorities.

Human tests and post-market monitoring

For the record, it must be said that very few tests on humans have been carried out up to now. Moreover, epidemiological studies are not feasible in America, since there is no organized traceability of GMOs anywhere on the continent, where, by far, most of edible GMOs are cultivated (97%). As a consequence, a post-market monitoring (PMM) is offered to the population. The Cartagena Biosafety Protocol identifying GMOs at the borders of a country has now been signed by over 150 countries, including the member states of the European Union. PMM may have some value in detecting unexpected adverse effects. It could therefore be considered as a routine need. This approach makes it possible to collect information related to risk management. It can be relied upon as a technique for monitoring adverse events or other health outcomes related to the

consumption of GM plant-derived foods, provided that the Toxotest approach, together with the SSC method, should have already been applied. The PMM should be linked with the possibility of detecting allergenicity reactions to GMOs in routine medicine, thanks to the very same routine cutaneous tests that should be developed prior to large-scale commercialization. A screening of serum banks of patients with allergies could be also put forward in order to search for antibodies against the main GMOs and not only their transgenic proteins, since they may induce secondary allergenic metabolites in the plant not visible in the substantial equivalence study.

The traceability of products from animals fed on GMOs is also crucial. The reason for this is because they can develop chronic diseases which are not utterly known today. Such possible diseases could be linked to the hepatorenal toxicity observed in some GMO-related cases (Table 1).

Moreover, labeling animals fed on GMOs is therefore necessary because some pesticide residues linked to GMOs could pass into the food chain and also because nobody would want to eat disabled or physiologically modified animals after long-term GMOs ingestion, even if pesticides residues or DNA fragments are not toxic nor transmitted by themselves.

Conclusion

Transcriptomics, proteomics and other related methods are not ready yet for routine use in the laboratories, and moreover they may be inappropriate for studying toxicity in animals, and could not in any way replace in vivo studies with all the physiological and biochemical parameters that are measured with organs weight, appearance, and histology. By contrast, afterwards, new approaches could well help to explain pathological results or action mechanisms of pesticides present in the GM plants or GM-fed animals, if found.

To obtain the transparency of raw data (including rat blood analyses) for toxicological tests, maintained illegally confidential, is crucial. It has also become crucial to apply objective criteria of interpretation like the criteria described here: sex-specific side effects or non-linear ones. Such data can be put online on the EFSA website with a view to provide a fuller review to the wider scientific community, and in order to better inform the citizen to make biotechnologies more socially acceptable. Since fundamental research is published on a regular basis, it should be the same for this kind of applied research on long-term health effects, as suggested by the CE/2001/18 and the corresponding 1829/2003 regulations.

We can conclude, from the regulatory tests performed today, that it is unacceptable to submit 500 million Europeans and several billions of consumers worldwide to the new pesticide GM-derived foods or feed, this being done without more controls (if any) than the only 3-month- long toxicological tests and using only one mammalian species, especially since there is growing evidence of concern (Tables 1 and 2). This is why we propose to improve the protocol of the 90-day studies to 2-year studies with mature rats, using the Toxotest approach, which should be rendered obligatory, and including sexual hormones assessment too. The reproductive, developmental, and transgenerational studies should also be performed. The new SSC statistical method of analysis is proposed in addition. This should not be optional if the plant is designed to contain a pesticide (as it is the case for more than 99% of cultivated commercialized GMOs), whilst for others, depending on the inserted trait, a case-by-case approach in the method to study toxicity will be necessary.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GES designed and coordinated the review. RM participated in the drafting of the manuscript and final version. EC, SG, JSV and DC helped the writing, compiling the literature, revising in details and proofreading the manuscript. All authors read and approved the final manuscript.

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References: **http://www.enveurope.com/content/23/1/10/#ins1**

24: Traces of GM Toxins in the blood of women

Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada.

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Abstract
Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3- MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

NOTE FROM GM WATCH:
Bt corn (maize) was developed by transferring cry1Ab from Bacillus thuringiensis (Bt) into corn. It is to be found in the most common GM corn - Monsanto's Bt MON810 (marketed with the trade name YieldGard) - a corn genetically engineered to resist corn borers by producing its own insecticide, the Cry1Ab toxin. Global production of Bt corn takes place on many millions of hectares worldwide and many different types of foods contain Bt corn. In the European Union, seven countries - Austria, Hungary, Greece, France, Luxembourg, Germany and Bulgaria have banned Mon810.
NOTE FROM GM-FREE CYMRU: This is potentially a very important study, since it incorporates the three main types of GM food crops, associated with Roundup or glyphosate (RR crops), Liberty or glufosinate (LL crops) and BT crops which have built-in toxins. Interestingly, the residues of Roundup and Liberty were found in the blood of non-pregnant women but not in the blood of pregnant women, whereas the reverse was true with respect to traces of BT toxins. This clearly requires further research.....
NOTE FROM A SPECIALIST IN THIS FIELD:
Here is my understanding of what the paper says:
Study participants: 30 pregnant women with vaginal deliveries and their just-born babies, and 39 healthy, fertile, non-pregnant women having tubal ligations. Age: average of 32.4 and 33.9 years respectively and there was no significant difference in age, or BMI between these two groups of women. Blood was taken from women pre- delivery and from babies from cord blood at delivery.
28 out of 30 (93%) pregnant women had Cry1Ab from GM corn in their blood and 24 out of 30 (80%) of their babies also had it in their blood. Of the women who had the Bt protein in their blood, 86% had passed it to their baby when the baby was in her uterus. 27 out of 39 (69%) of the non-pregnant women had it in their blood. A significantly higher proportion of pregnant women had the Bt protein in their blood than non-pregnant women (p=0.006).
My thoughts:
This Bt protein is not present in sweet corn (which can be eaten with minimum processing), it is only in certain varieties of GM maize such as MON810 and triple stack corn. Maize is usually strongly heated and/ or processed into corn chips, tacos, starch, high fructose corn syrup etc before it enters the human diet. Therefore, this paper shows that this GM protein can survive extensive food processing to enter the diet. It can then survive human digestion to enter the blood of the person eating it and then cross the placenta to enter the fetus.
The authors are suggesting that these women may have been exposed by eating meat contaminated with this protein. Therefore, they appear to be suggesting that GM corn, when fed to cattle, may survive digestion in the animal to enter the

meat of that animal. (There is already evidence that GM DNA can survive digestion in cows to enter their milk.) The GM protein in the meat then survives cooking, then survives the woman's digestive system to enter her blood, where it then crosses the placenta to enter the fetus.
If women have this in their blood, so would men. It indicates that between 69% and 93% of the Canadian population has this Bt toxin in their blood. The proportion is likely to be higher in the US population as their dietary exposure is likely to be higher. Not only does the US grow a great deal of GM corn, but they have a higher Hispanic population, which tends to have a higher maize consumption.
As it is unlikely that all of these women would have eaten this particular variety of GM corn within hours of their blood test, this indicates that once the Bt toxin enters the body, it may be quite long- lived there and may in fact accumulate in the body.

25: Roundup is an endocrine disruptor and has toxic effects

This paper below by Gasnier and others should be read alongside the paper by Orton et al on "endocrine activity." The latter authors selected 134 commonly used pesticides, and examined 37 of them for "receptor mediated anti-androgenic potency". Of these, 14 suspected of having AR antagonism were confirmed as anti-androgenic, together with 9 previously untested pesticides. In addition, 7 further compounds were conformed as being dangerous from the point of view of endocrine disruption. So 30 out of the 37 chemicals examined were potentially dangerous -- and were shown to have AR antagonism in vitro tests.
Glyphosate (number 22) was shown in the paper in the group of "known inactive androgen receptor antagonists", and one might assume therefore that the chemical is therefore harmless, or at least not damaging in this particular area. However, as Gasnier et al have pointed out in 2009, glyphosate is hardly ever sold as glyphosate, but normally as Roundup -- in various formulations. And these formulations, because of the adjuvants and other components used in the "recipes", are clearly toxic and are endocrine disruptors at sub- agricultural doses.
Both groups of authors call for urgent in vivo studies to confirm what they have found in the laboratory. There are two messages coming out of this work:
1. Roundup -- in various formulations -- is an endocrine disruptor with toxic effects, and it should be banned because of the residues known to exist in transgenic feed from HT (RR) crops.
2. Because 30 out of the 37 examined pesticides in the Orton et al study were found to have negative effects as endocrine disruptors, it is certain that there is a "cocktail" of these residues in the food supply chain, and that this must be linked to the decline in male reproductive health and sperm counts.
Danger signals, loud and clear -- but is anybody listening?

Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines

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Abstract
Glyphosate-based herbicides are the most widely used across the world; they are commercialized in different formulations. Their residues are frequent pollutants in the environment. In addition, these her- bicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue® , MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic (on ER��, ER��) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24 h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from

2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase tran- scription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbi- cides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.

Conclusion

In conclusion, according to these data and the literature, G-based herbicides present DNA damages and CMR effects on human cells and in vivo. The direct G action is most probably amplified by vesi- cles formed by adjuvants or detergent-like substances that allow cell penetration, stability, and probably change its bioavailability and thus metabolism (Benachour and Séralini, 2009). These deter- gents can also be present in rivers as polluting contaminants. The type of formulation should then be identified precisely in epidemi- ological studies of G-based herbicides effects (Acquavella et al., 2006). Of course to drive hypotheses on in vivo effects, not only dilution in the body, elimination, metabolism, but also bioaccumu- lation and time-amplified effects (Benachour et al., 2007b) should be taken into account. These herbicides mixtures also present ED effects on human cells, at doses far below agricultural dilu- tions and toxic levels on mitochondrial activities and membrane integrity. These doses are around residual authorized levels in transgenic feed, and this paper is the first clear demonstration of these phenomena in human cells. The in vivo ED classification of G- based herbicides with this molecular basis must be now carefully assessed.

http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info%3Adoi%2F10.1289%2Fehp.1002895

Widely Used Pesticides with Previously Unknown Endocrine Activity Revealed as in Vitro Anti-Androgens

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Abstract

Background: Evidence suggests that there is widespread decline in male reproductive health and anti-androgenic pollutants may play a significant role. There is also a clear disparity between pesticide exposure and endocrine disrupting data, with the majority of the published literature focused on pesticides that are no longer registered for use in developed countries.

Objective: The aim of this study was to utilise estimated human exposure data to select pesticides to test for anti-androgenic activity, focusing on highest use pesticides.

Methods: We used European databases to select 134 candidate pesticides based on highest exposure, followed by a filtering step according to known or predicted receptor mediated anti-androgenic potency, based on a previously published quantitative structure-activity relationship (QSAR) model. In total, 37 pesticides were tested for in vitro androgen receptor (AR) antagonism. Of these, 14 were previously reported to be AR antagonists ("active"), 4 were predicted AR antagonists using the QSAR, 6 were predicted to not be AR antagonists ("inactive"), and 13 with unknown activity, which were "out of domain" and therefore could not be classified with the QSAR ("unknown").

Results: All 14 pesticides with previous evidence of AR antagonism were confirmed as anti-androgenic in our assay and 9 previously untested pesticides were identified as anti-androgenic (dimethomorph, fenhexamid, quinoxyfen, cyprodinil, λ-cyhalothrin, pyrimethanil, fludioxonil, azinphos-methyl, pirimiphos-methyl). In addition, 7 compounds were classified as androgenic.

Conclusions:

Due to estimated anti-androgenic potency, current use, estimated exposure, and lack of previous data, we strongly recommend that dimethomorph, fludioxonil, fenhexamid, imazalil, ortho- phenylphenol and pirimiphos-methyl be tested for anti-androgenic effects in vivo. The lack of human biomonitoring data for environmentally relevant pesticides presents a barrier to current risk assessment of pesticides on humans.

26: Bt toxin has detrimental effect on GM plant growth & development

GM-Free Cymru Comment:

This is a very significant new study from India. It shows that GM plants (in this case those manipulated to be toxic to certain insects) are severely damaged by the GM process. "This is a completely unexpected finding," said the editor of the journal of Bioscience. Hmmm -- not sure about that. It has been well known for many years that Bt toxins in GM plants lose their efficacy in the killing of pests (both targeted and otherwise) over time -- which presumably means that the plant is seeking to reject the toxic inserts put into it by Monsanto and other GM scientists. Sorry -- I meant "GM technologists." Also, if a plant is bombarded with alien material it is bound to be stressed -- and in those circumstances, why should anybody be surprised that there will be abnormalities and mutations, that yields will drop off, and that the plants reproductive functions will also be compromised? GM plants are supposed to be distinctive, uniform and stable in order to pass the DUS test -- that is supposed to be mandatory in order for any GM variety to obtain approval in Europe. Is there a single GM variety on the market capable of passing the DUS test as it should be properly conducted? I doubt it..... since approvals are always based on the developer's own assurances and upon the absurd concept of "substantial equivalence."

GM watch comment:

A new study shows that expression of the Bt insecticidal toxin (cry1Ac) gene in GM Bt tobacco and cotton has a detrimental effect on the growth and development of the plant. The plants that express high enough levels of the insecticidal toxin to control pests grow and develop less well and are more likely to have abnormalities. The plants that express low levels of the toxin (and therefore are unlikely to be able to control pests) grow and develop better and are less likely to have abnormalities. It seems that GM Bt insecticidal plants may have an unintended built-in self-destruct mechanism. --- --- "The expression of Bt endotoxin Cry1Ac has detrimental effect on the in vitro regeneration as well as in vivo growth and development of tobacco and cotton transgenics"

by Rawat, P., A. K. Singh, et al. (2011). J Biosci 36(2): 363–376. **www.ias.ac.in/jbiosci/jun2011/363.pdf**

ABSTRACT High levels of expression of the cry1Ac gene from Bacillus thuringiensis cannot be routinely achieved in transgenic plants despite modifications made in the gene to improve its expression. This has been attributed to the instability of the transcript in a few reports. In the present study, based on the genetic transformation of cotton and tobacco, we show that the expression of the Cry1Ac endotoxin has detrimental effects on both the in vitro and in vivo growth and development of transgenic plants. A number of experiments on developing transgenics in cotton with different versions of cry1Ac gene showed that the majority of the plants did not express any Cry1Ac protein. Based on Southern blot analysis, it was also observed that a substantial number of lines did not contain the cry1Ac gene cassette although they contained the marker gene nptII. More significantly, all the lines that showed appreciable levels of expression were found to be phenotypically abnormal. Experiments on transformation of tobacco with different constructs expressing the cry1Ac gene showed that in vitro regeneration was inhibited by the encoded protein. Further, out of a total of 145 independent events generated with the different cry1Ac gene constructs in tobacco, only 21 showed expression of the Cry1Ac protein, confirming observations made in cotton that regenerants that express high levels of the Cry1Ac protein are selected against during regeneration of transformed events. This problem was circumvented by targeting the Cry1Ac protein to the chloroplast, which also significantly improved the expression of the protein.

Website: **http://www.gmwatch.org**

1. Gene alarm on GM crops The Telegraph (Calcutta, India) June 2 2011

http://bit.ly/iqVL2B

Indian scientists have discovered that the genetic modification of plants with a gene already used in crops worldwide may severely damage the plants, a surprising finding that may stir a debate on current crop biotechnology science. The scientists at the University of Delhi have shown that inserting a bacterial gene that makes a protein named Cry1Ac into genomes of plants appears to cause developmental defects, growth retardation and sterility in the plants. Several experimental and commercial genetically-modified plants, including GM cotton cultivated in India and other countries, make the Cry1Ac protein which is toxic to some insects. The insects die when they try to eat parts of these GM crops. The Delhi scientists have now shown through laboratory experiments that modifying cotton or tobacco with Cry1Ac has a detrimental effect on these plants. Their results have appeared in the Journal of Bioscience published this month by the Indian Academy of Sciences. "This is a completely unexpected finding," said Durgadas Kasbekar, a senior biologist with the Centre for Cellular and Molecular Biology in Hyderabad who was not associated with the study, but is the editor of the Journal of Bioscience. "Until this point, if you asked someone in the plant biotechnology community what the Cry1Ac toxin does in plants, they would say it kills insects. No one has yet demonstrated harm to plants as this study has done," Kasbekar told The Telegraph. The Delhi researchers say such observations may have been overlooked in the past as most previous studies were aimed at finding plant

varieties that can be genetically altered just enough so that they are suitable for cultivation. Independent studies have earlier shown that levels of Cry1Ac in some commercial GM cotton decline progressively over the life-cycle of the plant and are produced at such low levels in vulnerable parts of the crop that insects can continue to consume them. "We find a very strong correlation between the levels of Cry1Ac and abnormalities — higher the levels of Cry1Ac in the plants, the greater the damage," said Pradeep Burma, a plant geneticist at the University of Delhi South Campus, who led the study. Burma said these findings do not in any way suggest that GM crops are either unsafe for consumption or can cause damage to other crops or the environment. "But they reveal a previously unrecognised effect on GM plant development," Burma said. "It's a hurdle we need to overcome to improve insect-resistance in crops," he said. The researchers have themselves shown that if the plants are modified in such a way that the Cry1Ac is confined in their chloroplasts - the site of photosynthesis in plant cells — they do not show any developmental defects. "This could be a future strategy to protect plants from damage," Burma said. But scientists caution that the study describes observations and the mechanism of how the toxin harms host plants remains unclear. "We need to understand why the plants are being affected — and use that knowledge to make better plants," Kasbekar said. The Indian government had approved commercial cultivation of GM cotton containing Cry1Ac in 2002, and research groups have been trying to equip other plants with this protein. But a proposal to introduce GM brinjal with Cry1Ac has been stalled by the environment ministry amid concerns among sections of scientists and environmental activists about safety and environmental impact of edible GM crops. --- ---

2. BT gene in GM crops harmful for growth Deccan Chronicle June 3, 2011

http://bit.ly/jAiEOz

A team of Indian scientists has found that genetic modification (GM) will have a detrimental effect on the growth and development of plants. This is the first time that scientists have found that the Bt gene will trigger major problems in plants like stunted growth and sterility. Thus far, studies have centred on the toxicity of the Bt gene to animals and human beings. There has been considerable interest and activity in genetically engineering insect-resistant crop plants using 'Cry genes' encoding insect toxins from the soil bacterium Bacillus thuringiensis (Bt). The proteins encoded by these genes are called Bt-toxins and are thought to specifically affect only certain insects and not other organisms or the plants themselves. However, the team from the laboratory of Dr Pradeep Burma in the Department of Genetics at the University of Delhi, South Campus, has found that expression of the Bt-toxin 'Cry1Ac' in cotton and tobacco is detrimental to the growth and development of those plants. The study was published in the June issue of Journal of Biosciences. "Many of the transgenic plants obtained showed developmental defects comprising abnormal growth (stunting) and/or sterility. These symptoms suggest that expression of Cry1Ac could be causing growth defects in plants," the team observed. Consistent with this explanation, the researchers found that a majority of transgenic plants had very low or undetectable levels of Cry1Ac, and that all plants having appreciable levels of Cry1Ac showed developmental abnormalities. This indicates a correlation between the levels of Cry1Ac expression and the developmental defects in the plants. Plants release defence-related molecules to fight the toxicity induced in them through Bt technology. Though studies have not been conducted to establish whether these defence-related molecules will cause harm to human beings when they are consumed, scientists here feel that the toxins released may also be detrimental to human and animal health.

Website: **http://www.gmwatch.org**

27: BT proteins are toxic to human cells

Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide

Mesnage R., Clair E., Gress S., Then C., Székács A., Séralini G.-E Article first published online: 15 FEB 2012, Journal of Applied Toxicology

DOI: 10.1002/jat.2712

ABSTRACT

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified Bt insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac Bt toxins (10 ppb to 100 ppm) on the human embryonic kidney cell

line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000 ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified Bt toxins are not inert on nontarget human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.
PRESS RELEASE

GM maize: New indication of health risks Bt protein toxic to human cells

Caen/ München.

Insecticidal Bt toxins such as those produced in genetically engineered plants can be detrimental to human cells. This is a result of recent research led by researchers at the University of Caen (France). Their experiments showed that toxins produced in, for example, the genetically engineered maize MON810, can significantly impact the viability of human cells. The effects were observed with relatively high concentrations of the toxins, nevertheless there is cause for concern. According to companies like Monsanto, which produces genetically engineered maize with these toxins, the toxins are supposed to be active only against particular insects and should have no effect on mammals and humans at all. For the first time, experiments have now shown that they can have an effect on human cells. These kinds of investigations are not a requirement for risk assessment in Europe or in any other region.

Another finding of the researchers concerns a herbicide formulation sold under the brand name Roundup. Massive amounts of this herbicide are sprayed on genetically engineered soybean crops and its residues can be found in food and feed. According to the new publication, even extremely low dosages of Roundup (glyphosate formulations) can damage human cells. These findings are in accordance with several other investigations highlighting unexpected health risks associated with glyphosate preparations.

"We were very much surprised by our findings. Until now, it has been thought almost impossible for Bt proteins to be toxic to human cells. Now further investigations have to be conducted to find out how these toxins impact the cells and if combinatorial effects with other compounds in the food and feed chain have to be taken into account," says Gilles-Eric Séralini from the University of Caen, who supervised the experiments. "In conclusion, these experiments show that the risks of Bt toxins and of Roundup have been underestimated."

Bt toxins and tolerance to herbicides are broadly used in genetically engineered plants. Bt proteins only naturally occur in soil bacteria. By introducing the modified toxin gene into the plants, the structure of the toxins is modified and may thereby cause selectivity to be changed. The content of the proteins within the plants is highly variable. Many genetically engineered plants contain several Bt toxins at the same time. For example, SmartStax produces six different Bt toxins and therefore has a higher overall content of the proteins. In addition, it was made tolerant to herbicides. So far, there has been no investigation of the combinatorial effects of these toxins and residues from spraying, or their potential risks for human health, which was considered unlikely. The researchers have now shown that interactivity does occur. Under the specific conditions of their experiment, the modified Bt toxin lowered the toxicity of Roundup. Further investigations are necessary to examine other potential combinatorial effects under varying conditions.

"These results are pretty worrying. Risk assessment requirements for genetically engineered plants and pesticides need to be rigidly enforced. In the light of these findings, we think that the commercialization of these plants is not in accordance with EU regulations", says Christoph Then at Testbiotech. Testbiotech is closely following risk assessment at the European Food Safety Authority EFSA and has repeatedly brought attention to gaps in risk assessment.

The research was supported by GEKKO foundation (Germany). CRIIGEN Association (France) and Testbiotech (Germany) were involved in planning the experiments and the discussion of results.

Findings were published after peer review process. Mesnage R., Clair E., Gress S., Then C., Székács A., Séralini G.-E., 2012, Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide, Journal of Applied Toxicology**http://onlinelibrary.wiley.com/doi/10.1002/jat.2712/abstract**

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28: Glyphosate and Roundup toxicity in humans confirmed

Glyphosate Toxic to Mouth Cells & Damages DNA, Roundup Much Worse

Further evidence of genotoxic and cytotoxic effects – a prelude to cancer, birth defects and reproductive problems by Dr Eva Sirinathsinghji

ISIS Report 28/03/12

A fully referenced version of this articles is posted on ISIS members website

http://www.i-sis.org.uk/Glyphosate_Toxic_to_Mouth_Cells.php

New research finds that glyphosate causes cell and DNA damage to epithelial cells derived from the inside of the mouth and throat [1]. It raises concerns over the safety of inhaling glyphosate, one of the most common ways in which people are exposed to the herbicide.

Siegfried Knasmueller and his colleagues the Medical University of Vienna, Austria, found that Monsanto's formulated version of glyphosate called Roundup Ultra Max caused cellular damage and DNA damage including chromosomal abnormalities and ultimately killed the cells at higher concentrations. Importantly, DNA damage occurred at concentrations below those required to induce cell damage, suggesting that the DNA damage was caused directly by glyphosate instead of being an indirect result of cell toxicity.

These are not the first findings of glyphosate-based herbicides' cytotoxic and genotoxic effects. Numerous independent research teams have been documenting the hazards of glyphosate exposure over the last few years with in vivo, in vitro and clinical studies.

DNA damage was observed in blood samples from exposed residents in Argentina and Ecuador [2, 3]. Lab mice were found to harbour chromosomal and DNA damage in bone marrow, liver and kidney cells as well as lymphoid cells [4]. Similar effects were found in non-mammalian species, including sea urchins [5], goldfish [6, 7], eels [8], tilapia fish [9] as well as the fruitfly [10]. These experiments show that glyphosate herbicides are dangerous for humans and many other animals. Glyphosate is highly soluble in water, so impacts on aquatic wildlife may be of particular concern, especially following the recent report on the presence of glyphosate in rain water, groundwater, rivers and air [11, 12]. Its extreme toxic effects on amphibians such as frogs has already been shown (see [13] Roundup Kills Frogs, SiS 26). Cell damage has been documented in many cell types including those derived from the rat testis (see [14] Glyphosate Kills Rat Testes Cells, SiS 54), human placenta, umbilical cord, and embryo (see [15] Death by Multiple Poisoning, Glyphosate and Roundup, SiS 42), rat and carp neurones [16, 17], and liver [18, 19].

Multiple tests all show cellular damage in response to Roundup

To reflect occupational exposure, human buccal epithelial cells were exposed to glyphosate and Roundup for 20 minutes only at concentrations from 10 mg/L to 200 mg/L. The Roundup formulation used for the experiments contains 450 g/L of glyphosate and should be diluted according to the manufacturer's instructions to 1–3 % before use (final concentration 4 500–13 500 mg/l). The researchers found some significant effects with 10-20 mg/l, equivalent to a 225–1 350-fold dilution of the spraying solution.

Cell damage was assessed by the release of the membrane-bound enzyme lactose dehydrogenase into the culture medium. The integrity and viability of cells was indicated by their staining with neutral red as only healthy cells retain the dye. Mitochondrial function was assessed by measuring the activity of the enzyme mitochondrial dehydrogenase with the substrate XXT that gives a yellow colour product. And cell proliferation was measured by the total protein content of the cell cultures. The results showed that the cells were much more sensitive to the Roundup formulation than glyphosate. With Roundup, a significant effect was seen at a dose level of 40 mg/L with the XXT assay, while a clear increase of the lactose dehydrogenase levels was seen already with 10 mg/L. The cell proliferation and the neutral red assays were less responsive, with significant effects detected at 80 and 100 mg/L, respectively (still well below agricultural use levels). All effects were dose-dependent. With glyphosate, no significant effects were seen in 3 of the 4 assays, with only lactose dehydrogenase showing significant effects at over 80 mg/l.

Multiple tests show Roundup causes DNA damage

DNA damage was analysed by two methods. The first is the Single Cell Gel Electrophoresis (SCGE) assay, which reveals single or double-stranded breaks in DNA. The second is a special comprehensive assay of chromosome instability that picks up many DNA aberrations including chromosome breakage, DNA misrepair, chromosome loss, as well as cell death by either necrosis

(cell death that results from external stressors such as toxins), apoptosis (programmed cell-death) and cell growth. Different nuclear anomalies were measured including micronuclei, a biomarker of chromosomal damage, breakage or loss; nuclear buds, a biomarker of elimination of amplified DNA and/or DNA repair complexes; and nucleoplasmic bridges reflecting the formation of dicentric chromosomes (chromosomes with 2 instead of 1 centromere) , a marker of DNA misrepair and/or end-fusions of the chromosomes.

Significant effects on DNA integrity as assessed by the SCGE assay were seen at 20 mg/l of both Roundup and glyphosate, increasing in a dose-dependent manner.

Exposure of the cells for 20 minutes also led to a significant and dose-dependent increase of nuclear anomalies including increases in the total number of micronuclei beginning at 10 mg/L of Roundup, and 15 mg/L of glyphosate. The number of nuclear buds increased with exposure concentrations, starting at 10 mg/L with both glyphosate and Roundup. In the case of the nucleoplasmic bridges, the only significant effect was obtained with the highest dose of Roundup used (20 mg/L).

Apoptotic cells were observed following 20mg/L of Roundup but not glyphosate, while necrosis occurred in response to 20mg/L of both Roundup and glyphosate.

In summary, Roundup was cytotoxic at concentrations as low as 20 mg/L, while its active ingredient was not generally cytotoxic to buccal epithelial cells. Both glyphosate and Roundup elicited genotoxic effects at concentrations below the level required to induce cell damage. The different effects between the active ingredient and its commercial formulation is consistent with previous work, including experiments done on testicular, placental, embryonic and umbilical cord cells (see above). These results may explain some of the ailments observed in people who work with this herbicide and adds yet more weight to an outright ban of the herbicide [20] Ban Glyphosate Herbicides Now, SiS 43).

Death by Multiple Poisoning, Glyphosate and Roundup

Scientists pinpoint how very low concentrations of the herbicide and other chemicals in Roundup formulations kill human cells, strengthening the case for phasing them out, and banning all further releases of Roundup-tolerant GM crops

by Dr. Mae-Wan Ho and Brett Cherry

This article was submitted to the USDA on behalf of ISIS ISIS Press Release 11/02/09

http://www.i-sis.org.uk/DMPGR.php

Four different Roundup formulations of the herbicide glyphyosate manufactured by Monsanto are highly toxic to human cells, and at concentrations far below the recommended agricultural use levels. Researchers at the Institute of Biology in Caen , France published their latest results in the current issue of Chemical Research in Toxicology [1] .

Roundup formulations are lethal cocktails

The four Roundup formulations are mixtures of glyphosate with various adjuvants. (An adjuvant is 'helper' substance added to aid the effect of the active ingredient.) The Roundup formulations are currently the top non-selective herbicides worldwide and increasing, as more than 75 percent of genetically modified (GM) crops are Roundup tolerant. Glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA) are main contaminants in rivers. The adjuvants, not often measured in the environment, are usually considered 'inert' and protected as trade secret in manufacturing. Among them, the predominant one is polyethoxylated tallow amine (POEA) . POEA is used as a surfactant in Roundup formulations to improve solubility and penetration into plants.

Three human cell lines were tested: primary cell line HUVEC from umbilical cord vein epithelium, embryonic cell line 293 derived from kidney, and placenta cell line JEG3. All cells died within 24 hours of exposure to the Roundup formulations.

The Roundup formulations (Rs) contain different amounts of the active ingredient glyphosate: Roundup Express, 7.2 g/L (R7.2) ; Roundup Bioforce, 360 g/L (R360) ; Roundup Grand Travaux, 400 g/L (R400); and Roundup Grand Travaux Plus, 450 g/L (R450). They were compared with glyphosate (G), AMPA, and POEA . All Roundup formulations in the study, along with individual chemical ingredients, were tested at concentrations from10 ppm (parts per million) to 2 percent (the recommended agricultural usage level), which means that the Roundup formulations were diluted up to 100 000 times or more.

The researchers found that the presence of the other chemical ingredients in Roundup formulations, such as POEA, actually amplified glyphosate's toxic effects. The toxicities of the Roundup formulations were not proportional to the amount of glyphosate they contained, and are most likely due to POEA and other as yet undisclosed ingredient(s) present in all the formulations. POEA by itself is much more toxic than the Roundup formulations, while AMPA is more toxic than glyphosate.

Multiple targets in toxicity

The researchers tested Rs, G, AMPA, and POEA for effects on three targets that could kill the cell: damage to the cell membrane, poisoning of the mitochrondria (site of energy metabolism), and programmed cell death that results in

fragmentation of the DNA in the cell nucleus. They measured specific enzyme markers at different concentrations for each damage at 24 h of exposure, and also obtained images of the cell cultures under the microscope.
All Rs, as well as G, caused cells to die; the results are the same for all human cell types, but at different concentrations. Thus, R400, the most toxic formulation, killed all cells at 20 ppm, which is equivalent to 8ppm in G. However, 4-10 ppm G alone is non-toxic, its toxicity begins around 1 percent (10 000 ppm), and is not connected with the cell membrane. The R formulations damage the cell membrane, and also poison the mitochondria. In contrast, G poisons the mitochondria without damaging the cell membrane
Unexpectedly, R400 is more toxic than R450, the latter in turn more harmful than R360, R7.2 and G. However, the toxicities are not proportional to the concentration of G present. The cell killing power of R7.2 was almost the same as that of R360, and these results are consistent across all cell lines. This suggests other unknown substances are involved in the toxic effects. Thus AMPA and POEA also kill cells by poisoning the mitochrondia and damaging the cell membrane. POEA is so potent that it begins to damage the cell membrane in HUVEC and poison the mitochondria in 2 93 and JEG3 at 1 ppm. Roundup formulations are more toxic than either G or AMPA. AMPA itself destroys the cell membrane, however, which G does not do, though it is 3-8 times more toxic for the mitochondria than AMPA. But as cell membrane damage is more serious for the cell, AMPA is more toxic than G, while POEA is the most toxic of all.
What happens when these ingredients are combined? The researchers found that for HUVEC and 293 cells, combinations of G and POEA, G and AMPA, AMPA and POEA were all more toxic than the same concentration of the single ingredients
For programmed cell death, the action is quicker. The marker enzymes are activated from 6 h of exposure, with a maximum at 12 h in all cases. HUVEC was 60-160 times more sensitive than the other cell lines; G and R360 were effective at exactly the same concentration, from 50 ppm. The adjuvants do not seem necessary. G alone is 30 percent more potent here than Rs; it acted rapidly at concentrations 500 –1 000 times lower than agricultural use
Ban Roundup tolerant GM crops
These latest studies confirm a wealth of evidence on the toxicities of glyphosate and Roundup formulations [2] (Glyphosate Toxic & Roundup Worse , SiS 26), and pinpoint the different sites of action, all of which result in cell death. Epidemiological studies have previously linked glyphosate to spontaneous abortions, non-Hodgkin lymphoma, and multiple myeloma. Laboratory studies showed that glyphosate inhibits transcription in sea urchin eggs and delays development. Brief exposures to glyphosate in rats caused liver damage, and adding the surfactant in Roundup had a synergistic effect, causing greater liver damage. Roundup was also found to be much more lethal to frogs than to weeds, and could have contributed to the global demise of amphibians within the past decades [3] (Roundup Kills Frogs , SiS 26).
We have called for a new regulatory review on glyphosate and Roundup in 2005 [2]. There is a now a strong case for restricting, if not phasing out glyphosate and Roundup; in the first instance, by banning the release of Roundup tolerant GM crops worldwide. For the same reason, no further Roundup tolerant GM crops should be approved for commercial release.
References

- Benachour N and Séralini G-E.. Glyphosate formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells Chem. Res. Toxicol. , 2009, 22 (1), pp 97–105
- Ho MW and Cummins J. Glyphosate toxic and Roundup worse Science in Society 26, 12, 2005.

http://www.i-sis.org.uk/GTARW.php

- Ho MW. Roundup kills grogs Science in Society 26. 13, 2005.

29: German ban on MON810 is legal and based on sound science

This is a clearly written and very important article which defends the German ban on MON810, which was brought in, quite legally, on the basis of new science published in this article:

Bøhn T, Primicerio R, Hessen DO, Traavik T:

Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety.

Arch Environ ContamToxicol 2008, 55:584–592.
The article showed that in fields of MON810 maize, there were significant and negative long-term effects on a well-established aquatic arthropod model organism, Daphnia magna. When compared to its unmodified maize counterpart, it was

concluded that the tested Bt- transgenic maize had a lower quality as a feed source. The authors called for greater attention, not only to the runoff material from transgenic agricultural fields but also for the sensitivity of aquatic non-target organisms to transgenic plant products and Bt-transgenic crops.
Although it is obvious to any intelligent human being that Bt crops, with built-in toxins, will be harmful to non-target organisms, Monsanto has tried for years to pretend otherwise, and sought to have the German ban overturned in the courts. It lost its case.
In this article:
Ricroch A, Berge JB, Kuntz M:

Is the German suspension of MON810 maize cultivation scientifically justified?

Transgenic Research 2010, 19:1–12.
Agnes Ricroch and others sought to demonstrate that the Bøhn et al study was flawed -- and of course this latter study was then used tactically and politically by the GM industry to claim that bans on MON810 were flawed scientifically and therefore illegal. Now Thomas Bøhn and his colleagues have fought back, with a point-by-point refutation of the allegations made by their critics. They show convincingly that the study published in Transgenic Research contains i) serious scientific flaws, such as omitting core results and misrepresenting others; ii) inconsistency in how laboratory studies that show negative effects of GM plant exposure should be followed up; and iii) a systematic selection of particular results and/or studies that match their own arguments. Nothing new in any of that.....
This is yet another example of how corrupt practices and even scientific fraud can creep into GM science -- and another example of how the GM industry will stop at nothing to get their way and to achieve a total abandonment of the Precautionary Principle.
MON810 is currently banned in 6 countries within the EU. It should have been banned across Europe long since -- and in the light of this and other science it would be an outrage if EFSA and the EC give it a renewal of existing cultivation consent.
(Note: it looks as if the EC is beginning to see sense on this issue, since the latest news is that the Monsanto request for renewed authorisation -- dating back to 2009 -- is going nowhere. The Commission has sent the dossier back to EFSA for further safety evaluation -- in spite of EFSA stating that it has no reason to vary its original opinion about this controversial variety. Par for the course -- EFSA thinks that ALL GM varieties are perfectly safe, no matter what evidence may be brought to its attention.....)

The German ban on GM maize MON810: scientifically justified or unjustified?

Environmental Sciences Europe 2012, by Thomas Bøhn (thomas@genok.org), Raul Primicerio (raul.primicerio@uit.no) and Terje Traavik (terje.traavik@uit.no)
http://www.enveurope.com/content/24/1/22

Abstract
The ongoing controversies over genetically modified organisms (GMOs) in Europe remain intense. Assessing the risks associated with new technologies is crucial, and becomes particularly important for self-replicating GMOs used in open ecosystems. In general, scientific disagreement and debate is at the core of knowledge generation. However, in the GMO debate, it seems that pre-conceived conclusions can in some cases overshadow real data and factual results of investigations. In this article, we describe how the German ban on the cultivation of MON810 Bt-transgenic maize plant has been criticized for not having a sound scientific justification, and provide arguments for why we disagree with this perspective. We do this by demonstrating in detail how arguments put forward by Agnes Ricroch and colleagues in an article from Transgenic Research are based on i) serious scientific flaws, such as omitting core results and misrepresenting others; ii) inconsistency in how laboratory studies that show negative effects of GM plant exposure should be followed up; and iii) a systematic selection of particular results and/or studies that match their own arguments. We conclude that Ricroch et al. misrepresent and selectively scrutinize certain data only. The effect of this double standard is that those only reading or referring to Ricroch et al. will be seriously misinformed about our study as well as in the discussion on the 2009 German ban of the MON810 GM maize.

However, we do not claim that the ban was finally and irreversibly justified by the science referred to, including our own studies within the field. The German ban on MON810 was, and must be, a political decision, guided by valid scientific evidence.

30: GM soy is significantly different and highly variable in composition

Note: the findings in this paper do not constitute prima facie evidence that GM soy is harmful to the health of animals and humans -- but there are very solid grounds for assuming that it might be -- and that is why Monsanto set out, even before this paper was published, to neutralise its effect with a counter study of its own. it only does that sort of thing when it feels threatened by "uncomfortable" results.
"Alterations in Clinically Important Phytoestrogens in Genetically Modified, Herbicide-Tolerant Soybeans"
(Journal of Medicinal Food, (Vol 1., no. 4), July 1999)
by Marc A. Lappé, Ph.D., E. Britt Bailey, M.A., Chandra Childress, M.S., Kenneth D.R. Setchell, Ph.D.,

Abstract
The growing clinical interest and use of soybean-based food products or extracts to increase dietary phytoestrogen intake makes the precise composition of the key biologically active ingredients of soybeans, notably genistin and daidzin of substantial medical interest. Conventional soybeans are increasingly being replaced by genetically modified varieties. We analyzed the phytoestrogen concentrations in two varieties of genetically modified herbicide tolerant soybeans and their isogenic conventional counterparts grown under similar conditions. An overall reduction in phytoestrogen levels of 12-14 percent was observed in the genetically altered soybean strains. Most of this reduction was attributable to reductions in genistin and to a lesser extent daidzin levels, which were significantly lower in modified compared to conventional soybeans in both strains. Significant sample to sample variability in these two phytoestrogens, but not glycitin, was evident in different batches of genetically altered soybeans. Given the high biological potency of isoflavones and their metabolic conversion products, these data suggest genetically modified soybeans may be less potent sources of clinically relevant phytoestrogens than their conventional precursors. These observations, if confirmed in other soybean varieties, heighten the importance of establishing baselines of expected isoflavone levels in transgenic and conventional soy products to ensure uniformity of clinical results. Disclosure of the origins and isoflavone composition of soy food products would be a valuable adjunct to clinical decision-making.

31: Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence

M Antoniou, MEM Habib, CV Howard, RC Jennings, C Leifert, RO Nodari, CJ Robinson and J Fagan
J Environ Anal Toxicol S4:006. doi:10.4172/2161-0525.S4-006
http://www.omicsonline.org/2161-0525/2161-0525-S4-006.php?aid=7453
Note: This new study demonstrates corrupt science at the heart of the regulatory process, associated with the approval of Roundup in germany and the EU. The German authorities introduced irrelevant data to 'disappear' significant findings of birth defects associated with Roundup, and even went so far as to redefine a birth defect as a 'developmental variation'. According to Clare Robinson, one of the authors, "Germany set – and the EU authorities accepted – a 'safe' level for glyphosate exposure that may not be safe at all." The new study's findings back those of a separate study published in September by a team led by Professor Gilles-Eric Seralini. Seralini found increased incidence of tumours, mortality, and organ damage in rats fed with low levels of Roundup and a GM maize genetically engineered to tolerate the herbicide. The levels of Roundup that were toxic were well below levels permitted in drinking water and feed. We support calls for the toxicity of Roundup to be immediately reassessed -- there is now far too much evidence of serious toxicity in the public domain, and Roundup residues are everywhere in the food supply. Roundup should be banned.

Abstract

The publication of a study in 2010, showing that a glyphosate herbicide formulation and glyphosate alone caused malformations in the embryos of Xenopus laevis and chickens through disruption of the retinoic acid signalling pathway, caused scientific and regulatory controversy. Debate centred on the effects of the production and consumption of genetically modified Roundup Ready soy, which is engineered to tolerate applications of glyphosate herbicide. The study, along with others indicating teratogenic and reproductive effects from glyphosate herbicide exposure, was rebutted by the German Federal Office for Consumer Protection and Food Safety, BVL, as well as in industry-sponsored papers. These rebuttals relied partly on unpublished industry-sponsored studies commissioned for regulatory purposes, which, it was claimed, showed that glyphosate is not a teratogen or reproductive toxin. However, examination of the German authorities' draft assessment report on the industry studies, which underlies glyphosate's EU authorisation, revealed further evidence of glyphosate's teratogenicity. Many of the malformations found were of the type defined in the scientific literature as associated with retinoic acid teratogenesis. Nevertheless, the German and EU authorities minimized these findings in their assessment and set a potentially unsafe acceptable daily intake (ADI) level for glyphosate. This paper reviews the evidence on the teratogenicity and reproductive toxicity of glyphosate herbicides and concludes that a new and transparent risk assessment needs to be conducted. The new risk assessment must take into account all the data on the toxicity of glyphosate and its commercial formulations, including data generated by independent scientists and published in the peer-reviewed scientific literature, as well as the industry-sponsored studies.
(NB The full text with references is available online)
Conclusion Studies published in the peer-reviewed scientific literature have raised major concerns regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems. In addition, a debate has emerged over the reported effects on human health of herbicide application in regions that produce GM glyphosate-tolerant crops and about the safety of food and feed produced from these crops. Regulatory authorities and industry affiliates have defended the use of glyphosate largely by citing the industry-sponsored toxicological tests conducted for regulatory purposes, which they claimed showed no evidence of teratogenicity. However, the German authorities' draft assessment report revealed that even these industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity. Many of the malformations observed in these studies are of the type associated with the retinoic acid signalling pathway. Paganelli et al. [1] showed that this was the mechanism through which glyphosate and Roundup exercise their teratogenic effects. It is noteworthy that these industry tests were commissioned by the same companies that stand to profit from regulatory authorization. Regrettably, this system possesses an inherent risk of bias and makes it especially important that the regulatory assessment is rigorous. Yet in the EU, the evidence suggests that this was not the case. The significance of clear teratogenic effects of glyphosate in rabbits and rats found in tests commissioned by industry were minimized by German regulators. A scientifically rigorous assessment was further impeded by the outdated design of the standard tests, which are not sufficiently sensitive to detect effects from realistic exposures. As a result, the German authorities suggested, and the EU adopted, an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans. Another relevant factor is that the industry teratogenicity tests were on glyphosate, the presumed active ingredient of the herbicide, and not on the herbicide formulations as sold and used, even though studies indicate that the formulations are more toxic for certain endpoints than glyphosate alone. A substantial body of evidence demonstrates that glyphosate and Roundup cause teratogenic effects and other toxic effects on reproduction, as well as genotoxic effects. From an objective scientific standpoint, attempts by industry and government regulatory bodies to dismiss this research are unconvincing and work against the principle that it is the responsibility of industry to prove that its products are safe and not the responsibility of the public to prove that they are unsafe. The precautionary principle would suggest that glyphosate and its commercial formulations should undergo a new risk assessment, taking full account of the entirety of the peer-reviewed scientific literature as well as the industry-sponsored studies. Experience to date suggests that the new risk assessment should be conducted with full public transparency by scientists who are independent of industry.

32: BT crops: another nail in the coffin for substantial equivalence

After considering, in this long and detailed chapter, just how BT crops work in controlling insect pests, the authors conclude that their mode of operation is so different, in so many ways, that the idea of them being "substantially equivalent" to their isogenic or parent lines is scientifically nonsensical. No surprise there, then.....

ADVANCED TECHNOLOGIES FOR MANAGING INSECT PESTS

Eds: Isaac Ishaaya, Subba Reddy Palli and A Rami Horowitz
Chapter 10, p 197-230 Comparative Aspects of Cry Toxin Usage in Insect Control
by András Székács and Béla Darvas
Conclusions
Based on the above (text), Bt -based bioinsecticides and crops cannot be considered by far as equivalent technologies. Their application differs as Bt bioinsecticides allow singular applications, while Bt crops exert a continuous production of the Cry toxin. This results in higher environmental doses of the plant-expressed toxin(s) than in the case of the Bt bioinsecticide. For example a single treatment of Dipel bioinsecticide at the registered dosage (1 kg/ha) contains 4.8–60.2 mg/ha (average 20.6 mg/ha) of bioavailable Cry1Ab toxin, while the amount of bioaccessible amount of Cry1Ab toxin is 0.085–8.16 g/ha. In contrast, the production of plant-expressed Cry1Ab toxin was found to be 147–456 g Cry1Ab toxin/ha, representing 18–56 treatments with Dipel (on the basis of its maximally detected bioaccessible Cry1Ab toxin content, 8.16 g/ha). The level of plant-expressed Cry1Ab toxin can be further elevated by soil fertilization (2.3–6.8-fold) and the use of long maturation maize varieties (2.5–5.8-fold), representing, in worst case scenarios, in 625–1,930 treatments with Dipel. Moreover, it has to be mentioned that stacked genetic events may further elevate toxin production (twofold). These ratios are even higher if lower bioaccessible Cry1Ab protoxin content biopesticides or bioavailable Cry1Ab toxin contents are considered.
Beside toxin ratios, another characteristic difference is that while Bt bioinsecticides are composed of several crystalline toxins, single genetic event Bt crops express only a single toxin molecule. This has severe consequences in resistance development, which may be alleviated, yet not eliminated by the use of "pyramid" Bt event varieties, expressing several Cry toxins acting on the same insect order, as the evolutionary driving force remain the same. The active ingredient of Bt bioinsecticides are bacterial protoxins stabilized in crystalline form and requiring enzymatic activation, while Bt plants (e.g., MON 810) express a truncated form of the protoxin, so-called preactivated toxin. This has severe consequences in product registration, as the active ingredient toxin in the Bt crop is not the registered active substance of the corresponding Bt bioinsecticide, and the required toxicology studies have been carried out not with the plant-expressed preactivated toxin, but with the bacterial protoxin or the enzyme-activated active toxin. Moreover, commercial ELISA systems utilizing antibodies against the bacterial protoxin and analytical standards of that protoxin consistently underdetect actual toxin content in Bt plants due to their lower cross-reactivities to the plant-expressed preactivated toxin. As a result, all reported results obtained by protoxin-based ELISAs, including manufacturer documentation, are subject to correction. And fi nally, although Bt crops have been widely advocated to be included in integrated pest management (IPM) practices or even in ecological agriculture, Bt crops cannot fulfill the main ecological principle of IPM that any protection measures should be timed only to the period(s) when pest damage exceeds the critical level, and therefore, regardless how environmentally mild their active ingredient is, do not comply with IPM.
A. Székács and B. Darvas Authors: Department of Ecotoxicology and Environmental Analysis, Plant Protection Institute , Hungarian Academy of Sciences, Budapest, Hungary Contacts: e-mail: a.szekacs@cfri.hu ; drdarvas.bela@chello.hu

33: Toxic effects of Roundup on food micro-organisms

Emilie Clair, Laura Linn, Carine Travert, Caroline Amiel, Gilles-Eric Séralini, Jean-Michel Panoff.
Effects of Roundup(®) and Glyphosate on Three Food Microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus.
Curr Microbiol. 2012 Feb 24. Epub 2012 Feb 24. PMID: 22362186

Abstract:
Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup(®), a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms

(Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus) widely usedas starters in traditional and industrial dairy technologies. The presented results evidence that Roundup(®) has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiodiversity and microbial concentration observed in raw milk for many years.

34: Glyphosate damages beneficial micro-organisms in poultry

Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M.
The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro.
Curr Microbiol. 2012 Dec 9. [Epub ahead of print]

Abstract
The use of glyphosate modifies the environment which stresses the living microorganisms. The aim of the present study was to determine the real impact of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. The presented results evidence that the highly pathogenic bacteria as Salmonella Entritidis, Salmonella Gallinarum, Salmonella Typhimurium, Clostridium perfringens and Clostridium botulinum are highly resistant to glyphosate. However, most of beneficial bacteria as Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis and Lactobacillus spp. were found to be moderate to highly susceptible. Also Campylobacter spp. were found to be susceptible to glyphosate. A reduction of beneficial bacteria in the gastrointestinal tract microbiota by ingestion of glyphosate could disturb the normal gut bacterial community. Also, the toxicity of glyphosate to the most prevalent Enterococcus spp. could be a significant predisposing factor that is associated with the increase in C. botulinum-mediated diseases by suppressing the antagonistic effect of these bacteria on clostridia.

35: Bt maize pollen will harm butterflies in Europe

Thursday, 14 February 2013 11:44
http://www.gmwatch.org/index.php?option=com_content&view=article&id=14644:bt-maize-pollen-will-harm-butterflies-in-europe-study
NOTE: Below is the abstract of an important new study that concludes that the planting of Bt maize in some areas of Europe would cause increased mortality in the larvae of the protected peacock butterfly (Inachis io).
Interestingly the authors note that their study contradicts the findings of a previous study by Joe Perry and colleagues. Perry is the current chair and a long-time member of the EFSA GMO Panel, which assesses the risks posed by GMOs submitted for approval in the EU. Perry concluded there was negligible risk from Bt maize to the peacock butterfly and the two other species examined.
The authors of the new paper say they used more empirical data in their study than Perry and colleagues - in other words, hard data based on what actually happens to the butterfly and Bt maize pollen in nature, rather than assumptions.
The authors conclude that "a more comprehensive assessment is warranted of the risk implied to butterflies when and where Bt maize is grown." They add, "We contend that such an assessment is best carried out using empirical data, which invites scientific review and integration of knowledge, rather than on expert opinion, on which a qualified assessment is not possible."
This is a clear criticism of the lack of empirical data currently used in GMO risk assessments by EFSA, which are based heavily on flawed assumptions. It is also a criticism of the system of expert opinion that EFSA currently relies upon. This system is being replaced in some areas, such as evidence-based medicine, by more progressive risk assessment methods based on a systematic and replicable search and evaluation of all available data using pre-set transparent criteria. While far from perfect, these methods are increasingly being viewed as more reliable than expert opinion.

Increased mortality is predicted of Inachis io larvae caused by Bt-maize pollen in European farmland

Niels Holst, Andreas Lang, Gabor Lövei, Mathias Otto Ecological Modelling 250 (2013) 126–133http://www.sciencedirect.com/science/article/pii/S0304380012005315

Abstract

A potential environmental risk of the field cultivation of insect-resistant (Bt-toxin expressing) transgenic maize (Zea mays) is the consumption of Bt-containing pollen by herbivorous larvae of butterflies (Lepidoptera). Maize is wind-pollinated, and at flowering time large amounts of pollen can be deposited on various plants growing in the landscape, leading to inadvertent ingestion of toxic pollen with plant biomass consumed by these butterfly larvae. To examine the possible effect of this coincidence, we focused our study on the protected butterfly Inachis io and two regions of Europe. Using climatic records, maize and butterfly phenology data, we built a simulation model of the butterfly's annual life cycle, over- laid with the phenology of maize pollen deposition on the leaves of the food plant Urtica dioica, and linked these with the dose–response curve of I. io larvae to Bt-maize pollen (event MON810). The simulations indicated that in Northern Europe, where I. io is univoltine, Bt-maize pollen would not be present on the food plant at the same time as the I. io larvae. However, in Central and Southern Europe, where I. io is bivoltine, Bt-maize pollen and the second generation I. io larvae would coincide, and an increased mortality of the larvae was predicted. This prediction differs from earlier studies which predicted negligible effect of field-grown Bt-maize on I. io larvae. Our model is an improvement over previous efforts since it is based on more detailed, empirical data, includes more biological detail, and provides explicit estimation of all model parameters. The model is open-source software and is available for re-use and for modelling the effects on other species or regions.

36: New GM plants and pesticides are not being assessed for safety

Thursday, 21 March 2013 21:52

New kinds of genetically modified plants and pesticides not being assessed for safety Press release Centre for Integrated Research in Biosafety, University of Canterbury, New Zealand European Network of Scientists for Social and Environmental Responsibility (ENSSER) 21 March Contact: +64 3 354 2500, Fax: + 64 364 2590

Christchurch, NZ, 22.03.2013 - In a new peer-reviewed paper published by an international team from New Zealand, Brazil, and Australia in the prestigious journal Environment International, the researchers Jack A. Heinemann, Sarah Z. Agapito-Tenfen and Judy A. Carman have found that government safety regulators are failing to consider important risks of new kinds of genetically modified (GM) plants and some emerging co-technologies.

These plants are designed to make a form of genetic information called double-stranded RNA (dsRNA). While most existing GM plants are designed to make new proteins, these new GM plants make dsRNA in order to alter the way genes are expressed. Recent research has shown that dsRNAs can transfer from plants to humans and other animals through food. Potentially, they could also be transferred into people by inhaling dust from the plant (e.g., breathing in flour from GM wheat while baking with it), or by absorption through the skin.

The same technology is being developed for spraying directly onto plants as a type of pesticide spray. Another proposed use is to feed dsRNAs to insects such as bees to try to control bee viruses.

While RNA is a normal component of all cells, in dsRNA form it can have effects that depend on the species and tissues exposed to it. According to Adjunct Associate Professor Judy Carman of Flinders University and a co-author of the paper: "The dsRNA molecules in GM plants may work exactly as intended and have no other effects. On the other hand, they may have effects that were not predicted, both on their target organisms and other organisms such as people and wildlife. We won't know until we do thorough assessments, and these assessments have not yet been done."

The authors collectively reviewed three food or environment safety regulators with jurisdiction in three countries, Australia, Brazil and New Zealand. The regulatory decisions were on three different kinds of GM plants that do or may produce new dsRNA molecules and were intended for use as food or animal feed. The authors recorded their advice to the regulators and the responses from the regulators.

"Each regulator found reasons not to ask the product developers to specifically test for effects from dsRNA, and thus relied on assumptions rather than testing to determine safety," said co-author Sarah Agapito-Tenfen, a doctoral student at the Universidade Federal de Santa Catarina in Brazil.

"To our surprise, we found that there are no internationally agreed protocols or even guidelines for how to conduct a thorough and proper risk assessment on products with new dsRNA molecules in them," said Prof. Jack Heinemann of Canterbury University in New Zealand, member of ENSSER and the study's lead author. To fill this gap, the authors have developed the first formal assessment procedure for dsRNA-based products, whether they are living genetically modified organisms or agents that are sprayed onto plants.

Contacts for further comment

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Dr. Jack A. Heinemann is professor of Molecular Biology and Genetics in the School of Biological Sciences, and Director of the Centre for Integrated Research in Biosafety, at the University of Canterbury, New Zealand.

Sarah Z. Agapito-Tenfen has a masters degree in Plant Genetic Resources from the Universidade Federal de Santa Catarina, Brazil, and is currently a PhD student there.

Dr. Judy Carman is an adjunct associate professor in Health and the Environment, School of the Environment at Flinders University in South Australia and is also Director of the Institute of Health and Environmental Research. She has qualifications in biochemistry and epidemiology.

Environment International is an Elsevier journal ranked in the top 4% of environmental sciences journals by impact factor; A* by Excellence in Research for Australia, its highest standing; and A1 in the Brazilian/Capes ranking, also the highest standing.

The Centre for Integrated Research in Biosafety (INBI, previously NZIGE) was founded in 2001 as a research centre at the University of Canterbury. The Centre is dedicated to public good science with a focus on research in biotechnology of relevance to those with limited or no access to science research funding.

The European Network of Scientists for Social and Environmental Responsibility (ENSSER) brings together independent scientific expertise to develop public-good knowledge for the critical assessment of existing and emerging technologies. The objective of ENSSER is the advancement of public-good science and research for the protection of the environment, biological diversity and human health against adverse impacts of new technologies and their products. ENSSER advocates benign and peaceful use of scientific discoveries and technological developments, while expanding diverse approaches to assess their utility and safety in society. More information available at: http://www.ensser.org.

Open access (free download) **http://www.sciencedirect.com/science/journal/01604120**

Heinemann, J. A., Agapito-Tenfen, S. Z. and Carman, J. A. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int in press.

Abstract Changing the nature, kind and quantity of particular regulatory-RNA molecules through genetic engineering can create biosafety risks. While some genetically modified organisms (GMOs) are intended to produce new regulatory-RNA molecules, these may also arise in other GMOs not intended to express them. To characterise, assess and then mitigate the potential adverse effects arising from changes to RNA requires changing current approaches to food or environmental risk assessments of GMOs. We document risk assessment advice offered to government regulators in Australia, New Zealand and Brazil during official risk evaluations of GM plants for use as human food or for release into the environment (whether for field trials or commercial release), how the regulator considered those risks, and what that experience teaches us about the GMO risk assessment framework. We also suggest improvements to the process.

New paper on dsRNA risks - briefing for non-specialists

Friday, 22 March 2013 20:15

NOTE: The briefing document below is a summary for the lay person of the paper published yesterday, "A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessment" by Professor Jack Heinemann, Sarah Agapito-Tenfen and Adjunct Associate Professor Judy Carman.

Press release/abstract here: **http://www.gmwatch.org/index.php?option=com_content&view=article&id=14713**

The paper is open access (free download), thanks to sponsorship of the open access fee by the Safe Food Institute of Australia: **http://www.sciencedirect.com/science/journal/01604120/55** --- --- A briefing document for non-specialists

describing the lack of regulation of a new class of products and GM crops based on dsRNA technology by Adjunct Associate Professor Judy Carman, Professor Jack Heinemann and Sarah Agapito-Tenfen 21 March 2013

This is a briefing about the contents of a new, peer-reviewed scientific paper: "A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessment" by Professor Jack Heinemann, Sarah Agapito-Tenfen and Adjunct Associate Professor Judy Carman.

To date, most[1] genetically modified (GM) plants have been made by inserting a new piece of DNA into a plant so that the GM version makes a new protein. Most of these new proteins are designed to either kill insects that try to eat the plant or to make the plant resistant to a herbicide. The process works like this: the DNA is changed so that when a section of the DNA is read and copied, a new piece of messenger RNA (mRNA) is made. The mRNA then goes to another part of the cell and is read to make the new protein.

However, there is a new type of GM plant now being made. These are not designed to make a new protein, but to just make a new RNA molecule. However, the RNA molecule made is different to the single-stranded mRNA described earlier, because it is either double-stranded (dsRNA) or it is designed to find another single-stranded RNA molecule and bind to it to create a dsRNA molecule. These dsRNA molecules have important roles in cells. For example, they can silence or activate genes. For this to happen, the order of the nucleotide units in the dsRNA molecule is crucial. A different sequence can result in the dsRNA having different effects, and silencing or activating a different gene, or multiple other genes.

A number of GM plants have now been made using this technology. For example, Australia's CSIRO has developed GM wheat and barley varieties where genes have been silenced in order to change the type of starch made by the plant. Another example is biopesticide plants, which are designed to silence a gene in insects that eat the plant. That is, the insect eats the plant, the dsRNA in the plant survives digestion in the insect, travels into the tissues of the insect to silence a gene in the insect so that the insect dies as a result.

There is evidence that the gene silencing may be inherited by the offspring of some organisms that eat the dsRNA. Furthermore, there is massive, ongoing investment occurring to develop products that directly transfer dsRNA into the living cells of plants, animals and microbes via their food or by being absorbed through their "skin". This allows dsRNA molecules to be sprayed onto fields of crops to kill insects or to be delivered to beehives as oral medicine for bees.

Last year, a high profile scientific paper was published that showed that dsRNA molecules produced in non-GM plants can be taken into the bodies of people who eat the plant. The dsRNA from the plant was found circulating in blood, indicating that it survives cooking and digestion. Research has also shown that:

*at least one dsRNA produced in plants (called mir168a) can change the expression of genes in mice; and

*dsRNA (mir168a) can change the expression of a gene in human cells growing in tissue culture. Therefore, there is a real risk that the dsRNA produced by these new GM crops could survive digestion in people and change how those people's genes are expressed. These effects of dsRNA were predicted long ago by some scientists. The proof has now arrived.

So, are all dsRNA molecules safe?

A new paper has just been published in Environment International by Professor Jack Heinemann of New Zealand, Sarah Agapito-Tenfen of Brazil and Adjunct Associate Professor Judy Carman of Australia. These authors looked at how the safety of some plants, designed to produce new dsRNA, was determined. They reviewed their experience with three government safety regulators (for either food or the environment) in three different countries over the past ten years. They found that the safety of dsRNA molecules was usually not considered at all, and if it was considered in any way, the regulator simply assumed that any dsRNA molecules were safe, rather than requiring proof that they were safe.

The authors found that government regulators:

*dismissed any need for any assessment of the sequence of the nucleotides in the dsRNAs produced by GM plants; *seemed to assume that dsRNAs produced by these plants are much the same as the more fragile single-stranded RNAs (eg mRNA), and therefore would not survive cooking and digestion; and *claimed that these new dsRNA molecules are safe because humans and non-target animals would simply not be exposed to them.

However, the authors found many scientific studies showing that these assumptions were incorrect.

As a result, the regulators did not assess whether the dsRNAs could cause adverse effects in people or in the environment by, for example, silencing or activating genes in people that come into contact with the plant when it is grown commercially. Contact could include eating the crop or processed products derived from it, inhaling dust from the crop when harvesting it, or inhaling flour from the crop when baking with it. And regulators made that decision regardless of whether the dsRNA was generated intentionally or unintentionally by the crop. All three regulators decided that there were no risks to be considered, based on their own unproven and incorrect assumptions, rather than the scientific evidence.

As a result of their analysis, the authors developed and provided a safety testing procedure for all GM plants that may produce new dsRNA molecules, as well as for products where the active ingredient is dsRNA.

It is important to realise that our current understanding of dsRNA in GM plants is in its infancy and we are still trying to understand how dsRNA molecules may work and therefore how they may affect humans, animals and the environment. Even so, some GM plants using this technology have already been approved for human consumption, using the sorts of assumptions described earlier. Of these crops, several have been withdrawn from the market, while others are about to enter it.

Meanwhile, spraying dsRNAs directly onto crops can be expected to result in large exposures to dsRNA molecules in the environment. For example, we know that existing agricultural sprays can travel for several miles on the wind and can enter surface water and ground water due to run-off after rain. This will also happen with dsRNA molecules if they are sprayed onto crops. We also know that dsRNAs can persist for a long time in the environment.

GM plants and products based on dsRNA technology need a thorough, fit-for-purpose safety evaluation before we use them commercially. The authors provide a step-by-step procedure of how this could be done.

After all, we don't want to learn that one or more of these crops or sprays is toxic after millions of people have been exposed to them for years.

Notes

1. There are some extremely minor exceptions to this, such as virus-resistant papaya, some nutritionally- altered soybeans, and some other plants that are not yet on the market.

New paper on dsRNA type GMOs - Q&A with the authors

Friday, 22 March 2013 20:34

NOTE: In a new peer-reviewed paper published in the journal Environment International, the researchers Jack A. Heinemann, Sarah Z. Agapito-Tenfen and Judy A. Carman found that government safety regulators are failing to consider important risks of new kinds of GM plants and some emerging co-technologies.

These plants are designed to make a form of genetic information called double-stranded RNA (dsRNA). While most existing GM plants are designed to make new proteins, these new GM plants make dsRNA in order to alter the way genes are expressed. This is most commonly called RNAi for RNA interference, or post-transcriptional gene silencing. Recent research has shown that dsRNAs can transfer from plants to humans and other animals through food. - Zhang, L., et al. (2012). "Exogenous plant MIR168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA." Cell research 22(1): 107-126. http://www.ncbi.nlm.nih.gov/pubmed/21931358

Potentially, dsRNAs could also be transferred into people by inhaling dust from the plant (e.g., breathing in flour from GM wheat while baking with it), or by absorption through the skin - with unpredictable effects. The same technology is being developed for spraying directly onto plants as a type of pesticide spray. Another proposed use is to feed dsRNAs to insects such as bees to try to control bee viruses.

The paper's authors kindly agreed to answer some questions from GMWatch (below).

The paper "A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments" by J.A. Heinemann, S.Z. Agapito-Tenfen and J.A. Carman is published by Environment International. The paper is open access (free download), thanks to sponsorship of the open access fee by the Safe Food Institute of Australia: **http://www.sciencedirect.com/science/journal/01604120/55**

Interview with the authors of the dsRNA paper by GMWatch 22 March 2013

GMW: Why should we care about this paper?

Authors: The paper chronicles the systematic neglect by leading food and environmental safety regulators of important safety issues with GM crops, and emerging products containing molecules called double-stranded RNA (dsRNA). The record of neglect and the analysis of the failings have been verified through the judgment of rigorous blind peer-review.

The paper also establishes that all GM crops should be evaluated for the presence of unintended dsRNA molecules. That is, even crops not purposefully constructed to express these molecules need to be evaluated for them, because they are a common by-product of the engineering process. To date, GM crops have not been evaluated in this way.

Finally, the paper shows that the prevailing systems for evaluating the potential for adverse effects from dsRNA would fail. And for the first time, a robust process for testing GMOs or other products that may contain dsRNAs is suggested.

GMW: Won't industry and regulators say that the risks have been considered and GM crops have a clear track record of safety?

Authors: We show in the paper that the regulators have a priori denied the need to assess either the direct or important potential secondary effects of the dsRNA molecules. Instead they have resorted to flawed and outdated assumption-based

reasoning on the biochemistry of dsRNA. Thus, there is no public record of regulators ever having required or reviewed studies that provide evidence for no: (a) off-target effects of intended novel dsRNA molecules in the GMO; (b) effects of unintended novel dsRNA molecules in the GMO; and (c) production of unintended secondary dsRNA molecules in the GMO or in those exposed to the GMO (e.g., through ingestion, inhalation or absorption) – including non-target insects, wildlife and people. Consequently, there has never been an acute or chronic toxicity study done, for any commercial GMO, that has had the ability to detect any effect that could arise specifically from the primary or secondary dsRNA molecules that could be generated by the GMO .

There is no validated safety testing procedure for dsRNAs either for human food or the environment. And there are no international guidance documents that regulators can turn to for advice.

GMW: But surely, RNA is, and always has been, a part of the foods we eat.

Authors: In this and a previous paper (Heinemann and others 2011), we have shown that there is no basis for extrapolating the safety of novel dsRNA molecules from the history of safe use of dsRNA molecules in the cells of plants, animals, fungi and microorganisms that we eat. This is the key distinction: the adverse effects that might arise from dsRNA are determined by the actual sequence of nucleotides in the molecule (sequence-determined risks) and not the chemical nature of RNA. While there are also sequence-independent risks that should not be ignored, there is a difference between the sequence of novel dsRNA molecules in GM crops and those in nature, and that is why arguments about all dsRNAs being safe are dangerously flawed. An example that provides proof is corn engineered to resist the corn rootworm pest. Corn rootworm has always eaten maize roots and maize roots contain RNA (including forms of dsRNA). However, when Monsanto introduces a novel dsRNA of a specific sequence into the cells of the plant, the corn rootworm eating that RNA dies (Baum and others 2007; Gordon and Waterhouse 2007).

GMW: We're told though that it is very difficult to deliver dsRNAs to mammals, including people. That's what is stopping their use in medicine.

Authors: While this is true, it is irrelevant. Although researchers have not managed to find a pill or injectable form of dsRNA that works on people by design, it is now known that plant dsRNA molecules can be efficiently taken up through food to circulate through blood and alter gene expression in organs. Not all dsRNA molecules seem to be equally efficiently taken up, which indicates that there are processes involved that we still do not understand. Current thinking is that the way plants chemically modify dsRNA, and the presence of receptors for some dsRNAs on animal cells, determines the fate of dsRNA when it is taken up through food. It is also known that dsRNAs can be delivered to humans by breathing it into their noses.

GMW: But doesn't your paper overstate the risks? There are already safe GM products on the market using dsRNA.

Authors: The paper has a table of all the dsRNA food approvals that we know of (from our countries). It can be seen that most have either not been commercialized or have been withdrawn (e.g., Flavr Savr tomato, the G series of oleic acid soybeans, new leaf potato), and the remainder are in early commercial stages (e.g., Brazil's pinto bean, Monsanto's high oleic acid soybeans). The exceptions are boutique crops such as the Hawaiian papaya, which may not always make the dsRNA molecule the plant has been designed to make. Thus, we have almost no real experience upon which to base a track record of safety. Importantly, the range of companies, the kinds of traits, and the means of delivery are due to change rapidly. Thus, so will the sequence-determined risks because all the new novel dsRNA molecules will have unique sequences. For example: Australia's CSIRO (a government body that does commercially-oriented research) holds significant patents on food-borne delivery of dsRNAs intended to harm target insects, and is developing wheat with altered nutritional characteristics using dsRNA. A consortium of Alnylam Pharmaceuticals, Isis Pharmaceuticals, Monsanto, Genzyme and Sanofi (Aventis) is capturing THE patent space on chemical delivery systems for topical (i.e., absorption through skin or cell membranes) RNA applications. Monsanto (and probably other agrichemical companies) intends to develop pesticide sprays based on dsRNA (called its Biodirect line). These sprays are designed to transverse cell surfaces, so that they are absorbed by the organism and then transported through the tissues of that organism. The range of exposures, the scale of exposure, and the nature of the risk are without precedent.

Monsanto has purchased Beeologics, a company developing dsRNA molecules that are eaten by bees and mites through their preferred foods. The dsRNA molecules intended for mites are biocidal, the dsRNAs intended for bees are medicinal. Monsanto has also purchased the Rosetta Green company's "activity", which includes its work using dsRNA to manipulate a range of crops and traits.

GMW: You've proposed a risk assessment scheme for dsRNA products. Won't industry say it's too impractical, too expensive, and an unnecessary barrier to bringing food to poor and starving people?

Authors: The proposed safety scheme, illustrated in Figure 3 of the paper, is based on the proper application of cutting edge science. However, the capacity for this science is well within the expertise of both the industry and the academic community

and is not particularly expensive. For example, the bioinformatics techniques suggested require a personal computer, access to the internet and trained personnel (of a kind that are common now in molecular biology).
Meanwhile, the transcriptomic work is well within the industry's ability as illustrated by papers they publish, and an extension of the molecular work already done. The costs of this kind of work are in the same range as the costs needed to identify the intended dsRNAs for commercial development and are a minor part of the marketing, intellectual property rights registration, research and development program for the product.
Moreover, the products are not intended to feed poor people. The pesticide 'technology package' that is being delivered is designed for large industrial monoculture farms, which produce mainly animal feed and biofuel. For example, high oleic acid soybeans are promoted to food safety regulators as a 'safer' alternative to conventional soybean oil when in fact they are being developed to appeal to biofuel manufacturers (Graef and others 2009). Companies do not intend to sell these products in countries that do not recognise their intellectual property claims and for which they cannot extract a price premium, in other words, they are not intended for direct sale to the poor and starving.
GMW: Thinking back to the GM lobby's response to Prof Seralini's 2012 paper on GM maize and Roundup, won't they say that you are just anti-GM activists? And that the journal is obscure?
Authors: Environment International is an Elsevier journal ranked in the top 4% of environmental sciences journals by impact factor; A* by Excellence in Research for Australia, its highest standing; and A1 in the Brazilian/Capes ranking, also the highest standing.
All three authors are academics in good standing at recognised world-class public universities. They have extensive and credible publication records in the peer-reviewed literature and are biosafety experts of standing.
Dr. Jack A. Heinemann is professor of Molecular Biology and Genetics in the School of Biological Sciences, and Director of the Centre for Integrated Research in Biosafety, at the University of Canterbury, New Zealand.
Sarah Z. Agapito-Tenfen has a masters degree in Plant Genetic Resources from the Universidade Federal de Santa Catarina in Brazil and is currently a PhD student there.
Dr. Judy Carman is an adjunct associate professor in Health and the Environment, School of the Environment, at Flinders University in South Australia and is also Director of the Institute of Health and Environmental Research. She has qualifications and experience in biochemistry and epidemiology.
Paper is open access (free download) from **http://www.sciencedirect.com/science/journal/01604120**
References to the Q&A
Baum, J.A.; Bogaert, T.; Clinton, W.; Heck, G.R.; Feldmann, P.; Ilagan, O., et al. Control of coleopteran insect pests through RNA interference. Nat Biotechnol. 25:1322-1326; 2007
Gordon, K.H.J.; Waterhouse, P.M. RNAi for insect-proof plants. Nat Biotechnol. 25:1231-1232; 2007
Graef, G.; LaVallee, B.J.; Tenopir, P.; Tat, M.; Schweiger, B.; Kinney, A.J., et al. A high-oleic-acid and low-palmitic-acid soybean: agronomic performance and evaluation as a feedstock for biodiesel. Pl Biotechnol J. 7:411-421; 2009
Heinemann, J.A.; Kurenbach, B.; Quist, D. Molecular profiling — a tool for addressing emerging gaps in the comparative risk assessment of GMOs. Env Int. 37:1285-1293; 2011

37: New review: The Multiple Health Impacts of Glyphosate

This is a long peer-reviewed article, accompanied by 286 references, which summarises the multiple negative health effects of glyphosate and Roundup, which are widely dispersed through the environment and the food chain, especially in the United States. It makes for sobering reading. It will no doubt be viciously attacked by Monsanto and its friends, but with this much evidence now piling up, NOBODY can claim that Roundup is benign, and that it is perfectly OK for it to be present in the food supply, even in miniscule quantities. When are our political leaders going to wake up to the fact that in pushing the GMO agenda, they are conniving to precipitate a public health disaster?
Review article: Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases by Anthony Samsel and Stephanie Seneff , Entropy 2013, 15, 1-x manuscripts; doi:10.3390/e140x000xentropy ISSN 1099-4300 **www.mdpi.com/journal/entropy**
E-Mail: anthonysamsel@acoustictracks.net E-Mail: Seneff@csail.mit.edu; Tel.: +1-617-253-0451; Fax: +1-617-258-8642.
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Abstract:
Glyphosate, the active ingredient in Roundup®, is the most popular herbicide used worldwide. The industry asserts it is minimally toxic to humans, but here we argue otherwise. Residues are found in the main foods of the Western diet, comprised primarily of sugar, corn, soy and wheat. Glyphosate's inhibition of cytochrome P450 (CYP) enzymes is an overlooked component of its toxicity to mammals. CYP enzymes play crucial roles in biology, one of which is to detoxify xenobiotics. Thus, glyphosate enhances the damaging effects of other food borne chemical residues and environmental toxins. Negative impact on the body is insidious and manifests slowly over time as inflammation damages cellular systems throughout the body. Here, we show how interference with CYP enzymes acts synergistically with disruption of the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment in serum sulfate transport. Consequences are most of the diseases and conditions associated with a Western diet, which include gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease. We explain the documented effects of glyphosate and its ability to induce disease, and we show that glyphosate is the "textbook example" of exogenous semiotic entropy: the disruption of homeostasis by environmental toxins.
Extracts:
13. Discussion
Glyphosate is today the most popular herbicide in use in agricultural practices in the U.S., and, increasingly, throughout the world. Its usage rate has accelerated significantly in the last decade due mainly to two factors: (1) the patent expiration in 2000 led to greatly reduced cost, and (2) the adoption of genetically modified crops that are resistant to its toxic effects allows for higher exposure with little loss in harvest yield. The notion that glyphosate has minimal toxicity in humans, widely popularized by Monsanto, has prevented farmers from using caution in their application of this chemical to their crops. The recent rise in the rates of autism diagnoses in the United States is a cause for alarm. We have recently proposed that autism can be characterized as a chronic low-grade encephalopathy, where the cascade of events taking place in the brain is a process that enables the renewal of severely depleted sulfate supplies to the brain [277]. We identified a dysbiosis in the gut as a source of ammonia that initiates the encephalytic response, and we proposed glyphosate as one of the many environmental toxins that might be responsible for the dysbiosis and for sulfate depletion. A review of the literature on glyphosate has confirmed our suspicions that glyphosate might play a role, and, further, have led us to believe that glyphosate may be the most significant environmental toxin contributing to autism. While it is pervasive in our food supply, the fact that it is deemed by most regulators to be nontoxic makes it especially insidious. The key pathological biological effects of glyphosate -- disruption of the gut bacteria, impairment of sulfate transport, and interference with CYP enzyme activity—can easily explain the features that are characteristic of autism.
The term "developmental immunotoxicity" has been coined to describe permanent modifications to the immune function that take place early in life, leading to later development of allergies, asthma, and autoimmune diseases [278-280]. These authors have argued that prenatal and/or early life exposure to environmental toxins can lead to a phenotype that includes a hyperinflammatory response and disruption of cytokine networks, and they propose that an increased exposure to environmental toxins early in life may contribute to the increased incidence of these conditions observed today. It is significant that these problems often occur in association with autism [281].
Contrary to the current widely-held misconception that glyphosate is relatively harmless to humans, the available evidence shows that glyphosate may rather be the most important factor in the development of multiple chronic diseases and conditions that have become prevalent in Westernized societies. In addition to autism, these include gastrointestinal issues such as inflammatory bowel disease, chronic diarrhea, colitis and Crohn's disease, obesity, cardiovascular disease, depression, cancer, cachexia, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and ALS, among others. While glyphosate is obviously not the only environmental toxin to contribute to these diseases and conditions, glyphosate's ability to disrupt the gut bacteria, to impair serum transport of sulfate and phosphate, and to interfere with CYP enzymes, logically progresses to this multitude of diseased states, through well-established biological processes. And glyphosate's disruption of the body's ability to detoxify other environmental toxins leads to synergistic enhancement of toxicity. While genetics surely play a role in susceptibility, genetics may rather influence which of these conditions develops in the context of glyphosate exposure, rather than whether any of these conditions develops. We have explained the logical sequence of events leading to serotonin deficiency and subsequent pathologies, following glyphosate's disruption of tryptophan synthesis by gut bacteria [10,29], and its further sequestration into macrophages that infiltrate the intestinal tissues in order to detoxify lipopolysaccharides released from pathogenic bacteria, whose overgrowth is induced by glyphosate [35]. Sulfate depletion arises in the gut, both because of impaired transport of free sulfate in the bloodstream and impaired sulfate synthesis by eNOS [63,64]. Disruption of gut bacteria, exposure to toxic phenolic compounds necessary to enable sulfate transport, and deficient sulfate supply to the

mucopolysaccharides in the gut all contribute to the leaky gut syndrome that is a common feature in autism [51]. The evidence shows that glyphosate can interfere with development through its suppression of aromatase synthesis [110] and through its interference with the breakdown of retinoic acid [113] and its interference with CDKs and sulfate supplies. Glyphosate could also be a factor in the current epidemic in vitamin D3 deficiency [166] through its disruption of the CYP enzymes that activate this hormone in the liver [164,165]. The kosmotropic property of the glyphosate molecule combined with its disruption of CYP enzymes in the blood stream can lead to excess thrombosis and hemorrhaging, common problems today among the elderly. We propose that glyphosate's disruption of the synthesis of sulfate by the CYP orphan enzyme, eNOS, leads to widespread deficiencies in cholesterol and sulfate in the blood stream and all the tissues. We have previously described how disruption of eNOS' synthesis of sulfate would lead to diabetes and cardiovascular disease [177]. Glyphosate's induction of excess synthesis of ammonia in the gut, combined with depletion of zinc through impaired absorption, depletion of serotonin through dysbiosis of its substrate, tryptophan, depletion of dopamine through impaired synthesis of its substrate, tyrosine, depletion of vitamin D3, due to impairments in the CYP enzyme responsible for its activation, and depletion of sulfate through interference with its synthesis, can all lead to a multitude of pathologies in the brain, including autism, Alzheimer's disease, ADHD, Parkinson's disease, multiple sclerosis and ALS.

There is a substantial alignment among countries, worldwide, with low or decreasing birth rates, emerging obesity problems, and an increasing glyphosate burden. Given the arguments presented here, it is plausible that glyphosate is causal in these trends. It may also be possible to demonstrate strong correlations between glyphosate usage and both autism and breast cancer. Formal epidemiological studies should be conducted to look at these issues more closely.

In our opinion, it is imperative that governments around the globe unite in investing significant research funds to support independent studies evaluating the long-term effects of glyphosate. Other researchers should try to reproduce the results obtained in [9] showing tumorigenesis and premature death in rats with life-long exposure to glyphosate. The study on the gut microbiome of chickens [35] needs to be reproduced in other species, and the gene array study on E. coli [39] needs to be reproduced for other common gut bacteria. The novel idea that glyphosate disrupts sulfate transport through its kosmotropic effects, as predicted given biophysical laws, needs to be verified in specific studies among a variety of species. This could be done by comparing the levels of free sulfate in the blood under conditions of glyphosate exposure against controls. The study on glyphosate's effects on bees [126] should be reproduced by other researchers, along with further studies examining the impact of prior exposure to glyphosate on bees' resistance to pesticides. More refined and economical methods for detecting glyphosate in the food supply, such as in [282,283], and in the water supply [284], need to be developed, and then applied to a variety of different food items. Most critical in our view are the vegetable oils derived from GM crops – canola oil, soybean oil, corn oil, and cottonseed oil, as well as soy-derived protein, beet sugar, and high fructose corn syrup – ingredients that are pervasive in processed foods. Glyphosate is likely also present in meat, eggs, cheese, and other dairy products derived from animals fed glyphosate-contaminated grass, alfalfa, corn, and soy [285,286].

14. Conclusion

This paper presents an exhaustive review of the toxic effects of the herbicide, glyphosate, the active ingredient in Roundup®, in humans, and demonstrates how glyphosate's adverse effects on the gut microbiota, in conjunction with its established ability to inhibit the activity of cytochrome P450 enzymes, and its likely impairment of sulfate transport, can remarkably explain a great number of the diseases and conditions that are prevalent in the modern industrialized world. Its effects are insidious, because the long-term effects are often not immediately apparent. The pathologies to which glyphosate could plausibly contribute, through its known biosemiotic effects, include inflammatory bowel disease, obesity, depression, ADHD, autism, Alzheimer's disease, Parkinson's disease, ALS, multiple sclerosis, cancer, cachexia, infertility, and developmental malformations. Glyphosate works synergistically with other factors, such as insufficient sun exposure, dietary deficiencies in critical nutrients such as sulfur and zinc, and synergistic exposure to other xenobiotics whose detoxification is impaired by glyphosate. Given the known toxic effects of glyphosate reviewed here and the plausibility that they are negatively impacting health worldwide, it is imperative for more independent research to take place to validate the ideas presented here, and to take immediate action, if they are verified, to drastically curtail the use of glyphosate in agriculture. Glyphosate is likely to be pervasive in our food supply, and, contrary to being essentially nontoxic, it may in fact be the most biologically disruptive chemical in our environment.

38: Glyphosate linked to botulism and other animal health problems

Three very interesting papers from Monika Kruger and her team in Leipzig, where the health effects of glyphosate are under close scrutiny. The research is flagging up the effects which glyphosate has on the gut micro-organisma in animals, for example by damaging the beneficial bacteria while not having such a dramatic effect on other "harmful" bacteria. They say" "A reduction of beneficial bacteria in the gastrointestinal tract microbiota by ingestion of glyphosate could disturb the normal gut bacterial community." They have studied poultry and cattle -- and are homing in on the incidence of botulism. They show that the farmers who look after sick cattle with botulism often have botulism too. C. botulinum occurs in cows' and farmers' feces, and in cattle feeds. The researchers show that the humans are most likely getting infected not from the cattle but from the feeds, because the same type of botulinum is present in both humans and feeds, but the type of botulinum in the cattle is different. There is now a strong probability that glyphosate residues in animal feeds result in botulism in the cattle and also in related ailments in poultry.

Visceral botulism at dairy farms in Schleswig Holstein, Germany - Prevalence of Clostridium botulinum in feces of cows, in animal feeds, in feces of the farmers, and in house dust

by Monika Krüger, Anke Große-Herrenthey, Wieland Schrödl, Achim Gerlach, Arne Rodloff
Anaerobe 18 (2012) 221e223
Accepted 11 December 2011 Available online 21 December 2011

Abstract
From 41 dairy farms in Schleswig Holstein, Germany, 196 fecal specimens of diseased cows, 77 fecal specimens of farmers and family members from 26 of these farms, 35 animal feed specimens and 7 house dust specimens were investigated for Clostridium botulinum and its antigens, respectively. Four of the humans under study (one child, 8 month, and three adults) showed symptoms of infant/visceral botulism. Specimens were cultivated in reinforced clostridial medium (RCM). C. botulinum antigens were detected by ELISA. The aim of the study was to obtain information on the relationship of detected C. botulinum toxin-types in cows, in the feces of attending humans, and in the immediate environment. The results revealed that C. botulinum toxin-types were different for cows and humans. Toxin-type A was dominant in cow feces while type E was found in humans. Type E was also present in some animal feed specimens. Conversely, toxin-type A was prevalent in the house dust of farms. It may be assumed that the feeds were the source of human colonization with C. botulinum.

The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro

by Awad A. Shehata, Wieland Schro¨dl, Alaa. A. Aldin, Hafez M. Hafez , and Monika Kru¨ger
Curr Microbiol DOI 10.1007/s00284-012-0277-2 Received: 14 August 2012 / Accepted: 15 November 2012 Springer Science+Business Media New York 2012

Abstract
The use of glyphosate modifies the environment which stresses the living microorganisms. The aim of the present study was to determine the real impact of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. The presented results evidence that the highly pathogenic bacteria as Salmonella Entritidis, Salmonella Gallinarum, Salmonella Typhimurium, Clostridium perfringens and Clostridium botulinum are highly resistant to glyphosate. However, most of beneficial bacteria as Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis and Lactobacillus spp. were found to be moderate to highly susceptible. Also Campylobacter spp. were found to be susceptible to glyphosate. A reduction of beneficial bacteria in the gastrointestinal tract microbiota by ingestion of glyphosate could disturb the normal gut bacterial community. Also, the toxicity of glyphosate to the most prevalent Enterococcus spp. could be a significant predisposing factor that is associated with the increase in C. botulinum-mediated diseases by suppressing the antagonistic effect of these bacteria on clostridia.

Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum

by Monika Krüger, Awad Ali Shehata, Wieland Schrödl, Arne Rodloff.

Anaerobe 20 (2013) 74e78 Accepted 29 January 2013 Available online 6 February 2013

Abstract

During the last 10e15 years, an increase of Clostridium botulinum associated diseases in cattle has been observed in Germany. The reason for this development is currently unknown. The normal intestinal microflora is a critical factor in preventing intestinal colonisation by C. botulinum as shown in the mouse model of infant botulism. Numerous bacteria in the gastro-intestinal tract (GIT) produce bacteriocines directed against C. botulinum and other pathogens: Lactic acid producing bacteria (LAB) such as lactobacilli, lactococci and enterococci, generate bacteriocines that are effective against Clostridium spp. A reduction of LAB in the GIT microbiota by ingestion of strong biocides like glyphosate could be an explanation for the observed increase in levels of C. botulinum associated diseases. In the present paper, we report on the toxicity of glyphosate to the most prevalent Enterococcus spp. in the GIT. Ingestion of this herbicide could be a significant predisposing factor that is associated with the increase in C. botulinum mediated diseases in cattle.

39: Bt (Cry) proteins are toxic to the blood of mice

Thursday, 02 May 2013 22:07

Comment from GM-free Cymru: This is an interesting and potentially very important paper, demonstrating that BT proteins are toxic to mammals, in spite of frequent assurances and assumptions to the contrary. The study was an "in vivo" one, using live animals, rather than a computer-based study or even an "in vitro" laboratory study using animal tissues of cell cultures etc. What we still do not know is the concentration at which these Cry toxins start to have negative effects, and the extent to which these experiments mimic what will happen if a "whole food" from a BT crop is fed to mammals. Another interesting thing about this study is that it implicated an actual GMO crop as being potentially dangerous to health, rather than pointing the finger at glyphosate or Roundup, which are the chemicals associated with the use of herbicide-tolerant GM crops. This means that the health damage arises from the genetic modification itself -- as has been suggested in other studies in the past.

http://www.gmwatch.org/index.php?option=com_content&view=article&id=14803:bt-toxins-are-toxic-to-the-blood-of-mice

NOTE: A new study (abstract below) explores the toxicity of Bt proteins in mammals. The study shows that the Bt toxins Cry1Aa, Cry1Ab, Cry1Ac, or Cry2A have toxic effects in the blood of mice. The methodology is not clearly described but what is clear is that the presumed nontoxicity of Bt toxin to mammals, on which all regulatory approvals of Bt crops are based, is false.

In insects, Bt toxins exercise their toxic effects by breaking holes in the gut and rupturing the cells. In the mice in this experiment, Bt toxins caused red blood cells to rupture.

The study says, "It has been reported that Cry toxins exert their toxicity when activated at alkaline pH of the digestive tract of susceptible larvae, and, because the physiology of the mammalian digestive system does not allow their activation, and no known specific receptors in mammalian intestinal cells have been reported, the toxicity [of] these MCAs [microbial control agents] to mammals would negligible. However, our study demonstrated that Bt spore-crystals genetically modified to express individually Cry1Aa, Cry1Ab, Cry1Ac, or Cry2A induced hematotoxicity, particularly to the erythroid lineage. This finding corroborates literature that demonstrated that alkali-solubilized Bt spore-crystals caused in vitro hemolysis in cell lines of rat, mouse, sheep, horse, and human erythrocytes and suggested that the plasma membrane of susceptible cells (erythrocytes, in this case) may be the primary target for these toxins [33]...

"In conclusion, results showed that the Bt spore-crystals genetically modified to express individually Cry1Aa, Cry1Ab, Cry1Ac, or Cry2A can cause some hematological risks to vertebrates, increasing their toxic effects with long-term exposure. Taking into account the increased risk of human and animal exposures to significant levels of these toxins, especially through diet, our results suggest that further studies are required to clarify the mechanism involved in the hematotoxicity found in mice, and to establish the toxicological risks to non-target organisms, especially mammals, before concluding that these microbiological

control agents are safe for mammals."
The toxicity of Bt proteins in mammalian cells was also the subject of an in vitro (test-tube) study (Mesnage et al., 2012; http://onlinelibrary.wiley.com/doi/10.1002/jat.2712/abstract). In this study, Bt toxin Cry1Ac was found to be toxic to human cells. --- --- Mezzomo, B. P., et al. (2013). Hematotoxicity of Bacillus thuringiensis as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac, or Cry2Aa in Swiss albino mice. J Hematol Thromb Dis 1(1).
Study available here in full: **http://esciencecentral.org/journals/JHTD/JHTD-1-104.pdf**

Abstract:
Formulated and sporulated cultures of Bacillus thuringiensis (Bt) have been widely used against insect pests, but after the advent of genetically modified plants expressing δ-endotoxins, the bioavailability of Cry proteins has been increased. For biosafety reasons their adverse effects should be studied, mainly for non-target organisms. Thus, we evaluated, in Swiss albino mice, the hematotoxicity and genotoxicity of four Bt spore-crystals genetically modified to express individually Cry1Aa, Cry1Ab, Cry1Ac, or Cry2A, administered alone by gavage with a single dose of 27 mg/ Kg, 136 mg/Kg or 270 mg/Kg, 24 h, 72 h or 7 days before euthanasia. Binary combinations of these four spore-crystal proteins were also assayed at 270 mg/Kg with a single administration 24 h before euthanasia. Control mice received filtered water or cyclophosphamide at 27 mg/kg. For hematotoxicity evaluations, blood samples were drawn by cardiac puncture and processed in a multiple automated hematology analyzer; for genotoxicity analyses, micronucleus test was carried out in mice bone marrow cells. Spore-crystal administrations provoked selective hematotoxicity for the 3 exposure times, particularly for erythroid lineage. A significant reduction in bone marrow cell proliferation demonstrated cytotoxic but not genotoxic effects. These effects persisted for all exposure times, becoming more evident at 7 days. Similar results were observed for binary combinations at 24 h, suggesting that further studies are required to clarify the mechanism involved in the hematotoxicity found in mice, and to establish the toxicological risks to non-target organisms, especially mammals, before concluding that these microbiological control agents are safe for mammals.

40: New paper on toxicity: glyphosate bad, Roundup worse for aquatic invertebrates

Date Added to website 20th June 2013
Comment from GM-Free Cymru:
This is an important new paper which demonstrates (yet again) the toxicity of glyphosate and Roundup when present in minute amounts in the aquatic environment. It also makes the point (yet again) that the regulators within the EU and USA are guilty of severe negligence since they assume that tests on glyphosate are adequate to demonstrate the environmental impacts of Roundup. This latter commercial formulation, as pointed out several times by Seralini and his colleagues, is MUCH more dangerous than glyphosate on its own. How much more experimental evidence do EFSA and the EC actually need before they accept that their assumptions about the safety of Roundup are based upon false assumptions and scientific fraud?

Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna

by Marek Cuhra, Terje Traavik and Thomas Bøhn
Ecotoxicology (2013) 22:251–262 DOI 10.1007/s10646-012-1021-1
Accepted: 15 November 2012 / Published online: 6 December 2012

Abstract
Low levels of glyphosate based herbicide induced significant negative effects on the aquatic inverte- brate Daphnia magna. Glyphosate herbicides such as brands of Roundup, are known to be toxic to daphnids. However, published findings on acute toxicity show significant dis- crepancies and variation across several orders of magnitude. To test the acute effects of both glyphosate and a commercial formulation of Roundup (hereafter Roundup), we conducted a series of exposure experiments with different clones and age-classes of D. magna. The results demonstrated EC50 (48) values in the low ppm-range for

Roundup as well as for the active ingredient (a.i.) isopropylamine salt of glyphosate (glyphosate IPA) alone. Roundup showed slightly lower acute toxicity than glyphosate IPA alone, i.e. EC50 values of 3.7–10.6 mg a.i./l, as compared to 1.4–7.2 mg a.i./l for glyphosate IPA. However, in chronic toxicity tests spanning the whole life-cycle, Roundup was more toxic. D. magna was exposed to sublethal nominal concentrations of 0.05, 0.15, 0.45, 1.35 and 4.05 mg a.i./l for 55 days. Significant reduction of juvenile size was observed even in the lowest test concentrations of 0.05 mg a.i./l, for both glyphosate and Roundup. At 0.45 mg a.i./l, growth, fecundity and abortion rate was affected, but only in animals exposed to Roundup. At 1.35 and 4.05 mg a.i./l of both glyphosate and Roundup, significant negative effects were seen on most tested parameters, including mortality. D. magna was adversely affected by a near 100 % abortion rate of eggs and embryonic stages at 1.35 mg a.i./l of Roundup. The results indicate that aquatic invertebrate ecology can be adversely affected by relevant ambient concentrations of this major herbicide. We conclude that glyphosate and Roundup toxicity to aquatic invertebrates have been underestimated and that current European Commission and US EPA toxicity classification of these chemicals need to be revised.

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41: A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM corn maize diet

Date Added to website September 2nd
(2013) by Dr Judy Carman, Howard Vlieger, Dr Larry Ver Steeg, Veryln Sneller, Dr Garth Robinson, Dr Kate Clinch-Jones, Dr Julie Haynes and Dr John Edwards. Journal of Organic Systems, Vol 8. No 1 (2013), pp 38-54

Abstract:
A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a longterm toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).

A long-term toxicology study on pigs fed a mixed GM diet. Adverse effects of GM crops found.
By Dr. Judy Carman 11 June 2013
This is a briefing about the contents of a new, peer-reviewed scientific paper titled: A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM corn maize diet, by Dr Judy Carman, Howard Vlieger, Dr Larry Ver Steeg, Veryln Sneller, Dr Garth Robinson, Dr Kate Clinch- Jones, Dr Julie Haynes and Dr John Edwards.
At a commercial piggery in the US, we took 168 just-weaned pigs and fed them a typical diet for the piggery, containing soy and corn, for 22.7 weeks (over 5 months) until the pigs were slaughtered at their usual slaughter age. Half of the pigs were fed widely-used varieties of GM soy and GM corn (the GM-fed group) for this whole period and the other half of the pigs were fed an equivalent non-GM diet (the control group). The GM diet contained three GM genes and therefore three GM proteins. One protein made the plant resistant to a herbicide and two proteins were insecticides. We chose a mixed diet instead of a single crop because this is usually what pigs and people eat. Regulators do not require animal feeding studies on mixtures of GM genes and their proteins, regardless of whether the genes are all "stacked" into the one plant or spread across several plants that are eaten in the same meal. We chose pigs because they have a similar digestive system to humans, and because some of the investigators had been observing reproductive and digestive problems in pigs fed GM crops. We took blood

from the pigs a few days before they were slaughtered to do standard biochemistry tests. Autopsies were done by qualified veterinarians who didn't know if a given pig was fed the GM diet or not, so their observations were completely unbiased. Some of the investigators had previously seen a reduced ability to conceive and higher rates of miscarriage in piggeries where sows were fed a GM diet, and a reduction in the number of piglets born if boars were used for conception rather than artificial insemination. Artificial insemination guarantees the presence of a certain number of viable sperm. Because male pigs were neutered at 3 days of age in order to provide meat free of boar-taint, we were only able to look at the female reproductive system in these pigs. We found that, on average, the weight of the uterus of pigs fed the GM diet, as a proportion of the weight of the pig, was 25% higher than the control pigs. We found that this biologically significant finding was also statistically significant. We list some of the pathologies that could be occurring in these uteri in the paper. Some of the investigators had also previously seen higher rates of intestinal problems in pigs fed a GM diet, including inflammation of the stomach and small intestine, stomach ulcers, a thinning of intestinal walls and an increase in haemorrhagic bowel disease, where a pig can rapidly "bleed-out" from their bowel and die. We weren't able to look inside the intestines, due to the amount of food in them, but we were able to look inside the stomach. We found that the level of severe inflammation in stomachs was markedly higher in pigs fed the GM diet. Pigs on the GM diet were 2.6 times more likely to get severe stomach inflammation than control pigs. Males were more strongly affected. While female pigs were 2.2 times more likely to get severe stomach inflammation when on the GM diet, males were 4 times more likely. These findings are both biologically significant and statistically significant. We found that these key findings were not reflected in the standard biochemistry tests that are done in GM feeding studies, probably because standard biochemistry tests provide a poor measure of inflammation and matters associated with uterus size. We did however find a marginally significant change on a measure of liver health in the blood of GM-fed pigs.

42: Genetic damage found in soybean workers in Brazil

Date Added to website 2nd September 2013
A new peer-reviewed study has found DNA damage and elevated cell death of blood cells in soybean workers exposed to fungicides, herbicides, and insecticides in Brazil. Glyphosate and 2,4-D were among the herbicides used by the exposed group. 2,4-D is increasingly used to combat glyphosate-resistant weeds in GM soybean fields.
This research comes as no surprise, given the revelations about the health damage done to farm workers and poor people who happen to live in the spray zones around plantings of GMO soy in particular. But anecdotal evidence is one thing -- careful studies like this are needed to underpin the horrendous images of the "collateral damage" associated with GM crop plantings. --- ---
Benedetti, D., et al. (2013). "Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays." Mutat Res 752(1-2): 28-33.http://www.sciencedirect.com/science/article/pii/S138357181300003X

Abstract
Soybean cultivation is widespread in the State of Rio Grande do Sul (RS, Brazil), especially in the city of Espumoso. Soybean workers in this region are increasingly exposed to a wide combination of chemical agents present in formulations of fungicides, herbicides, and insecticides.
In the present study, the comet assay in peripheral leukocytes and the buccal micronucleus (MN) cytome assay (BMCyt) in exfoliated buccal cells were used to assess the effects of exposures to pesticides in soybean farm workers from Espumoso. A total of 127 individuals, 81 exposed and 46 non-exposed controls, were evaluated. Comet assay and BMCyt (micronuclei and nuclear buds) data revealed DNA damage in soybean workers. Cell death was also observed (condensed chromatin, karyorhectic, and karyolitic cells). Inhibition of non-specific choline esterase (BchE) was not observed in the workers.
The trace element contents of buccal samples were analyzed by Particle-Induced X-ray Emission (PIXE). Higher concentrations of Mg, Al, Si, P, S, and Cl were observed in cells from workers. No associations with use of personal protective equipment, gender, or mode of application of pesticides were observed. Our findings indicate the advisability of monitoring genetic toxicity in soybean farm workers exposed to pesticides.

43: Glyphosate found in human urine across Europe

Date Added to website 2nd September 2013

NOTE: Read the full report, "Determination of Glyphosate residues in human urine samples from 18 European countries", by Medical Laboratory Bremen, here:

https://www.foeeurope.org/sites/default/files/glyphosate_studyresults_june12.pdf

Weedkiller found in human urine across Europe Friends of the Earth Europe, 13 June 2013 **http://www.foeeurope.org/weed-killer-glyphosate-found-human-urine-across-Europe-130613**

People in 18 countries across Europe have been found to have traces of the weed killer glyphosate in their urine, show the results of tests commissioned by Friends of the Earth Europe and released today [1].

The findings raise concerns about increasing levels of exposure to glyphosate-based weed killers, commonly used by farmers, public authorities and gardeners across Europe. The use of glyphosate is predicted to rise further if more genetically modified (GM) crops are grown in Europe [2].

Despite its widespread use, there is currently little monitoring of glyphosate in food, water or the wider environment. This is the first time monitoring has been carried out across Europe for the presence of the weed killer in human bodies.

Friends of the Earth Europe's spokesperson Adrian Bebb said: "Most people will be worried to discover they may have weed killer in their bodies. We tested people living in cities in 18 countries and found traces in every country. These results suggest we are being exposed to glyphosate in our everyday lives, yet we don't know where it is coming from, how widespread it is in the environment, or what it is doing to our health.

"Our testing highlights a serious lack of action by public authorities across Europe and indicates that this weed killer is being widely overused. Governments need to step-up their monitoring and bring in urgent measures to reduce its use. This includes rejecting any genetically modified crops that would increase the use of glyphosate."

Friends of the Earth Europe is calling on the European Union to urgently investigate how glyphosate is finding its way into people's bodies; to increase the levels of monitoring in the environment and in food and water; and to introduce immediate restrictions on the use of glyphosate.

Friends of the Earth Europe commissioned laboratory tests on urine samples from volunteers in 18 countries across Europe and found that on average 44% of samples contained glyphosate. The proportion of positive samples varied between countries, with Malta, Germany, the UK and Poland having the most positive tests, and lower levels detected in Macedonia and Switzerland.

All the volunteers who provided samples live in cities, and none had handled or used glyphosate products in the run-up to the tests which were carried out between March and May 2013.

Glyphosate is used on many genetically modified crops. 14 new GM crops designed to be cultivated with glyphosate are currently waiting for approval to be grown in Europe. Approval of these crops would inevitably lead to a further increase of glyphosate spraying in the EU.

The biggest producer of glyphosate is Monsanto which sells it under the brand name 'Roundup'. Two weeks ago the US Department of Agriculture announced that it had found GM wheat developed by Monsanto that has not been approved anywhere in the world growing in a field in Oregon, leading to some countries restricting or testing US wheat imports and farmers in the US starting legal cases against the company.

Notes:

[1] Urine samples were collected from volunteers in Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, France, Georgia, Germany, Hungary, Latvia, Macedonia, Malta, Poland, Spain, Switzerland, The Netherlands, and the UK. A total of 80/182 samples tested were found to contain glyphosate. Volunteers were all city-dwellers and included vegetarian and non-vegetarian diets. No two samples were tested from the same household. The samples were analysed by Dr Hoppe at Medical Laboratory Bremen in Germany.

The full results of "Determination of Glyphosate residues in human urine samples from 18 European countries" by Medical Laboratory Bremen are available online:

44: Horizontal gene transfer from food into human blood

Date Added to website 2nd September 2013

Another important article which demonstrates the reality of horizontal gene transfer and **https://www.foeeurope.org/sites/default/files/glyphosate_studyresults_june12.pdf**which shows that the advice from the FSA in this country and from EFSA in Europe is far wide of the mark. We support GM Watch on this, and demand that the FSA advice on horizontal gene transfer is updated as follows:

"Biologically active genes and proteins are common constituents of food and feed. Digestion in both animals and humans degrades the DNA to varying degrees, depending partly on disease status of the consumer. Degradation can be incomplete and fragments large enough to carry complete genes can avoid degradation and enter the human circulatory system. A high level of uptake of DNA fragments from the intestinal tract into the circulatory system appears to be correlated with the presence of inflammatory diseases."

http://www.gmwatch.org/index.php/news/archive/2013/15007-complete-genes-can-pass-from-food-to-human-blood-study

Complete genes can pass from food to human blood - study

A new study shows that, contrary to reassurances from industry and regulators, complete genes - including GM genes - can pass from food into human blood.

For many years, the public and scientists have been concerned that GM genes might be incorporated into animal products like meat and milk and consumed by humans.

In 2007 the European Food Safety Authority issued a reassuring statement to the effect that "a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids, or edible products of farm animals like broilers, cattle, pigs, or quails".

http://www.food.gov.uk/policy-advice/gm/gmanimal#.UgnoAryE7gP

Even in 2007, EFSA's statement had already been proven false by some scientific studies:**http://earthopensource.org/files/pdfs/GMO_Myths_and_Truths/GMO_Myths_and_Truths_1.3b.pdf** (section 3.8)

When reviewing the issue in the same year, EFSA seemed to be preparing the ground to change its advice, noting that "the recombinant [DNA] sequence is present in the GM plant only as a single or low copy number, which makes the potential absorption a rare event and therefore difficult to detect", and that "when more studies are carried out with more sensitive detection methods, such recombinant DNA fragments may be more frequently found in the future".**http://www.food.gov.uk/policy-advice/gm/gmanimal#.UgnoAryE7gP**

Thus EFSA could blame its change of mind on better detection methods rather than its own poor knowledge of the science.

This year, in the face of a sizeable and growing number of scientific studies showing that DNA from food, be it GM or non-GM, can and does end up in animal tissues and milk products that people eat, the UK Food Standards Agency updated its advice. The FSA admitted, "It is … possible that DNA fragments derived from GM plant materials may occasionally be detected in animal tissues, in the same way that DNA fragments derived from non-GM plant materials can be detected in these same tissues." **http://www.food.gov.uk/policy-advice/gm/gmanimal#.UgnoAryE7gP**

Now the FSA and EFSA will have to revise their advice once again, as a new study by Hungarian researchers on human blood serum samples shows that meal-derived DNA fragments which are large enough to carry complete genes can avoid degradation in the digestive tract and through an unknown mechanism enter the human circulation system.

These were not "fragments" of DNA, but stretches of plant DNA that are complete enough to enable the researchers to identify the exact plants that the human subjects ate, such as soy, maize, and oilseed rape. The researchers even found that in one of the blood serum samples the relative concentration of plant DNA was higher than the human DNA.

Interestingly, the highest concentrations of plant DNA were found in people with inflammatory diseases such as inflammatory bowel disease and Kawasaki disease, an autoimmune disease in which the blood vessels become inflamed.

In light of this study, the FSA might especially like to update its earlier, quite inaccurate, advice that "Biologically active genes and proteins are common constituents of food and feed, but digestion in both animals and humans is known to rapidly degrade their DNA, and the subsequent uptake of DNA fragments from the intestinal tract into the body is a normal physiological process."

The FSA might more accurately say something like:

"Biologically active genes and proteins are common constituents of food and feed. Digestion in both animals and humans degrades the DNA to varying degrees, depending partly on disease status of the consumer. Degradation can be incomplete and fragments large enough to carry complete genes can avoid degradation and enter the human circulatory system.
"A high level of uptake of DNA fragments from the intestinal tract into the circulatory system appears to be correlated with the presence of inflammatory diseases."
We've always said that science would eventually prove the truth of the dictum, "You are what you eat". Now it is time for regulatory authorities admit it. --- --- Spisak, S., et al. (2013). Complete genes may pass from food to human blood. PLoS ONE 8(7): e69805. **http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0069805**

Our bloodstream is considered to be an environment well separated from the outside world and the digestive tract. According to the standard paradigm large macromolecules consumed with food cannot pass directly to the circulatory system. During digestion proteins and DNA are thought to be degraded into small constituents, amino acids and nucleic acids, respectively, and then absorbed by a complex active process and distributed to various parts of the body through the circulation system. Here, based on the analysis of over 1000 human samples from four independent studies, we report evidence that meal-derived DNA fragments which are large enough to carry complete genes can avoid degradation and through an unknown mechanism enter the human circulation system. In one of the blood samples the relative concentration of plant DNA is higher than the human DNA. The plant DNA concentration shows a surprisingly precise lognormal distribution in the plasma samples while non-plasma (cord blood) control sample was found to be free of plant DNA.

45: Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans

Date Added to website 9th Jan 2014

Full Paper Here: **http://www.sciencedirect.com/science/article/pii/S0308814613019201**
Authors: T. Bøhn, M. Cuhra, T. Traavik, M. Sanden, J. Fagan, R. Primicerio
Highlights

- Glyphosate tolerant GM soybeans contain high residues of glyphosate and AMPA.
- Soybeans from different agricultural practices differ in nutritional quality.
- Organic soybeans showed a more healthy nutritional profile than other soybeans.
- Organic soy contained more sugars, protein and zinc, but less fibre and omega-6.
- This study rejects that GM soy is "substantially equivalent" to non-GM soybeans.

Abstract
This article describes the nutrient and elemental composition, including residues of herbicides and pesticides, of 31 soybean batches from Iowa, USA. The soy samples were grouped into three different categories: i) genetically modified, glyphosate-tolerant soy (GM-soy); ii) unmodified soy cultivated using a conventional "chemical" cultivation regime; and iii) unmodified soy cultivated using an organic cultivation regime. Organic soybeans showed the healthiest nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soy. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soy. GM-soy contained high residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively). Conventional and organic soybean batches contained none of these agrochemicals. Using 35 different nutritional and elemental variables to characterise each soy sample, we were able to discriminate GM, conventional and organic soybeans without exception, demonstrating "substantial non-equivalence" in compositional characteristics for 'ready-to-market' soybeans.
Note: This is a very important paper which gets rid of the myth -- once and for all -- that glyphosate /Roundup simply sits on the surface of plants and gets washed away in the rain. There is no doubt that glyphosate is absorbed into the plant and that it is easily traceable in the plant following harvest. It also accumulates over time. The authors argue -- very convincingly -- that GM Soy treated with Roundup is SUBSTANTIALLY DIFFERENT from conventional or organically produced soy. Thus the use of the term "substantially equivalent" is misleading and scientifically nonsensical -- and should be abandoned forthwith. Furthermore, the organic soy is shown in these tests to be more nutritional than the GM soy, according to a number of

different measures. On these grounds as well, the idea of substantial equivalence has to be abandoned.

46 and 47: Damage to rats from MON810 (Ajeeb YG) in 3-month study

Date Added to website 14th January 2014

These two papers from Egyptian researchers show that there are substantial and significant differences which appear in animals fed on a Monsanto 810 variety called Ajeeb YG (for Yieldguard) and in those fed on normal Ajeeb grains. Histopathologocal changes were apparent in rats after 91 days, as were organ changes, and other morphological and biochemical changes. Once again, the evidence stacks up of actual harm to mammals that are fed on GMO corn. When is somebody going to sit up and take notice?

Histopathological Changes in Organs of Male Rats Fed on Genetically Modified Corn

(Ajeeb YG) by El-Shamei, Z. S., Gab-Alla, A.A., Shatta, A. A, Moussa, E. A. and Rayan, A. M. ammrayan@yahoo.com

Abstract:
Ajeeb YG is a genetically modified (GM) insect resistant corn produced by incorporated the MON 810 (Monsanto) borer resistance trait in the best corn germplasm Ajeeb. The safety of Ajeeb YG corn was assessed by comparison of toxicology response variables in rats consuming diets containing Ajeeb YG with those containing Ajeeb corn grains. Corn grains from Ajeeb YG or Ajeeb were incorborated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 91 days. An additional negative control group of rats (n= 10/group) were fed AIN93G diets. Rats fed on GM corn showed histopathological changes. Liver displayed cytoplasmic vacuolation of centrolobular hepatocytes and fatty degeneration of hepatocytes. Kidneys showed congestion of renal blood vessels and cystic dilatation of renal tubules. Testes revealed necrosis and desquamation of spermatogoneal germ cells lining seminiferous tubules. Spleen showed slight lymphocytic depletion and splenic congestion. Small intestine showed hyperplasia, hyperactivation of mucous secretory glands and necrosis of intestinal villi were detected. Due to these observations, we suggest that the risk of GM crops cannot be ignored and deserves further investigations in order to identify possible long-term effects, if any, of GM food consumption that might help in the post market surveillance.
http://www.academia.edu/3405345/Histopathological_Changes_in_Some_Organs_of_Male_Rats_Fed_on_Genetically_Modified_Corn_Ajeeb_YG_
[El-Shamei, Z. S., Gab-Alla, A.A., Shatta, A. A, Moussa, E. A. and Rayan, A. M. Histopathological Changes in Some Organs of Male Rats Fed on Genetically Modified Corn (Ajeeb YG). J Am Sci 2012;8(10): 684-696]. (ISSN: 1545-1003).**http://www.jofamericanscience.org**. 93

Morphological & Biochemical Changes in Male Rats Fed on Genetically Modified Corn

(Ajeeb YG) by Gab-Alla, A. A., El-Shamei, Z.S., Shatta, A.A., Moussa, E.A., and Rayan, A.M. ammrayan@yahoo.com

Abstract :
This study was designed to evaluate the safety of genetically modified (GM) corn (Ajeeb YG). Corn grains from Ajeeb YG or its control (Ajeeb) were incorporated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 45 and 91 days. An additional negative control group of rats (n= 10/group) was fed AIN93G diets. General conditions were observed daily, total body weights were recorded weekly. At the termination of the study periods, some visceral organs (heart, liver, kidneys, testes and spleen) and serum biochemistry were measured. The data showed several statistically significant differences in organs/body weight and serum biochemistry between the rats fed on GM and/or Non-GM corn and the rats fed on AIN93G diets. In general, GM corn sample caused several changes by increase or decrease organs/body weight or serum biochemistry values. This indicates potential adverse health/toxic effects of GM corn and further investigations still needed.
http://www.academia.edu/3138607/Morphological_and_Biochemical_Changes_in_Male_Rats_Fed_on_Genetically_Modified_Corn_Ajeeb_YG_

[Gab-Alla, A. A., El-Shamei, Z. S., Shatta, A. A., Moussa, E. A., and Rayan, A. M. Morphological and Biochemical Changes in Male Rats Fed on Genetically Modified Corn (Ajeeb YG). J Am Sci 2012;8(9):1117- 1123]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 152

48: MON810 corn is substantially different from its isoline

Date Added to website 17th January 2014

This is an interesting study undertaken in Egypt, using a traditional non-Gm corn called Ajeeb, on the one hand, and a Yieldguard variety called Ajeeb YG on the other hand -- the latter bred by Monsanto using MON810 as its breeding stock. This is of course a BT variety, with inbuilt insect resistance. So the study is a straight GM v non-GM study of plant characteristics. We are assured by one of the authors as follows: "……this is to inform you that genetically modified corn (Ajeeb YG) and control grain (Ajeeb; the same background genetics but lacked the MON-00810-6 coding sequence) were grown at the same time (2011) and in the same county in Egypt (Hehia, Sharkia Governorate). Trials were harvested from side-by-side plots, to remove any possible effect of environment and climate or any cross-contamination." This looks like a pretty secure trial -- and the result is not at all unexpected -- the BT corn is SUBSTANTIALLY DIFFERENT from its non-GM counterpart.

Chemical Analysis of BT corn "Mon-810: Ajeeb-YG®" and its counterpart non-Bt corn "Ajeeb" by Abdo E.M., Barbary O.M., Shaltout O.E. (Department of food science, faculty of Agriculture Saba Basha, Alexandria University, Egypt)
IOSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736. Volume 4, Issue 1 (Mar. – Apr. 2013), PP 55-60**www.iosrjournals.org**

Abstract:
Commercialization of biotech crops has started since 1996, where the cultivated area of these crops was increased from 1.7 million hectares in 1996 to 170.3 million hectares in 2012 according to the latest statistics in 2012. Bt corn "MON810: Ajeeb YG®" is one of these crops that express endotoxin from Bacillus thuringiensis (Bt) throughout the whole plant. This study was designed to assess the safety of Bt corn by comparing its compositional chemical analysis with its conventional counterpart "Ajeeb". Moisture content, crude fat, total saccharides, starch & crude fiber were determined; sodium, potassium, magnesium, calcium and phosphorous content were measured, tannins & phytic acid were determined as anti-nutrients. Amino acids and fatty acids profiles were also evaluated. Results indicated the presence of significant differences between both of Bt corn and its counterpart.

49 and 50: Pesticides used with GMOs are much more toxic than stated

Date Added to website 6th February 2014

Major pesticides are more toxic to human cells than their declared active principles

Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini G-E. . Biomedical Research International, 2014.
http://downloads.hindawi.com/journals/bmri/aip/179691.pdf

Abstract
Pesticides are used throughout the world as mixtures called formulations. They contain adjuvants, which are often kept confidential and are called inerts by the manufacturing companies, plus a declared active principle (AP), which is usually tested alone. This is true even in the longest toxicological regulatory tests performed on mammals. We tested the toxicity of 9 pesticides, comparing active principles and their formulations, on three human cell lines (HepG2, HEK293 and JEG3). We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. Glyphosate, isoproturon, fluroxypyr, pirimicarb, imidacloprid, acetamiprid, tebuconazole, epoxiconazole and prochloraz constitute respectively the active principles of 3 major herbicides, 3 insecticides and 3 fungicides. Fungicides were the most toxic from concentrations 300-600 times lower than agricultural dilutions, followed by herbicides, and then insecticides, with very similar profiles in all cell types. The human placental JEG3 cells appeared to be the most sensitive. Despite its relatively benign reputation, Roundup was by

far the most toxic among the herbicides and insecticides tested. Most importantly, 8 formulations out of 9 were several hundred times more toxic than their active principle. Our results challenge the relevance of the Acceptable Daily Intake for pesticides because this norm is calculated from the toxicity of the active principle alone. The study of combinatorial effects of several APs together may be of only secondary importance if the toxicity of the combinations of each AP with its adjuvants is neglected or unknown. Chronic tests on pesticides may not reflect relevant environmental exposures if only one ingredient of these mixtures is tested alone.

Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity.

R. Mesnage, B. Bernay, G.-E. Séralini. Toxicology, Volume 313, Issues 2–3, 16 November 2013, Pages 122–128
http://dx.doi.org/10.1016/j.tox.2012.09.006

Abstract
Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is an herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls we used a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate. This was performed after 24 h exposures on hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. The compositions in adjuvants were analyzed by mass spectrometry. Here we demonstrate that all formulations are more toxic than glyphosate, and we separated experimentally three groups of formulations differentially toxic according to their concentrations in ethoxylated adjuvants. Among them, POE-15 clearly appears to be the most toxic principle against human cells, even if others are not excluded. It begins to be active with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 ppm, at environmental/occupational doses. We demonstrate in addition that POE-15 induces necrosis when its first micellization process occurs, by contrast to glyphosate which is known to promote endocrine disrupting effects after entering cells. Altogether, these results challenge the establishment of guidance values such as the acceptable daily intake of glyphosate, when these are mostly based on a long term in vivo test of glyphosate alone. Since pesticides are always used with adjuvants that could change their toxicity, the necessity to assess their whole formulations as mixtures becomes obvious. This challenges the concept of active principle of pesticides for non-target species.

51: Glyphosate induces damage to liver & blood in mice

Date Added to website 6th February 2014

Yet more evidence of damage to mammals associated with glyphosate in drinking water -- and still Monsanto pretends that it is benign or harmless, while marketing it as "the ultimate killing machine".....

Oxidative stress and comet assay in tissues of mice administered glyphosate and ampa in drinking water for 14 days.

Mañas, F., Peralta, L., Ugnia, L., Weyers, A., García Ovando, H., & Gorla, N. (2013). BAG. Journal of basic and applied genetics, 24(2), 67-75.
http://www.scielo.org.ar/scielo.php?pid=S1852-62332013000300007&script=sci_arttext

Abstract
Excessive amounts of the herbicide glyphosate are incorporated daily to the soil and the ecosystems. AMPA is its major environmental breakdown product. In this study we determined the levels of thiobarbituric acid reactive substances (TBARs); quantified superoxide dismutase (SOD) and catalase (CAT) activity in liver, kidney, lung and heart, and performed the comet assay in blood and liver of mice administered glyphosate (40 or 400 mg/kg/day) or AMPA (100 mg/kg/day) in drinking water

for 14 days. Exposure to glyphosate 400 mg/kg induced a statistically significant ($p < 0.05$) decrease of SOD activity in heart and an increase in CAT activity in kidney. In the comet assay there were statistically significant differences in all the treatments and tissues studied in comparison to control animals ($p = 0.01$). The major results of this study were that mice administered glyphosate or AMPA in drinking water for 14 days induced a significant increase in DNA damage in liver and blood but minor effects on oxidative stress parameters. DNA effects on liver and blood indicate that these compounds could be of concern in terms of their potential to damage the genetic material, and that oxidative stress does not seem to be the mechanism causing that effect.

52: Field-evolved resistance by western corn rootworm to multiple Bt toxins in transgenic maize

Date Added to website 21st March 2014
by Aaron J. Gassmann, Jennifer L. Petzold-Maxwell, Eric H. Clifton, Mike W. Dunbar, Amanda M. Hoffmann, David A. Ingber, and Ryan S. Keweshan Proc Nat Acad USA, 2014, doi:
10.1073/pnas.1317179111 http://www.pnas.org/content/early/2014/03/12/1317179111

Significance

Crops genetically engineered to produce insecticidal toxins derived from the bacterium Bacillus thuringiensis (Bt) kill pest insects and reduce the use of conventional insecticides. However, the evolution of Bt resistance can diminishes these benefits. The western corn rootworm is a serious pest of maize and is managed with Bt maize. Beginning in 2009, western corn rootworm with resistance to maize producing the Bt toxin Cry3Bb1 imposed severe injury to Cry3Bb1 maize in Iowa. We show that cross-resistance exists between Cry3Bb1 maize and mCry3A maize and is associated with severe injury to Bt maize in farmers' fields. These results illustrate that Bt crops producing less than a high dose of toxin against target pests may select for resistance rapidly; consequently, current approaches for managing Bt resistance should be reexamined.

Abstract

The widespread planting of crops genetically engineered to produce insecticidal toxins derived from the bacterium Bacillus thuringiensis (Bt) places intense selective pressure on pest populations to evolve resistance. Western corn rootworm is a key pest of maize, and in continuous maize fields it is often managed through planting of Bt maize. During 2009 and 2010, fields were identified in Iowa in which western corn rootworm imposed severe injury to maize producing Bt toxin Cry3Bb1. Subsequent bioassays revealed Cry3Bb1 resistance in these populations. Here, we report that, during 2011, injury to Bt maize in the field expanded to include mCry3A maize in addition to Cry3Bb1 maize and that laboratory analysis of western corn rootworm from these fields found resistance to Cry3Bb1 and mCry3A and cross-resistance between these toxins. Resistance to Bt maize has persisted in Iowa, with both the number of Bt fields identified with severe root injury and the ability western corn rootworm populations to survive on Cry3Bb1 maize increasing between 2009 and 2011. Additionally, Bt maize targeting western corn rootworm does not produce a high dose of Bt toxin, and the magnitude of resistance associated with feeding injury was less than that seen in a high-dose Bt crop. These first cases of resistance by western corn rootworm highlight the vulnerability of Bt maize to further evolution of resistance from this pest and, more broadly, point to the potential of insects to develop resistance rapidly when Bt crops do not achieve a high dose of Bt toxin.

• E-mail: aaronjg@iastate.edu. Author contributions: A.J.G. and J.L.P.-M. designed research; J.L.P.-M., E.H.C., M.W.D., A.M.H., D.A.I., and R.S.K. performed research; A.J.G. and J.L.P.-M. analyzed data; and A.J.G. and J.L.P.-M. wrote the paper.
Conflict of interest statement: A.J.G. has received research funding related to this project from Monsanto and has received funding not related to this project from AMVAC, Dow AgroSciences, DuPont-Pioneer, Monsanto, Syngenta, and Valent. A.J.G. has filed for a patent of the plant-based bioassay described in this article.
This article is a PNAS Direct Submission.
This article contains supporting information online at
www.pnas.org/lookup/suppl/doi:10.1073/pnas.1317179111/-/DCSupplemental.

53: Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka?

Date Added to website 21st March 2014

by Channa Jayasumana, Sarath Gunatilake, and Priyantha Senanayake,
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Int. J. Environ. Res. Public Health 2014, 11(2), 2125-2147; doi:10.3390/ijerph110202125
http://www.mdpi.com/1660-4601/11/2/2125
Received: 17 December 2013; in revised form: 22 January 2014 / Accepted: 27 January 2014 / Published: 20 February 2014

Abstract:
The current chronic kidney disease epidemic, the major health issue in the rice paddy farming areas in Sri Lanka has been the subject of many scientific and political debates over the last decade. Although there is no agreement among scientists about the etiology of the disease, a majority of them has concluded that this is a toxic nephropathy. None of the hypotheses put forward so far could explain coherently the totality of clinical, biochemical, histopathological findings, and the unique geographical distribution of the disease and its appearance in the mid-1990s. A strong association between the consumption of hard water and the occurrence of this special kidney disease has been observed, but the relationship has not been explained consistently. Here, we have hypothesized the association of using glyphosate, the most widely used herbicide in the disease endemic area and its unique metal chelating properties. The possible role played by glyphosate-metal complexes in this epidemic has not been given any serious consideration by investigators for the last two decades. Furthermore, it may explain similar kidney disease epidemics observed in Andra Pradesh (India) and Central America. Although glyphosate alone does not cause an epidemic of chronic kidney disease, it seems to have acquired the ability to destroy the renal tissues of thousands of farmers when it forms complexes with a localized geo environmental factor (hardness) and nephrotoxic metals.

54: Detection of Glyphosate Residues in Animals and Humans.

Date Added to website 6th April 2014

Detection of Glyphosate Residues in Animals and Humans.

by Krüger M, Schledorn P, Schrödl W, Hoppe HW, Lutz W, et al. (2014)

J Environ Anal Toxicol 4:210. doi: 10.4172/2161-0525.1000210
http://omicsonline.org/open-access/detection-of-glyphosate-residues-in-animals-and-humans-2161-0525.1000210.php?aid=23853

Abstract
In the present study glyphosate residues were tested in urine and different organs of dairy cows as well as in urine of hares, rabbits and humans using ELISA and Gas Chromatography-Mass Spectroscopy (GC-MS). The correlation coefficients between ELISA and GC-MS were 0.96, 0.87, 0.97and 0.96 for cattle, human, and rabbit urine and organs, respectively. The recovery rate of glyphosate in spiked meat using ELISA was 91%. Glyphosate excretion in German dairy cows was significantly lower than Danish cows. Cows kept in genetically modified free area had significantly lower glyphosate concentrations in urine than conventional husbandry cows. Also glyphosate was detected in different organs of slaughtered cows as intestine, liver, muscles, spleen and kidney. Fattening rabbits showed significantly higher glyphosate residues in urine than hares. Moreover, glyphosate was significantly higher in urine of humans with conventional feeding. Furthermore, chronically ill humans showed significantly higher glyphosate residues in urine than healthy population. The presence of glyphosate residues in both humans and animals could haul the entire population towards numerous health hazards, studying the impact of glyphosate residues on health is warranted and the global regulations for the use of glyphosate may have to be re-evaluated.

55: Glyphosate is probably carginogenic to humans

Date Added to website 23rd June 2015

This short report from the Cancer Monograph Working Group of the IARC and WHO has caused quite a stir, because it confirms a probable link between glyphosate and cancer in humans. Many people have been saying this for a very long time, but this is the highest-profile committee so far to have come out and stated the link is such powerful terms. The working group declares tht the evidence of a glyphosate - cancer link in animals is "sufficient" and that there is limited evidence of glyphosate causing non-Hodgkin lymphoma and genotoxicity and oxidative stress in humans. Inevitably, when the report appeared in The Lancet, there was global media coverage, and Monsanto and the rest iof the GMO "establishment" has sought to cast doubt on the report, questioning the authors' impartiality and expertise! Monsanto went way over the top, referring to the report as "junk science"..... But the WHO has refused to be intimidated, and the full Report will probably appear next year. In the meantime, the battle of words continues.

"Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate"
Kathryn Z Guyton, Dana Loomis, Yann Grosse, Fatiha El Ghissassi, Lamia Benbrahim-Tallaa, Neela Guha, Chiara Scoccianti, Heidi Mattock, Kurt Straif,
on behalf of the International Agency for Research on Cancer Monograph Working Group,
IARC, Lyon, France
Published Online: 20 March 2015
The Lancet Oncology

www.thelancet.com/journals/lanonc/article/PIIS1470-2045%2815%2970134-8/abstract
DOI: http://dx.doi.org/10.1016/S1470-2045(15)70134-8

Extract:

Glyphosate is a broad-spectrum herbicide, currently with the highest production volumes of all herbicides. It is used in more than 750 different products for agriculture, forestry, urban, and home applications. Its use has increased sharply with the development of genetically modifi ed glyphosate-resistant crop varieties. Glyphosate has been detected in air during spraying, in water, and in food. There was limited evidence in humans for the carcinogenicity of glyphosate. Case-control studies of occupational exposure in the USA,14 Canada,6 and Sweden7 reported increased risks for non-Hodgkin lymphoma that persisted after adjustment for other pesticides. The AHS cohort did not show a signifi cantly increased risk of non-Hodgkin lymphoma. In male CD-1 mice, glyphosate induced a positive trend in the incidence of a rare tumour, renal tubule carcinoma. A second study reported a positive trend for haemangiosarcoma in male mice.15 Glyphosate increased pancreatic islet-cell adenoma in male rats in two studies. A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice. Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Soil microbes degrade glyphosate to aminomethylphosphoric acid (AMPA). Blood AMPA detection after poisonings suggests intestinal micro bial metabolism in humans. Glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells in vitro. One study reported increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying of glyphosate formulations.16 Bacterial mutagenesis tests were negative. Glyphosate, glyphosate formulations, and AMPA induced oxidative stress in rodents and in vitro. The Working Group classifi ed glyphosate as “probably carcinogenic to humans” (Group 2A).

For the very petulant response of Monsanto and the Glyphosate task Force, see these:
Monsanto seeks retraction for report linking herbicide to cancer
By Carey Gillam, Reuters
http://www.reuters.com/article/2015/03/24/us-monsanto-herbicide-idUSKBN0MK2GF20150324
The response by the pesticide industry association, the Glyphosate Task Force, is here:
http://www.wmcactionnews5.com/story/28574811/statement-of-the-gtf-on-the-recent-iarc-decision-concerning-glyphosate

56: GMO plants accumulate formaldehyde and are not "substantially equivalent"

Date Added to website 18th July 2015

[Comment from GM-Free Cymru: this new study, involving a systems biology approach, uses data from over 6,000 experiments to suggest that GMO plants are stressed by the GM process and thereby lose the ability to control or resist other stresses -- leading to a build-up of formaldehyde and glutathione to unacceptable levels. In other words GMO plants can become toxic whereas parent line are not. This destroys the concept of substantial equivalence and undermines the whole regulatory process in the US and Europe. The research also provides support for the findings of Ewen and Pusztai in 1998-99, who found that the transformation of potatoes from normal to GMO apparently induced toxicity which had a negative effect on the health of animals. For this, the authors were vilified and Pusztai's research team was dismantled in one of the most despicable acts of scientific vandalism and victimisation of recent decades. The text of the new paper can be accessed from the site referred to below. Let us see where this latest research leads.....]

Systems Biology Group, International Center for Integrative Systems: GMO Soy Accumulates Formaldehyde & Disrupts Plant Metabolism, Suggests Peer-Reviewed Study, Calling For 21st Century Safety Standards

Study Concludes FDA GMO Approval Process is Flawed, Outdated, and Unscientific

http://www.prnewswire.com/news-releases/systems-biology-group-international-center-for-integrative-systems-gmo-soy-accumulates-formaldehyde--disrupts-plant-metabolism-suggests-peer-reviewed-study-calling-for-21st-century-safety-standards-300112959.html

WASHINGTON, July 14, 2015 /PRNewswire/ --

A new study published today in the peer-reviewed journal AGRICULTURAL SCIENCES reveals genetic engineering of soy disrupts the plant's natural ability to control stress, and invalidates the FDA's current regulatory framework of "substantial equivalence" used for approval of genetically engineered food (GMOs).

The study, led by Dr. V.A. Shiva Ayyadurai, Ph.D., an MIT-trained systems biologist, utilizes his latest invention, CytoSolve, a 21st century systems biology method to integrate 6,497 in vitro and in vivo laboratory experiments, from 184 scientific institutions, across 23 countries, to discover the accumulation of formaldehyde, a known carcinogen, and a dramatic depletion of glutathione, an anti-oxidant necessary for cellular detoxification, in GMO soy, indicating that formaldehyde and glutathione are likely critical criteria for distinguishing the GMO from its non-GMO counterpart.

Dr. Ayyadurai stated, "The results demand immediate testing along with rigorous scientific standards to assure such testing is objective and replicable. It's unbelievable such standards for testing do not already exist. The safety of our food supply demands that science deliver such modern scientific standards for approval of GMOs."

"The discovery reported by Dr. Ayyadurai reveals a new molecular paradigm associated with genetic engineering that will require research to discover why, and how much formaldehyde and glutathione concentration, and what other cellular chemicals relevant to human and animal health, are altered. We need the kinds of standards Dr. Ayyadurai demands to conduct such research," stated Dr. Ray Seidler, a former EPA Senior Scientist. "Formaldehyde is a known class1 carcinogen. Its elevated presence in soybeans caused by a common genetic engineering event is alarming and deserves immediate attention and action from the FDA and the Obama administration. Soy is widely grown and consumed in the U.S., including by infants fed baby food products, with 94% of soy grown here being genetically engineered," declared Seidler.

The study concludes the U.S. government's current standards for safety assessment of GMOs, based on the principle of "substantial equivalence," is outdated and unscientific for genetically engineered food since it was originally developed for assessing the safety of medical devices in the 1970s. The current criteria for assessing "equivalence" considers only basic nutritional and superficial characteristics such as taste, sight, smell and touch, for declaring GMOs safe for human consumption, allowing them to be fast-tracked to market without independent scientific testing. If formaldehyde and glutathione were criteria, then the GMO would likely not be deemed "equivalent" to its non-GMO counterpart. This finding calls into question the FDA's food safety standards for the entire country.

The publication of the paper coincides with release of a bulletin by the Obama Administration on July 2, 2015, calling for "Improving Transparency and Ensuring Continued Safety in Biotechnology."

Ayyadurai shares, "This is not a pro- or anti-GMO question. But, are we following the scientific method to ensure the safety of our food supply? Right now, the answer is 'no'. We need to, and we can, if we engage in open, transparent, and collaborative

scientific discourse, based on a systems biology approach."
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Nathan Nye: nnye@fenton.com, (910)876-2601;
Alison Channon: achannon@fenton.com, (202)789-7752

57: Safety tests carried out for GMO / chemicals approvals are mostly worthless due to feed contamination

Date Added to website 19th July 2015
Laboratory rodent diets contain toxic levels of environmental contaminants: Implications for regulatory tests. Robin Mesnage and Nicolas Defarge*, Louis-Marie Rocque, Joel Spiroux de Vendomois, and Gilles-Eric Seralini. Plos One, 2 July 2015
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0128429
This important paper by Mesnage et al was finally published on 2nd July. It shows that contamination of laboratory animal feed is the norm rather than the exception. This means that most GMO "safety studies" are worthless, since both the control groups and the test groups have been given feed with GMOs and other toxins. Please read the paper and share our press release as widely as possible..
http://www.gmfreecymru.org/news/Press_Notice17June2015b.html

The authors of the recent paper on contaminated lab feed used in GMO and chemical safety testing have signalled their disappointment at the exclusion of two key sentences from their abstract as published in the journal PlosOne. The paper was due to be published on 18th June, but on the day before that the Editors delayed publication (thereby screwing up the press launch already fixed for Paris and effectively blocking off media coverage in the UK and elsewhere). The paper was finally published on 2nd July, accompanied by rather feeble excuses from the journal Editors about inadequate declarations of interest and inadequate listing of financial contributors to the study. Anyway, with the paper now safely in print, the GRIIGEN research team led by Prof Seralini has put out a statement specifying which two sentencs were removed by the Editors. They are as follows:
"This [work] invalidates the use of external controls (historical data) in regulatory tests, consisting of comparisons of toxicological effects to control rats from other experiments, because these control rats are fed different mixtures of pollutants. This also questions the use of 50 rats per group in carcinogenicity studies to increase the statistical power lost due to the elevated pathological background."
They have also flagged up their own take on the significance of the research: "Tests carried out for the commercialization of chemicals and GMOs are invalidated by the diets of laboratory rats."
Much as the GMO industry would like Seralini and his team to shut up or go away, they have no intention of doing either!
http://www.criigen.org/…/Tests-carried-out-for-the-commerci…

Monday 6 July 2015
The paper accepted by PLOS ONE has been published finally on july 2nd and not on june 17th. No change in results, but the abstract has been shortened by the editor on the regulatory consequences and with precisions of our funding for our other works.
Laboratory rats are frequently used for testing chemicals and genetically modified (GMO) foods, as the last step before commercialization in order to determine effects on mammalian health and predict risk in humans. Such chemicals include pesticides (which often are endocrine disruptors or toxic to the nervous system), plasticizers, and food additives. Some are suspected of being carcinogenic, and others are gradually being banned after having poisoned people and the ecosystem. However, health agencies consider that a high proportion of laboratory animals are predisposed to developing many diseases, based on industrial data archives known as "historical control data". According to these data, 13–71% of the animals would spontaneously or naturally present mammary tumors and 26–93% pituitary tumors, and the kidney function of these animals would frequently be deficient. This prevents the attribution of observed toxic effects to the products tested, and requires the sacrifice of a large number of animals in an attempt to observe statistically significant results in carcinogenicity tests, for example. But often, doubt persists and the product remains on the market. Do these diseases originate from genetic or environmental factors?

To investigate this question, the team of Professor Gilles-Eric Seralini of the University of Caen, supported by CRIIGEN, analyzed the dried feed of laboratory animals using standard methods and with the help of accredited laboratories. These animal feeds, sourced from five continents, are generally considered balanced and hygienic. The study was exceptionally wide-ranging; it investigated 13 samples of commonly used laboratory rat feeds for traces of 262 pesticides, 4 heavy metals, 17 dioxins and furans, 18 PCBs and 22 GMOs.

The results were overwhelming. All the feeds contained significant concentrations of several of these products, at levels likely to cause serious diseases and disrupt the hormonal and nervous system of the animals. This hides the effects of the products tested. For example, residues of the most widely used pesticide in the world, consisting of glyphosate and highly toxic adjuvants, such as Roundup, were detected in 9 of the 13 diets. Eleven of the 13 diets contained GMOs that are grown with large amounts of Roundup.

It should be noted that one of these feeds was used in DuPont's regulatory study on GM Roundup-tolerant oilseed rape. The type of feed given to the control animals in the DuPont study was found to contain significant amounts of Roundup residues, at levels known to cause toxic effects. The study concluded that the oilseed rape in question was safe, yet it is obviously flawed.

It therefore appears that the long-term consumption of contaminated feed interferes with good experimental practice and that the cause of diseases and disorders found in laboratory rats has been too quickly attributed to the genetic characteristics of the species used. Contrary to the assertions of the health agencies, these diseases cannot be called "spontaneous or natural". Further, the new study shows that the results of a number of regulatory toxicology tests conducted to date are highly questionable. Does the new study bring us a step closer to understanding the compromises and laxity of the methods of some experts?

In this way, countless industrial products that are potentially dangerous for public health have been helped onto the market.

The following text has been accepted first by the editor, and censored in the abstract the day before publication. It is important for the authors : This [work] invalidates the use of external controls (historical data) in regulatory tests, consisting of comparisons of toxicological effects to control rats from other experiments, because these control rats are fed different mixtures of pollutants. This also questions the use of 50 rats per group in carcinogenicity studies to increase the statistical power lost due to the elevated pathological background.

Published in PLOS ONE

Laboratory rodent diets contain toxic levels of environmental contaminants: Implications for regulatory tests. Robin Mesnage and Nicolas Defarge*, Louis-Marie Rocque, Joel Spiroux de Vendomois, and Gilles-Eric Seralini.

* These authors contributed equally to this work and should both be considered as first authors

Contact: Professor Gilles-Eric Seralini, Caen University, Institute of Biology and Network on Risks, Quality and Sustainable Environment. Tel. +33 (0)2 31 56 56 84

58: Glyphosate is an endocrine disruptor below "safety limits"

Date Added to website 18th August 2015

Potential toxic effects of glyphosate and its commercial formulations below regulatory limits

R. Mesnage, N. Defarge, J. Spiroux de Vendômois, G.E. Séralini

Food and Chemical Toxicology (2015), doi: 10.1016/j.fct.2015.08.012

http://www.sciencedirect.com/science/article/pii/S027869151530034X

Abstract

Glyphosate-based herbicides (GlyBH), including Roundup, are the most widely used pesticides worldwide. Their uses have increased exponentially since their introduction on the market. Residue levels in food or water, as well as human exposures, are escalating. We have reviewed the toxic effects of GlyBH measured below regulatory limits by evaluating the published literature and regulatory reports. We reveal a coherent body of evidence indicating that GlyBH could be toxic below the regulatory lowest observed adverse effect level for chronic toxic effects. It includes teratogenic, tumorigenic and hepatorenal effects. They could be explained by endocrine disruption and oxidative stress, causing metabolic alterations, depending on dose and exposure time. Some effects were detected in the range of the recommended acceptable daily intake. Toxic effects

of commercial formulations can also be explained by GlyBH adjuvants, which have their own toxicity, but also enhance glyphosate toxicity. These challenge the assumption of safety of GlyBH at the levels at which they contaminate food and the environment, albeit these levels may fall below regulatory thresholds. Neurodevelopmental, reproductive, and transgenerational effects of GlyBH must be revisited, since a growing body of knowledge suggests the predominance of endocrine disrupting mechanisms caused by environmentally relevant levels of exposure.

Review by GM Watch
17 August 2015
New review casts doubt on assumptions of Roundup safety
A new review of the scientific literature shows that glyphosate herbicides may be toxic below regulatory safety limits.
Dr Robin Mesnage and co-authors examined a number of different types of toxic effects to arrive at their conclusions, including liver and kidney toxicity, neurotoxicity, carcinogenicity, reproductive toxicity, and teratogenicity (ability to cause birth defects).
Unlike regulatory authorities, the researchers considered studies from the independent literature, as well as the few industry toxicity studies, conducted in support of regulatory approvals, that have been made public. They shared this approach of considering the entirety of the published literature with the World Health Organization's cancer agency IARC, which recently concluded that glyphosate is a probable carcinogen.
The new review shows that endocrine (hormone) disruptive effects can occur below the doses deemed not to cause any toxic effects in industry studies performed for regulatory approvals. Endocrine disruption may increase the risk of certain types of cancer. Yet regulatory studies do not test low dose exposures for endocrine disruptive effects.
Neither the US nor the EU regulators have finalised their endocrine disruption testing requirements. That's in spite of the fact that US Congress mandated the US EPA to develop an endocrine disruptor screening and testing programme in 1996 – yet it still has not done so. In Europe, legislation on endocrine disruptors has been delayed by industry lobbying, apparently helped along by the actions of the former EU chief scientific advisor, Anne Glover.
The conclusions of the new review stand in contrast with those of several Monsanto-sponsored reviews, which all concluded that the herbicide is safe.
Dr Robin Mesnage commented:

"This is the first independent systematic and peer-reviewed review to balance the dozens of Monsanto-sponsored reviews of Roundup and glyphosate toxicity, which have concluded that these substances are safe to use. Our review shows that there is a coherent body of evidence showing that toxic effects can occur below regulatory safety limits.

"Contrary to many claims, the toxicity of glyphosate herbicides has not been thoroughly assessed. For instance, lifelong toxicity tests in laboratory animals have never been done with the complete glyphosate formulations as sold and used. Even glyphosate alone has never been tested throughout the entire lifespan, because chronic tests are not carried out for the whole natural lifespan of the animals but are begun on young adults that are then killed before they get old. Yet clearly human beings are not euthanised at 60 years old. We need a clearer picture of how toxins affect the old as well as the young.

"Our study shows that glyphosate toxicity thresholds determined in regulatory assessments can be controversial. Thus a precautionary approach should be applied and glyphosate should be banned for all unnecessary uses. For example, there should be a moratorium on glyphosate spraying of crops for pre-harvest desiccation (drying down). This makes harvesting easier but is not necessary for the crop's survival or growth."

59: Analysis of endocrine disruption effect of Roundup® in adrenal gland of male rats

Date Added to website 18th August 2015

Aparamita Pandey, Medhamurthy Rudraiah,
Toxicology Reports 2 (2015) 1075–1085
http://www.sciencedirect.com/science/article/pii/S221475001530041X (open access)

Abstract

The effect of Roundup® on adrenal gland steroidogenesis and signaling pathway associated with steroid production was investigated. Doses of 10, 50, 100 and 250 mg/kg bw/d Roundup® were administered for two weeks to adult male rats. The 10 mg/kg bw/d dose which reduced circulatory corticosterone levels, but did not change food consumption and body weight, was selected for further study. The expression of cholesterol receptor (low density lipoprotein receptor), de novo cholesterol synthesis enzyme (3-hydroxy-3-methylglutaryl-coenzyme A synthase), hormone-sensitive lipase, steroidogenic acute regulatory protein (StAR) mRNA and phosphorylated form was decreased. Adrenocorticotropic hormone receptor (ACTH), melanocortin-2 receptor, expression was not changed but circulatory ACTH levels and adrenal cortex protein kinase A (PKA) activity were reduced. Surprisingly, exogenous ACTH treatment rescued steroidogenesis in Roundup®-treated animals. Apoptosis was evident at 250 mg/kg bw/d, but not at 10 mg/kg bw/d dose. These results suggest that Roundup® may be inhibitory to hypothalamic–pituitary axis leading to reduction in cyclic adenosine monophosphate (cAMP)/PKA pathway, StAR phosphorylation and corticosterone synthesis in the adrenal tissue.

http://us6.campaign-archive1.com/?u=29cbc7e6c21e0a8fd2a82aeb8&id=a5cc132f9b&e=2350a21e83

Study shows shortcomings of regulatory system

The glyphosate-based herbicide Roundup is an endocrine (hormone) disruptor in adult male rats, a new study shows.

The lowest dose tested of 10 mg/kg bw/d (bodyweight per day) was found to reduce levels of corticosterone, a steroid hormone produced in the adrenal glands. No other toxic effects were seen at that dose, so if endocrine disruption were not being specifically looked for, there would be no other signs that the dose was toxic.

The researchers didn't test below that level, so we don't know if when it comes to endocrine disruption, there are "safe" lower doses of Roundup. In technical parlance, this means that no NOAEL (no observed adverse effect level), was found.

How does this level relate to safety limits set by regulators?

One problem with trying to work out how the endocrine disruptive level of 10 mg/kg bw/d relates to "safe" levels set by regulators is that the experiment looked at Roundup, the complete herbicide formulation as sold and used, but regulators only look at the long-term safety of glyphosate alone, the supposed active ingredient of Roundup. Safe levels for Roundup chronic exposure have never been tested or assessed for regulatory processes. This is a serious omission because Roundup has been shown in many tests to be more disruptive to hormones than glyphosate alone.

Given this yawning data gap, let's for a moment assume that the regulatory limits set for glyphosate alone can be used as a guide for the safe level of Roundup.

The endocrine disruptive level of Roundup found in the experiment, of 10 mg/kg bw/d, is is well above the acceptable daily intake (ADI) set for glyphosate in Europe (0.3 mg/kg bw/d) and the US (1.75 mg/kg bw/d). But this isn't a reason to feel reassured, since with endocrine effects, low doses can be more disruptive than higher doses.

Another worrying factor is that 10 mg/kg bw/d is well below the NOAEL (no observed adverse effect level) for chronic toxicity of glyphosate: 500 mg/kg bw/d for chronic toxicity, according to the US EPA. In other words, the level of 500 mg/kg bw/d – a massive fifty times higher than the level of Roundup found to be endocrine disruptive in the experiment – is deemed by US regulators not to cause chronic toxicity. This experiment shows they are wrong by a long shot. They failed to see toxicity below that level because they failed to take endocrine disruptive effects from low doses into account and industry does not test for them.

Interestingly, the NOAEL for glyphosate in industry's 3-generation reproductive studies in rats was much lower than that for chronic toxicity – 30 mg/kg bw/day for adults and 10 mg/kg bw/day for offspring. The latter figure is the same level of glyphosate as the level of Roundup found to be endocrine disruptive in the new study. These results show that the reproductive processes of the rats are sensitive to low doses that are apparently not overtly toxic. This in turn may suggest that the reproductive toxicity findings are due to endocrine disruptive effects.

Regulatory tests still do not include tests for endocrine disruption from low doses, in spite of the fact that scientists have known about the syndrome since the 1990s.

In the final section of the new study, the researchers discuss its implications. They note that the effects seen in the Roundup-treated rats were similar to adrenal insufficiency in humans and that "This condition manifests as fatigue, anorexia, sweating, anxiety, shaking, nausea, heart palpitations and weight loss. Chronic adrenal insufficiency could be fatal, if untreated. A progressive increase in its prevalence has been observed in humans…"

Since no safe dose has been established for Roundup with regard to endocrine disrupting effects, it should be banned.

60: MON810 effects on Daphnia magna show it is not substantially equivalent

Date Added to website 18th August 2015

Chronic Responses of Daphnia magna Under Dietary Exposure to Leaves of a Transgenic (Event MON810) Bt-Maize Hybrid and its Conventional Near-Isoline.
Ferreira Holderbaum, Cuhra M, Wickson, Orth A, Nodari RO, Bøhn T.
J Toxicol Environ Health A. 2015;78(15):993-1007.
doi: 10.1080/15287394.2015.1037877.

Abstract
Insect resistance is the second most common trait globally in cultivated genetically modified (GM) plants. Resistance is usually obtained by introducing into the plant's genome genes from the bacterium Bacillus thuringiensis (Bt) coding for insecticidal proteins (Cry proteins or toxins) that target insect pests. The aim of this study was to examine the hypothesis that a chronic, high-dose dietary exposure to leaves of a Bt-maize hybrid(GM event MON810, expressing a transgenic or recombinant Cry1Ab toxin), exerted no adverse effects on fitness parameters of the aquatic nontarget organism Daphnia magna (water flea) when compared to an identical control diet based on leaves of the non-GM near-isoline. Cry1Ab was immunologically detected and quantified in GM maize leaf material used for Daphnia feed. A 69-kD protein near Bt's active core-toxin size and a 34-kD protein were identified. The D. magna bioassay showed a resource allocation to production of resting eggs and early fecundity in D. magna fed GM maize, with adverse effects for body size and fecundity later in life. This is the first study to examine GM-plant leaf material in the D. magnamodel, and provides of negative fitness effects of a MON810 maize hybrid in a nontarget model organism under chronic, high dietary exposure. Based upon these results, it is postulated that the observed transgenic proteins exert a nontarget effect in D. magna and/or unintended changes were produced in the maize genome/metabolome by the transformation process, producing a nutritional difference between GM-maize and non-GMnear-isoline.

Long-term exposure to GM maize leaves harms water fleas
http://us6.campaign-archive2.com/?u=29cbc7e6c21e0a8fd2a82aeb8&id=3753f622cc&e=2350a21e83
New study confirms GM maize MON810 is not substantially equivalent to non-GM maize
Long-term exposure to leaves of MON810 GM Bt maize causes reduced growth and fertility in Daphnia magna (water flea), compared with the level of the non-GM parent variety (isoline), a new study has found. The authors suggest that the transgenic protein and/or unintended changes in the maize due to the GM process were the causes of the observed effects. Daphnia magna is an important indicator species for environmental toxicity.
The study shows that this GM maize is not substantially or biologically equivalent to the non-GM parent variety (yet it was approved by regulators worldwide on the basis that it is) and is more toxic.
In addition, unique characteristics were found in the GM Cry1Ab insecticidal toxin of MON810 Bt maize, as opposed to bacterially produced Cry1Ab. Yet bacterially produced Bt toxins are routinely used in many regulatory tests to assess the safety of GM Bt crops. The authors call for the use of Bt toxins as actually produced in the plant in regulatory tests.

61: Glyphosate, pathways to modern diseases IV: cancer and related pathologies

Date Added to website 7th November 2015

Anthony Samsel and Stephanie Seneff.
Journal of Biological Physics and Chemistry 15 (2015), 121–159.
https://www.researchgate.net/profile/Anthony_Samsel

Abstract
Glyphosate is the active ingredient in the pervasive herbicide, Roundup, and its usage, particularly in the United States, has increased dramatically in the last two decades, in step with the widespread adoption of Roundup®-Ready core crops. The World Health Organization recently labelled glyphosate as "probably carcinogenic." In this paper, we review the research literature, with the goal of evaluating the carcinogenic potential of glyphosate. Glyphosate has a large number of tumorigenic effects on biological systems, including direct damage to DNA in sensitive cells, disruption of glycine homeostasis, succinate dehydrogenase inhibition, chelation of manganese, modification to more carcinogenic molecules such as N-nitrosoglyphosate and glyoxylate, disruption of fructose metabolism, etc. Epidemiological evidence supports strong temporal correlations between glyphosate usage on crops and a multitude of cancers that are reaching epidemic proportions, including breast cancer, pancreatic cancer, kidney cancer, thyroid cancer, liver cancer, bladder cancer and myeloid leukaemia. Here, we support these correlations through an examination of Monsanto's early studies on glyphosate, and explain how the biological effects of glyphosate could induce each of these cancers. We believe that the available evidence warrants a reconsideration of the risk/benefit trade-off with respect to glyphosate usage to control weeds, and we advocate much stricter regulation of glyphosate.

Note: For the first time the authors, Anthony Samsel and Stephanie Seneff, present in tabulated form the data contained in secret Monsanto studies conducted in the period 1980 - 1990 which showed unequivocally that animals exposed to very small quantities of glyphosate in their food supply developed tumorigenic growth in multiple organs. Both Monsanto and the American EPA knew of these and other deleterious effects, but the EPA agreed to refer to these early studies as "trade secrets" and prevented public scrutiny. The results were considered inconvenient, and so they were ignored. To make matters worse, the EPA then agreed to further Monsanto-sponsored studies which used inappropriate control group data to create "experimental noise" and to mask carcinogenic and other effects in the animal test groups. Other fraudulent practices have also been subsequently revealed, including the non-reporting of test group deaths, the fabrication of data tables, and the falsification of experimental data.

Dr Samsel is the first independent researcher to have been given access to the full Monsanto / EPA dossier of research reports, and the new paper itemises the key research findings in these early papers and presents a number of detailed appendices of the results.

The authors conclude:
"In this paper, we have reviewed the research literature on glyphosate and on the biological processes associated with cancer, and we have provided strong evidence that glyphosate is likely contributing to the increased prevalence of multiple types of cancer in humans. Monsanto's own early studies revealed some trends in animal models that should not have been ignored. Forty years of glyphosate exposure have provided a living laboratory where humans are the guinea pigs and the outcomes are alarmingly apparent."

See also:
http://www.gmfreecymru.org/documents/monsanto_knew_of_glyphosate.html
http://www.gmfreecymru.org/news/Press_Notice06Nov2015.html

62: Co-formulants of glyphosate herbicides are endocrine disruptors

Date Added to website 29th February 2016

New research shows regulatory "safe" limits for glyphosate may not be safe at all

The approval process for glyphosate herbicide is disputed because the commercial herbicide formulations as sold and used contain additives or co-formulants, which are more toxic than glyphosate alone. Yet glyphosate alone is tested to calculate the ADI or acceptable daily intake, the level that is supposedly safe to consume over the long term. That's in spite of the fact that

we are exposed to the formulations, not the isolated presumed active ingredient glyphosate.

A new study shows that the acceptable daily intake (ADI), the supposedly safe level, for glyphosate is unreliable in terms of assessing the risks of the complete commercial formulations that we are actually exposed to.

The co-formulants were shown in the new study to have a far more powerful endocrine-disrupting effect at lower doses than the isolated active ingredient, glyphosate.

The complete formulations were also found to have much greater endocrine disrupting effects at lower doses than glyphosate alone.

The research shows that the ADI should be calculated from toxicity tests on the commercial formulations as sold and used.

In the new study, published in the International Journal of Environmental Research and Public Health, the researchers measured the endocrine disruptive effects of the co-formulants of six glyphosate herbicides. They measured the activity of aromatase, a key enzyme for the balance of sex hormones, in human placental cells, using a method validated by the OECD to assess endocrine disruptors.

The aromatase activity was significantly decreased both by the co-formulants alone and by the formulations, from doses 800 times lower than the agricultural dilution. But glyphosate alone only showed such an effect from one-third of the agricultural dilution (in other words, glyphosate was much less of an endocrine disruptor than the co-formulants and the formulations).

According to a press release distributed by the research group CRIIGEN, which supported the work, the new study is the first ever demonstration that the endocrine-disrupting effects of glyphosate-based herbicides are not only attributable to glyphosate, the declared active ingredient, but above all to the co-formulants.

(Source: CRIIGEN)
http://www.gmwatch.org/news/latest-news/16742

Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels

by Nicolas Defarge, Eszter Takács, Verónica Laura Lozano, Robin Mesnage, Joël Spiroux de Vendômois, Gilles-Eric Séralini, and András Székács
International Journal of Environmental Research and Public Health 2016, 13, 264
doi:10.3390/ijerph13030264
http://www.mdpi.com/1660-4601/13/3/264 (open access)

Abstract
Pesticide formulations contain declared active ingredients and co-formulants presented as inert and confidential compounds. We tested the endocrine disruption of co-formulants in six glyphosate-based herbicides (GBH), the most used pesticides worldwide. All co-formulants and formulations were comparably cytotoxic well below the agricultural dilution of 1% (18–2000 times for co-formulants, 8–141 times for formulations), and not the declared active ingredient glyphosate (G) alone. The endocrine-disrupting effects of all these compounds were measured on aromatase activity, a key enzyme in the balance of sex hormones, below the toxicity threshold. Aromatase activity was decreased both by the co-formulants alone (polyethoxylated tallow amine—POEA and alkyl polyglucoside—APG) and by the formulations, from concentrations 800 times lower than the agricultural dilutions; while G exerted an effect only at 1/3 of the agricultural dilution. It was demonstrated for the first time that endocrine disruption by GBH could not only be due to the declared active ingredient but also to co-formulants. These results could explain numerous in vivo results with GBHs not seen with G alone; moreover, they challenge the relevance of the acceptable daily intake (ADI) value for GBHs exposures, currently calculated from toxicity tests of the declared active ingredient alone.

63: GMO Crops -- more costs than benefits

Date Added to website 2nd December 2015

An interesting new report from Canada, which shows that the widespread introduction of GMOs in Canada is based more on hype than on a realistic assessment of actual costs and benefits. The only real "benefit|" coming from GMOs is more effective weed management -- at least in the early days of GMP planting, before super-weeds (and super-bugs) kick in. And in a world where "farm productivity" is the obsession, farmers have swallowed the line promoted by the biotechnology industry, just in order to hold down their farm production costs including labour requirements. But there are massive costs that are conventionally ignored, especially since many of these costs are not carried by the farming industry at all -- but are pushed onto society as a whole, in the form of human and animal health damage, environmental degradation etc. Many of us have been saying these sorts of things for years -- but in this report the real costs of GMO farming are carefully documented, with a very full list of supporting references.

New Report:
ARE GM CROPS BETTER FOR FARMERS?

Canadian Biotechnology Action Network, November 2015

http://gmoinquiry.ca/wp-content/uploads/2015/11/Are-GM-crops-better-for-farmers-E-web-singles.pdf

SUMMARY

This fourth report of GMO Inquiry 2015 investigates the impacts and risks of genetically modified (GM; also called genetically engineered or GE) crops on farms and famers over the past twenty years, with a focus on Canada.

The use of patented GM traits has helped facilitate corporate consolidation in the seed market. Markets for GM crops are dominated by a few seed and agrochemical companies. This high level
of corporate concentration in the seed market has meant higher prices, limited choices for farmers, a narrowing of genetic diversity in crops, and stagnating innovation. Legal control over seeds has also increased, in the form of patents on genetic sequences and other mechanisms that prevent farmers from saving, exchanging and reusing seed. GM crops have diminished the choices available to farmers, while strengthening the control of a few companies.

Yields in GM and non-GM crops have increased at a similar rate in Canada, and there are no clear patterns to show that GM crop yields have increased more than those of non-GM crops. In fact, research comparing GM crops in North America and non-GM varieties of the same crops grown in Europe has shown that non-GM crop yields have increased as much, or more. GM traits are added to plant varieties that are already high- yielding due to background genetics developed through non-GM breeding methods. It is these pre-existing characteristics, along with other factors, that have determined yield increases in the past decades, not GM traits.

Growing GM crops is not putting more money into the pockets of Canadian farmers. Although gross farm income in Canada has increased over the past two decades, realized net income (the income remaining after farm expenses are paid) has not changed significantly. Farm expenses have increased substantially, in part because of the rising prices of seeds and other inputs. GM crops have fed into this pattern; GM seeds are significantly more expensive than non-GM seed, in Canada and other countries.

The major benefit that GM herbicide-tolerant crops offered farmers was simplified weed management. However, the increased use of herbicides has led to the emergence and spread of herbicide- resistant weeds, which are reversing this benefit and creating new costs and complications for farmers. The biotechnology industry's solution to this problem is to sell

new GM crops that are tolerant to different herbicides, an approach that will further drive up herbicide use and speed up the spread of herbicide-resistant weeds.

GM contamination can also present serious costs for farmers. The examples of GM flax contamination, which closed Canada's export markets, and GM canola contamination, which meant that most Canadian organic farmers were forced to stop growing canola, stand testament to these costs. Despite these experiences, new GM crops such as the GM alfalfa are being commercialized. If released in Canada, GM alfalfa contamination will have serious and irreversible impacts, the brunt of which will be borne by organic and other non-GM farmers.

The Canadian government does not assess the agronomic and economic impacts of GM crops or evaluate the benefits or risks they pose, and farmers are not consulted before GM crops are approved for growing. The experiences of the past twenty years show us that there is an urgent need for a democratic decision-making process to assess what role, if any, GM crops should play in our food and farming systems.

CONCLUSION

The high level of corporate consolidation in the seed market has been partly facilitated by the use of GM technology. This corporate concentration has meant that seed prices have risen at a faster rate than other farm inputs, while farm incomes in Canada have not increased. The choices available to farmers in the market have decreased, and legal control, in the form of patents that prevent farmers from reusing seed, has increased.

Farmers have not yet benefitted from increased yields or rising net incomes because of GM traits. The benefits that GM herbicide-tolerant crops may have offered farmers are now being reversed due to the new management costs of herbicide-resistant weeds.

In Canada, there is no assessment of the potential economic consequences of introducing new GM crops. For example, potential GM contamination is only assessed in relation to a narrow set of questions about environmental impacts, not in relation to potential economic costs, despite the fact that farmers can pay a high price. Farmers in Canada are not consulted before genetically modified crops are approved, for field trials or commercial release.

Twenty years of GM crops have benefitted the companies that sell GM seeds, but have not always benefitted farmers.

Quote: "Farmer experiences regarding this technology have yet to be fully studied for Canada, the United States, and Argentina as the first countries to commercialize GM crops, or are restricted to the benefits. The role and potential contribution of farmer knowledge also has yet to be systematically evaluated for any GM crops and, indeed, risk research as a whole" — Mauro and McLachlan, 2008

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The GMO Inquiry 2015 is a project of the Canadian Biotechnology Action Network (CBAN). CBAN is a campaign coalition of 17 organizations that researches, monitors and raises awareness about issues relating to genetic engineering in food and farming. CBAN members include farmer associations, environmental and social justice organizations, and regional coalitions of grassroots groups. CBAN is a project on the shared platform of Tides Canada.

64: GMOs -- twenty years of hype for a worthless technology

Date Added to website 1st March 2016

This is a great article by Pat Thomas, summarising the hype and the hubris surrounding the GMO enterprise over the last twenty years -- with virtually nothing of any use actually delivered, and a vast amount of harm done on many different fronts.....

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20 years ago today… What have we learned since the GMO Flavr Savr tomato?
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February 5, 2016 by Pat Thomas
http://beyond-gm.org/20-years-ago-today-what-have-we-learned-since-the-gmo-flavr-savr-tomato/

"It was 20 years ago today…" as the song goes – and in many ways the sparkly side-show that is genetically modified food is as lacking in substance as the eternal showmen of Sgt Pepper's band.

On this day in 1996 the first GM product – a tomato puree made from the Flavr Savr tomato hit UK shops.

The product was produced by Zeneca from tomatoes that were grown and processed in California and genetically engineered to ripen more slowly. It was introduced to the UK by supermarkets Sainsbury's and Safeway. The paste was competitively priced and clearly labelled and more than 1.8 million cans were sold from 1996 through early 1999.

For a while the GMO tomato paste even out-sold conventional tomato paste at many locations, but sales declined dramatically in 1998 as consumers became more aware of just what was at stake. It was withdrawn from the market in 1999.

The Flavr Savr tomato isn't just a footnote in GMO history. It was a turning point, which showed that even when a product was sold at a cut rate price, consumers were simply not prepared to gamble with the unknown consequences of eating GM foods.

Although an army of vested interests, rent-a-quote 'scientists' and' bought' politicians remains as vocal as ever about the promises of genetically modified crops, the reality, two decades on, is that GMOs have consistently failed to deliver.

A damaging, outdated approach to farming

What this anniversary highlights is that genetic engineering of food crops is an outdated approach to food production. It has its roots in a world that still believed in the Green Revolution and the idea that industrialised monoculture chemical-heavy farming was the way to feed the world.

No matter how much money has been thrown at GMOs from government, private investors and corporations, no matter how much PR spin is put on the story, the fact remains that the promises that genetically modified food would revolutionise our world, feed the hungry, boost the yields and therefore the incomes of farmers, cure disease and more recently fight climate change remain spectacularly unfulfilled.

In fact, the original intent of GMOs wasn't to do any of these things. It was, arguably, more focused on creating a more easily controlled international market of standardised food products. Indeed the issue of climate change had not yet come to the fore when the first GMO crops were being introduced.

This factory farming paradigm, which is rooted in the notion that yield is the most important measurement of success and that

perceived agricultural problems arise as a result of some genetic flaw in nature, is a worn out concept and GM technology is increasingly looking like a relic of a bygone age.

Continuing to promote it as solution to current and future problems is rather like trying to walk forward whilst looking backwards.

20 years of failure documented

For our launch late in 2014 Beyond GM produced The Letter from America – a plea from US citizens to citizens of the UK and EU not to embrace GMOs the way the Americas have.

The fully referenced letter detailed 20 years of failure and we urge everyone who wants a quick primer in GMOs to read it (it is now available in 8 languages) and we urge US citizens to continue to sign it and UK citizens to send a copy to their MPs from the site.

The Letter showed that in that time GMO crops have, in fact, increased pesticide use. The US Government's own data shows that herbicide us has increased dramatically over the last decade and that total volumes of glyphosate applied to the three biggest GM crops in the US – maize, cotton and soybean – have increased tenfold.

They have also failed to consistently increase yields. They haven't reduced costs to farmers nor increased profits. They haven't created an international market – most of the world rejected GMO crops and a recent UN report even suggested that GMOs were responsible for a rise in trade disputes (when shipments of grain are found to be contaminated with GMOs).

Embracing the GMO agenda has had direct consequences, in that it both supports and accelerates an outmoded factory farm mentality, and indirect consequences, in terms of environment, health and culture but also science and democracy.

GM Nation?

In 2002/2003, long after the Flavr Savr had been forgotten, the Labour party conducted the only really comprehensive investigation into GMOs in the UK, GM Nation.

That suite of investigations included public debates and research into consumer attitudes, it included an economic analysis (which included looking at liability for economic and environmental damage) and an environmental analysis.

The economic review concluded there was no benefit to UK plc from growing GM crops; the environmental review found severe damage to the countryside and biodiversity; and the public debate showed was widespread mistrust of government and multinational companies, with participants expressing a very strong interest to be 'better informed' about GM issues.

In fact, only 2% of the more than 30,000 people who took part in the consultation said they would be happy with GM foods in any circumstances. A staggering 93% thought that GM technology was "driven by profit rather than the public good".

Since that time no government, no regulator and very few scientists, has asked the really important question about GM crops and foods:

- Not just who benefits from the technology, but who loses?
- What new facts have emerged about the environmental and health consequences?
- What productive and sustainable alternatives have fallen by the wayside in the push for GMOs?
- How does the technology affect what we are producing and how we are producing it – and to whose needs does it respond?
- What are the social goals and ethical criteria that guides research?
- What are the social and agronomic goals?

A world in need of repair

Some people may not like it, but we know most of the answers about whether GMOs are worth it already.

As the current Conservative government pushes ahead with its irrational, anti-science, pro-GM agenda we would argue that any party that wishes to be influential in the future, needs to have a rational sustainable food policy which acknowledges that, from an agricultural point of view, we are living in a damaged world in need of deep repair.

It is a world that is facing challenges that could not have been anticipated 20 years ago when GM technology first entered the marketplace and many these challenges are the result of our own short-termism and greed.

In order to begin the process of repair several things urgently need to be done. We believe that these things are best addressed in the space of a continuing moratorium on the planting of GM crops in the UK.

Changing the framework

Most urgently, the regulatory framework by which GMO crops and the chemicals used on them and GM foods are assessed its completely inadequate:

• There is a lack of transparency with regard to the decisions taken around GMOs
• There is no requirement for independent testing
• There is a lack of post marketing surveillance

There are new challenges too in that biotechnology companies are lobbying to have newer forms of plant breeding technologies, which clearly fall under the heading of GMOs, regulated as non-GM.

The problem of co-existence and environmental contamination and degradation have never been adequately addressed. There is still a complete lack of laws covering environmental and economic liability. These have to be addressed with more than just bland reassurances before crops are planted.

There is a better way

In spite of its promises of drought tolerant and salt tolerant crops – neither of which has materialised – or food crops that will contain extra nutrients – again none exist commercially – it has dismally failed to anticipate and to paint a compelling picture of what our farming and food future should be.

Other people, scientists and farmers, have painted that picture though. The 2008 IAASTD Report which was sponsored by a number of major international organisations, including the United Nations, the World Bank, the UN Food and Agricultural Organization, and UNESCO concluded that industrial farming was too dependent on cheap oil and too damaging to the wider environment and was not a suitable technology for alleviating hunger because it does not benefit small and subsistence farmers, and it is these farmers that provide 70% of the world's food.

The 2010 UN report into Agroecology and the Right to Food concluded that organic and sustainable small scale farming could double food production in the parts of the world where hunger is the biggest issue.

Rebuilding public trust in food and farming

In the space of the de facto moratorium on GMOs which has been in effect over the last two decades the government could usefully and effectively have directed funds into studies and projects around agroecological methods of farming. It could have engaged the public at the level that it did in 2003 on the issue of GMOs – because the public are important stakeholders in this issue. A continuing moratorium would allow space to get it right the second-time around.

Public trust in our food system is at an all-time low. None of us should have to live in a world where words like 'poison', 'scare', 'insecurity', 'crisis' and 'waste' are consistently being paired with the word 'food'. And yet that is the world that is

being created by a bull-headed adherence to the myth that GMOs and techno-farming will save the world.

Public concerns on every level, not just about the science but about the ethics, and the 'rightness' of GMOs are legitimate and deeply felt, rooted in rational science, and should be taken seriously. Why not make this anniversary of the GMO Flavr Savr tomato a turning point where we can begin to get beyond the GM paradigm and onto a more positive and productive way of thinking about food?

65: Safety assessments of GMOs are non-existent

Date Added to website 7th October 2015

[This is an excellent extended interview which reveals that proper safety assessments of GMOs are never done, and that the assumptions about GMO crop and food safety are based upon commercial expectations, politics and spin rather than on science.]

Vishwanath Kulkarni, with Michael Hansen, Hema Yadav, Ray Seidler, and Shiva Ayyadurai
in The Hindu -- Business Line

http://www.thehindubusinessline.com/opinion/safety-assessments-of-gmos-are-nonexistent/article7674558.ece

US-based systems biologist Shiva Ayyadurai created a controversy recently by claiming that his studies had found that genetically modified soya bean plants had less capacity to get rid of toxins such as formaldehyde compared to non-GM counterparts. The idea that GM technology disrupts a plant's natural metabolism was seized upon as vindication by anti-GM activists even as it came under criticism from others. In a wide-ranging interview, BusinessLine spoke via conference call to Shiva Ayyadurai, whose current research focuses on developing systems biology methods to understand bio-molecular phenomena, and three other scientists who make a strong pitch for increased regulation and transparency in GM foods — Michael Hansen, senior scientist at the non-profit Consumer Union working on consumer-related policy issues; Ray Seidler, a former senior scientist at the US Environmental Protection Agency (EPA); and Hema Yadav, an agricultural expert who has worked on capacity-building for farmers and managers in India and Africa. Excerpts:

In a nutshell, the essence of your hypothesis seems to be: Like all plants, genetically modified soya bean produces formaldehyde, a carcinogen; but unlike non-GM soya bean, it depletes glutathione, a key anti-oxidant that helps plants remove formaldehyde and other toxins from their cells. As a result, it is wrong to suggest that GM soy is equivalent to non-GM soy and, by implication, that GM plants are equivalent to non-GM plants. Does this make for a fair description?

Shiva Ayyadurai: Yes, our systems analysis shows that GM soy is substantially different from non-GM soy. The critical point is that we used a systems biology approach — the first of its kind — to look at a critical molecular system called C1 Metabolism, which occurs in all plants, bacteria and fungi. In that molecular system, there are three sub-systems: methionine biosynthesis, methylation and formaldehyde detoxification. The goal of this systems approach was to explore if GM plants are the same as, or "substantially equivalent" to non-GM plants. We based the study on integrating molecular pathway information from 6,497 wet lab experiments done in 184 institutions, across 23 countries, on what occurs from genetic engineering to produce Roundup Ready Soy (RRS), the GM soy, and whether such GM causes disruption to C1 Metabolism. We found that there is a significant disruption, particularly to formaldehyde detoxification, following insertion of the foreign gene.

Our analysis concludes that, in GM soy, oxidative stress is caused by the GM, resulting in glutathione, a natural anti-oxidant, being depleted and formaldehyde accumulating. Formaldehyde does exist in all plants, at various levels, and detoxification occurs at different stages. However, in the GM soy, since the formaldehyde detoxification pathway is perturbed, this analysis shows that the levels of formaldehyde and glutathione will vary between GM soy and non-GM soy.

Your research did not involve testing actual plants. So why rely wholly on a 'systems biology' approach? Why didn't you bother to validate this by testing a few transgenic and non-transgenic varieties of soy? Wouldn't that have strengthened your

hypothesis?

Michael Hansen: Clearly, such testing needs to be done and that will be in a forthcoming paper. By the way, this series of four papers is based on thousands of wet lab tests and is not "just a model". This paper is focused on using modern systems biology methods to provide a foundation, at the molecular level, for all researchers to understand, with full transparency, how GM may perturb complex molecular systems. There is an active effort towards conducting wet lab tests on the results indicated from this systems biology analysis. However, conducting such experiments is extremely difficult, given the lack of transparency from those who own, manufacture, and control those GM seeds, be it soy or others. For example, there are legal constraints in the US to even obtain the seeds to conduct such testing.

Ayyadurai: The systems biology approach is the most important contribution of this series of four papers, and aims to advance the scientific method in a far more transparent and integrative manner to get an accurate view of what is going on in GM versus non-GM. Today, scientists primarily do individual, single experiments using the scientific method, where they first begin with a hypothesis, then do a single experiment, and then gather and organise the data from that experiment. The data is then analysed to build a model, which makes a prediction. The predictions are published, which motivates others to do more experiments, and this cycle of the scientific method is repeated to generate more data and new predictions, until there is consensus on the predictions.

The biggest breakthrough in biology took place after the human genome project ended in 2003, where biologists realised that humans have the same number of genes as a worm, motivating biologists to recognise that the complexity of an organism is not a function of the number of genes, but can only be understood by interconnecting the complexity of molecular pathways derived from multiple experiments, and recognising the need to interconnect molecular pathway information, so we move away from what is called reductionist biology to a systems biology. Reductionist biologists are like guys looking at pieces of an elephant (just the trunk, the ears, tusk, and so on) and each making assumptions on what they think it is, leading them often to erroneous and biased conclusions. Systems biologists attempt to look at the connections across the whole organism, and put it all together to get a more accurate, unbiased view. So, that's what we did across the series of four papers, step-by-step:

Paper I: Aggregates over 11,000 papers to identify the fundamental molecular pathways of C1 Metabolism (published in Agricultural Sciences).

Paper II: Interconnects the molecular pathways of C1 Metabolism, in normal condition, using a systems approach. This paper shows that formaldehyde is detoxified, and glutathione is maintained in normal plants (published in American Journal of Plant Sciences).

Paper III: Identifies the molecular pathways of oxidative stress in plants, connects them and integrates them with the C1 Metabolism system of Paper II. Oxidative stress occurs when plants experience "stress" such as a drought or weather changes. This integration shows that under stress, plants deplete glutathione, resulting in the accumulation of formaldehyde (published in American Journal of Plant Sciences).

Paper IV: Shows that GM soy is different from non-GM soy based on the differences in the levels of glutathione and formaldehyde. We found in the GM soy, the Roundup Ready version, five molecules are disturbed, based on data from actual wet lab experiments. This molecular disturbance causes oxidative stress, which (as shown from Paper III) results in C1 Metabolism (from Paper I and II) being disturbed, resulting in glutathione being depleted, and formaldehyde accumulating (published in Agricultural Sciences).

Hansen: The research effort is now in the midst of collecting transgenic and non-transgenic varieties of soy. John Fagan, who is part of this effort, has access to soy plants but they are a bit old. The goal is to get the freshest supply of soy from recently harvested plants. The harvest of Argentine soybeans occurs in April to June. In September, the US harvest comes. By testing at harvest time, it is possible to have access to freshest transgenic and non-transgenic materials. As part of that testing, the standards for testing will be defined. None of the people are attacking the research, done by Dr Ayyadurai and his team, in the typical manner when one publishes a systems paper. People typically tend to question the model and rate constants and

molecular pathways. No one is questioning the rate constants, or the thoroughness of the research in those four papers. No one is questioning how the modelling was done, and source of those papers. The only question is how the testing will be done. We believe that setting standards, so anyone can replicate and validate these experiments, within that standardised framework is extremely important to such testing.

Ayyadurai: Those standards are what is missing in the scientific field of GM research, and the reason for the ongoing controversies. Without those standards, results from our testing are guaranteed to be questioned. So, as a part of defining those standards, we want to bring together people from both sides, pro- and anti-GM, into an International Standards Committee that, openly and transparently, defines the standards within the framework of systems biology. This need for standards is one of the main conclusions of Paper IV. In the US, given how the system is set up, it is very difficult to even do such testing today. In fact, one may violate the licensing laws of GM seed manufacturers if one tries to test without their consent. We want to use this opportunity to perform such testing in an open, transparent manner, based on Standards.

What do you have to say about the charge that your paper was published in a 'pay-to-play' journal or of somewhat not established repute? Surely you must have known your credibility would have been far higher if a reputed journal had published it.

Ayyadurai: Let us first talk about reputed journals and non-reputed journals, because there's an assumption here. Who's deciding which is reputed and which is not? Randy Sheckman, an eminent scientist who won the Nobel Prize in Medicine and has published extensively in major and "reputed" journals such as Nature, Science and Cell, wrote a scathing article exposing how these major journals are damaging science based on the false measure of "impact factor" and the collusion that takes place to promote their journals as a brand. Reputed media organisations such as The Guardian and The Hindu have shared Scheckman's exposition. Scheckman believes that "There is a better way, through the new breed of Open Access journals that are free for anybody to read, and have no expensive subscriptions to promote. Born on the web, they can accept all papers that meet quality standards, with no artificial caps. Many are edited by working scientists, who can assess the worth of papers without regard for citations. It is the quality of the science, not the journal's brand that matters."

Open Access journals are the future, particularly because of the massive online audience. Thousands of journals, many of high repute, are Open Access. Before Open Access, the reader or poor graduate student had to pay $30, $40, $100 or more to download a single paper. Now the reader gets it for free, and it allows far greater access. Therefore, saying someone's research is "Pay to Play" is essentially a derogatory way of characterising Open Access, to arbitrarily put down the findings of a paper one doesn't like. In fact, MIT and Harvard, and the biggest institutions now support Open Access because it is becoming too expensive for the end-reader. So, yes, the authors pay. But to dismiss the quality of all research papers published in those journals as "Pay to Play" is the same as dismissing all papers in Nature, Cell and Science based on the collusion that Sheckman has exposed.

We chose these journals because that is where we saw other leaders, even those that disagree with us, publishing. The journals were Open Access, and since we were confident our work was high-quality, we wanted to ensure that as many people as possible in the field got access to our work. In fact, we've now had nearly 40,000 — and growing — accesses of our papers, when a typical paper gets only 1,000 views at best.

What you are basically saying is that people you violently disagree with have published in the very same journal, which is not exactly a great advertisement for the journals.

Ayyadurai: Two of four papers were published in Agricultural Sciences and the other two in The American Journal of Plant Sciences. Monsanto and USDA, as well as multiple departments of the University of Florida in Gainesville (UFG) have published in these journals.

As a systems biologist, I typically publish in engineering and biomedical sciences journals. The important point here is that the foundational CytoSolve systems biology methodology, used across all our papers, has also been published in multiple peer-reviewed journals such as IEEE, CELL's Biophysical Journal, etc., and cited by many other major journals including the "eminent" and "reputed" Nature. Remember, no one is disputing our methodology. What we did experience, however, was

deliberate disinformation to distract the public and other scientists, to dismiss the findings of our work by attacking the reputation of the journals. Kevin Folta, representing himself as an "unbiased scientist", is the one who raised this issue, but he should probably attack his own colleagues at UFG, his home institution, who've also published multiple times in the journals we published in. This is just irrational behaviour. Mr Folta, by the way, is far from being an "unbiased scientist". Recent and alarming evidence in the The New York Times — documented across hundreds of emails, now made public following a freedom-of-information request — shows that Mr Folta was paid by Monsanto and worked closely with their public relations agency to actively spread pro-GM information. It's deplorable and exposes the most egregious academic collusion. He led the rabid attacks on our work in the blogs of a not-for-profit organisation called "Genetic Literacy Project", which is also widely known to be favourable for genetic engineering and conventional agriculture.

Randy Scheckman doesn't have this kind of baggage, and yet he is saying that "eminent" journals like Science, Cell and Nature, which he himself has published in, are damaging science. Who are you going to believe? The real issue, therefore, is the quality of our research. Our work did go through a peer review process. We got feedback. We got comments. Moreover, we have many eminent international scientists, including from Harvard and MIT, who are signatories in support of our findings as well as the systems biology approach we took.

Does it surprise you that a body like the USFDA has not responded to your work?

Ray Seidler: The FDA is a regulatory body and doesn't really respond or comment on published scientific work. That is really not in their purview. Remember, the FDA also has not taken any official position on GM foods, but rather provides guidelines, such as substantial equivalence, from which they perform "safety consultations" and allow manufacturers to be self-regulating.

Hansen: This is something that is often forgotten and not explicitly shared in the media. There is a big myth that the USFDA regulates and does safety assessment of GM foods. This is simply not true. The fact is that the FDA does not take a position on the safety of GM foods. A GM manufacturer simply self-reports the safety analysis of their product versus the non-GM counterpart, and the FDA simply sends them a standard letter, based on a "safety consultation", to acknowledge the self-reported safety results. On the FDA website, you can see these standard "safety consultation" letters, representing over 101 consultations, which have been completed to date.

Ayyadurai: That typical USFDA letter issued to the "Company" has a consistent paragraph, which clearly shows the self-reporting nature of this process for the GM product created by the company. Here is an exemplar paragraph from those letters:

"Based on the safety and nutritional assessment the Company has conducted, it is our understanding that the Company has concluded that food and feed from GM product are not materially different in safety, nutrition, composition, or other relevant characteristics from food and feed from apples currently on the market, and that GM product do not raise issues that would require premarket review or approval by FDA… It is the Company's continuing responsibility to ensure that foods marketed by the firm are safe, wholesome, and otherwise in compliance with all applicable legal and regulatory requirements."

The FDA simply blesses the self-reporting results for the particular GM product, and the USFDA does neither safety assessment testing nor verify the test results of the company.

Hansen: China, for example, in November 2013 decided it was no longer going to import GM corn from the US, because China had not authorised the import of a particular variety until they could do their own safety assessment. Since the US had not done any safety assessments, this made it much more difficult for the US to complain to the World Trade Organisation (WTO) that China was setting an artificial trade barrier… the truth is that the US and the FDA do not do safety assessment of GM foods that are compliant with Codex guidelines.

But the FDA says that it clears these products in terms of allergenicity, toxicity. Also that nutritionally, GM products are the same as conventional ones.

Seidler: The FDA does not carry out allergenicity or toxicity assessments on GM foods. The US has no safety assessment

model for GM but provides a "safety consultation". The FDA does not carry out analysis to say whether a GM product is substantially the same as the non-GM version. The reason other countries are stopping imports of US GM products is because the US methods for safety are inadequate relative to international standards... a loosey-goosey approach and, at best, unscientific relative to real biosafety assessment. The USFDA is not even close to commenting on safety of GM foods.

Hansen: There is global agreement that there should be safety assessment for GM products. The global standard comes from Codex Alimentarius, which is the United Nations food standard agency jointly run by the WHO and the FAO. From 2000 to 2008, Codex convened an intergovernmental taskforce for foods derived from modern biotechnology. The taskforce met in Japan during this period to have a global process for risk analysis of modern biotechnology. There were several documents on how to do safety assessment of foods derived from genetically engineered plants, animals and micro-organisms. In 1995, when WTO was set up, any food standards or guidelines, etc., from Codex were considered to be trade legal. This gave a country the legal right not to import GM products if they had not been assessed for safety in that country. Since 2003, when Codex approved the first standard for foods derived from genetically engineered plants, the US has been unable to meet the global standards for such safety assessment of GM products.

You raised Codex. One of the things Codex does is labelling. In this connection, what about the legislation in Vermont in favour of mandatory labelling of GM food? Although it is still pending, do you think other states may join the campaign in the US?

Hansen: You are right. Food labelling is part of Codex. Sixty-four countries, including India, require labelling of GM food products. Over 90 per cent of the American public wants labelling. In the last two years, three states — Connecticut (CT), Maine (ME) and Vermont (VT) — have passed mandatory labelling laws, but in CT and ME they don't go into effect until four other states pass such laws. The VT bill passed in 2014 and was challenged in Vermont federal court by Grocery Manufacturers Association (GMA), with support from big agribusiness. Their lawsuit failed in April 2015, and the GMA is appealing it to a higher court. Vermont is set to start labelling in 2016, unless there is further court action. While all this was taking place, a bill was passed in the US House of Representatives to make it unlawful for states to require GM labelling in July 2015. Critics have dubbed this bill the DARK (Deny Americans the Right to Know) Act. This Act now proceeds to the US Senate for debate. This bill is a desperate move by the biotech and food industry to stop the labelling movement. There are also bills pending in three other states: New York, Massachusetts, and Rhode Island to enact labelling at the state level.

Where do you see India in this context?

Ayyadurai: As of today it is inconclusive, according to the consensus of scientists globally, whether GM crops are safe or not. The January 2015 paper (published in Environmental Sciences Europe), of which Dr Hansen is one of the co-authors, gathered 300 signatories including scientists from India, (and) clearly concluded that there is "No Consensus on GMO safety". In fact, in our opinion, the Indian scientific community, relative to American scientists, has been far more prudent on GM crop safety. In the Indian Parliamentary Committee Report of 2009 and the Indian Supreme Court Technical Expert Committee (TEC) Report of 2012, Indian scientists concluded the following key points:

India should not be using GM crops until at least ten years of safety assessment are done;

Except for the Bt variety (cotton), currently approved, any new Bt variety should also go through proper development and safety standards over ten years;

The US has huge factory farms. India is still 70 per cent small farms. The use of herbicide-tolerant GM crops does not make any sense in the Indian context; and, finally,

Given the biodiversity of crops in India, there should be a ban on all GM crops because it could significantly affect this biodiversity from GM crop contamination, and this will be irreversible for India.

Given the relatively more open and conducive environment for GM science in India, we are working to conduct the first meeting of International Standards Committee in India, to develop the standards for objective safety testing of GM crops

versus their non-GM counterparts. We think the Indian scientific community, as well as the Indian media are currently far more open to discuss this in an objective and scientific manner relative to the US, in which lobbying and moneyed interests influence and control major scientific and academic institutions to disseminate propaganda. One such propaganda is that third world and developing nations such as India and Africa must have GM crops since they don't have enough arable land.

Yadav: I can speak specifically about India. The truth is India has enough arable land. With 157 million hectares, India holds the second-largest arable land in the world. We are also the second-largest producer of foodgrains. What India needs is irrigation technology, better education for farmers to increase productivity of crops, as well as building the capacity of stakeholders to understand the market. India, for example, has already taken a lead in organic agriculture… Sikkim's initiative for becoming a 100 per cent organic state is an example. This can give India a competitive advantage. Instead of going for GM crops, India needs to focus on enhancing productivity. The argument that India needs GM crops because of declining land and rising population is simply not correct. Vast tracts have not been brought into cultivation in the northeast of India.

Hansen: Indian scientists have been far more cautious and wise on the issue and reflect the global opinion, as against the US, which is allowing massive use of GM crops without any real safety assessment that complies with international guidelines.

But what are the alternatives? We have new risks every year — climate change, diminishing productivity. And the returns from the Green Revolution are diminishing?

Yadav: There are many alternatives, and we need to move to real science, engineering and public policy to implement those alternatives. Given that there is no consensus on the safety of GM crops and given that the Indian scientific community has clearly said that it is premature to grow them in India, we need to address systemic issues such as farmer training, productive use of existing land, irrigation, and better mechanisation. GM crops are not a necessary part of this solution; in fact, they and the massive use of pesticides may likely be key contributors to the risks you noted such as destruction of soil, diminishing productivity and the need for more water. In fact, there is growing evidence that GM crops, Bt cotton in India, for example, require more water than the non-GM variety. Why would a developing country like India, where water is a precious resource, want to go for more water-consuming crops?

Are we exaggerating the power of the pro-GM media and playing down the power on the other side? At the end of the day it is always easier to raise an alarm.

Hansen: What we are starting to see is that major food companies are adjusting to the consumer's authentic demand for organic, less-processed and non-GM foods. In fact, even Kraft foods, based on this growing demand, decided to stop using preservatives and food colouring. From pure business considerations, food suppliers are listening to the consumers and adjusting their products. Chipotle, one of the fastest growing restaurants, is going to stop using GM ingredients, also in response to such public and consumer feedback and demand for healthier foods. The pro-GM folks, however, have resorted to massive disinformation in the media to try to reverse this tide of public opinion.

Seidler: The key point is that the broad mass of the American people are against GM, and want to see labelling. They don't want preservatives, and they don't want damaging chemicals in their food. The recent declaration by the World Health Organisation's International Agency for Research on Cancer (IARC) that glyphosate is a probable human carcinogen has resulted in limitations and/or bans of glyphosate in numerous countries. More recently a similar declaration of glyphosate carcinogenicity was announced by the California Environmental Protection Agency. This follows on the heels that glyphosate is omnipresent in virtually all GMO crops, foods, water, air, human urine, and even human breast milk. Such declarations have added significantly to this groundswell of public demand for organic and healthy foods.

Ayyadurai: It is in reaction to this groundswell that the pro-GM lobby has been spending massive amounts of money on using the media to misinform the public. The recent disclosures, in the NY Times, of Monsanto and its PR agency directly funding and collaborating with the University of Florida at Gainesville and the Chairman of its Horticultural Department, in creating propaganda to deliberately manipulate public opinion, exemplifies this desperate behaviour in a very unfortunate and dangerous way.

Some form of genetic modification takes place in nature and some of it occurs through breeding. So should there be a big deal made about the targeted genetic modification taking place with GM foods?

Seidler: Let's start with why there are major differences between recombinant genetic engineering, what we refer to as "genetic modification" (GM), and natural plant breeding. They are entirely different. The term "genetic modification" originally was used in Europe to refer to human-induced recombinant genetic engineering, and then began to be misused in a dilettante manner in the US, to broadly refer to the genetic changes that occur during natural plant breeding. And this is very misleading, as it allows those in favour of GM to confuse the public by saying that since GM is just another form of natural plant breeding, GM is safe.

With regard to GM (genetic engineering), to be clear, this typically requires a sterile laboratory environment and typically involves the asexual transfer of three, four or five (more or less in that range) genes, typically from one or more organisms, to produce a new product that is not natural. This means that the resulting new product has never been found, discovered or evolved naturally on the planet. Whereas, during the natural process of sexual plant breeding, there is a complete transfer of up to 20,000 genes, and the resulting organism is a natural product and a natural complement. We should not, therefore, be too surprised that recombinant genetic engineering of DNA, GM, disturbs the natural biochemical and physiological parameters in of itself. To me that is what is exciting, which Shiva's research has uncovered, using a systems biology approach, and shows that GM is substantially different from the natural process of plant breeding. So when you read or hear that genetic engineering, GM, is comparable to natural breeding, it is simply not true.

Roughly, 3-4 per cent of the arable lands on the planet have genetically engineered, GM, crops. The other 96-97 per cent of the crops on arable land is the result of natural processes that occur from pollinators that are wind or other natural events and, on occasion, through human intervention via plant breeding on a magnitude where 10,000 to 20,000 genes are transferred. This has been the natural process for millennia, but that is completely different from GM.

Hansen: By the way, except in the US, there is a clear consensus globally that GM is different from natural plant breeding. There's no debate on this issue as long as we properly agree that GM refers to the genetic engineering process/biotechnology as defined in the international Codex guidelines, which are referenced by WTO.

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Pusztai vindicated by the courts despite GM Corporations best attempt to discredit him and his work.

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Fifteen years ago Dr Arpad Pusztai was mercilessly attacked by many prominent members of the scientific establishment, forced out of his job at the Rowett Institute in Aberdeen on grounds of "malpractice" and made to look on as his research team was dismantled and his laboratory closed (1). In a major departure from the norms of scientific discourse, he was accused of incompetence and even scientific fraud by people who knew next to nothing about GMOs or animal feeding trial protocols, and placed under a binding gagging order.
And the cause of this extraordinary and shameful string of events? A few words in a TV interview for "World in Action" in August 1998 -- in which Pusztai referred to the results of a feeding trial in which rats were fed on a diet which included specially bred lines of GM potatoes. The study threw up 39 statistically significant differences between various tissues and organs, in rats fed on the GM, the non-GM, or the non-GM potatoes supplemented with the gene product. Only four or five of these differences could be explained by random variation. He said: "The effect (on feeding trial rats) was slight growth retardation and an effect on the immune system. One of the genetically modified potatoes, after 110 days, made the rats less responsive to immune effects." Later he said that he would not personally eat the GM food and expressed the view that it was "very, very unfair to use our fellow citizens as guinea pigs". That was all. Pusztai made no extravagant claims about cancer or about the long-term safety or otherwise of GM foods to lab animals or human beings. But he upset Prime Minister Tony Blair and -- reputedly -- President Clinton, and he upset a scientific establishment that had already decided that GMOs were

perfectly safe, on the basis of virtually no evidence. So he was chosen to be the sacrificial lamb, and he had to go (2). In an attempt to justify its actions, the Rowett Institute published an audit criticizing Pusztai's results, and sent the raw data to six anonymous reviewers who also attacked Pusztai's work. The UK's premier scientific body, the Royal Society, also set up a "rebuttal unit" which was briefed to discredit the Rowett team's research; and this unit became involved in a heavily biased external audit or "peer review process" which was quite unprecedented in the biotechnology field.

When a short note summarising the GM potato research (3) was published by Pusztai and his colleague Stanley Ewen in "The Lancet" on October 1999, there was another campaign of intimidation and vilification, this time directed partly at the journal editor Dr Richard Horton, and involving prominent fellows of the Royal Society including Peter Lachmann. Five of the six referees recommended publication, but nonetheless Horton says that he was actually threatened, maybe partly because of his determination to publish in the public interest and partly because he had had the temerity to accuse the Royal Society for its "breathtakingly arrogant" approach to risk research on GM safety. One of the paper's referees, John Pickett, tried to prevent publication and even published a "spoiler article" in a daily newspaper before it was published. Two other Royal Society fellows, Derek Burke and Mike Gasson, published a riposte to the Ewen / Pusztai paper which was fraudulently portrayed by the Royal Society as an original piece of research. Burke seeks to maintain that pretence to this day (5).

It is indisputable that what has become known as "The Pusztai Affair" did immense damage to the standing of science and the reputation of scientists and scientific establishments in the UK. The attacks on Pusztai were vitriolic, even though his statements on the record were tentative and responsible, and even though they had been approved and supported initially by the head of the Rowett Research Institute, Professor Philip James. Throughout twelve months or more of sustained attacks Dr Pusztai maintained his dignity while lesser scientists -- including many Fellows of the Royal Society -- prowled and snarled around him and lied about his work in briefings to the media. To this day they have never found anything fundamentally wrong with his research, and none of them have ever sought to repeat it -- probably because none of them has the competence. Pusztai -- the small man mercilessly attacked by the scientific establishment -- became the first "GM martyr" -- lauded throughout the world simply because he spoke the truth. The Royal Society became a laughing stock because of its pathetic and frenzied attempts to find fault with Pusztai's project -- which had after all been set up after a competitive tendering process (6) and whose protocols had been subject to intense and ongoing peer review and scrutiny. Senior UK scientists had all too visibly allowed themselves to be swayed by political and commercial pressures into a systematic misrepresentation of a careful and deeply worrying (from a public health point of view) piece of safety research. And the furore caused a mild concern about GM crops and foods in the UK to deepen into a solid antipathy, which continues to this day.

Were the Pusztai findings anomalous or unexpected?

The answer to that question is "No." During the 1990's there were many research findings which suggested physiological damage to organisms which consumed GMOs. Some of these findings came from SCRI scientists who were working within the large team coordinated by Arpad Pusztai (7). Concurrently Hilbeck and others discovered that the toxins intended for incorporation into Bt crops damaged "non-target" insects including lacewing larvae and lady beetles (8). And in 1999 John Losey and colleagues showed that transgenic pollen harmed the larvae of the monarch butterfly. Some of these findings were published BEFORE the Ewen / Pusztai paper appeared in The Lancet in October 1999. Pusztai has explained subsequently that the testing of the GMO potatoes on rats was agreed on by the whole research team, on the basis that they had seen toxic effects already on both aphids and lady beetles.

With respect to the feeding of GMOs to mammals, the Rowett Institute research team must have been aware of earlier work which had indicated possible toxic effects. In 1993 research on the Calgene Flavr Savr GM tomato had shown (10) that when it was fed to rats some of them developed "gross lesions" or "gastric erosions" in their stomachs. These have been described as "pinprick bleedings" and linked to cellular changes which clearly needed to be explained. In one trial, seven of the forty rats fed on the GM tomatoes died, and the deaths were simply ignored by the Calgene researchers. In another feeding experiment, this time involving Bt tomatoes, similar lesions were observed but not reported in 1995 (11) by a team including Harry Kuiper, who went on the play a key role in EFSA. These findings must have been known to the Rowett Institute team, and were hypothesised -- perfectly reasonably -- as possible immune responses from animals whose digestive systems were having to cope with foreign or unrecognisable proteins. It was also perfectly reasonable that that hypothesis should be tested in the GMO potato feeding experiments with rats. Running concurrently with the Rowett Institute research, an Egyptian study showed that Bt toxin expressed in potatoes caused major changes in the small intestine of mice (12). With the work of Vazquez-Padron and others, it was demonstrated that Bt toxins bind not only to the insect gut but also to the mammalian gut, leading to various immunity problems (13).

When Pusztai described the cell changes / immune system responses in the feeding study rats, he was not describing an aberration or anything outrageous. He was simply confirming something already described by other scientists and perfectly easy to explain biologically. Indeed, Professor Philip James, in an interview for the Scottish Daily Record in February 1998, warned about GM foods which were inadequately tested, stating that "scientists could be stocking up serious health problems for the future." (14) He also warned, on the record, "The perception that everything is totally straightforward and safe is utterly naive. I don't think we fully understand the dimensions of what we're getting into." On the evening of the TV broadcast of the Pusztai interview, which had been given prior authorisation by James, he put in a complimentary phone call to Pusztai, to congratulate him on the modest way in which he had presented the evidence on the programme. Next day James took charge of a media campaign designed to flag up the importance of the research results for a worldwide audience. And on the day after that, in an extraordinary act of betrayal of a respected scientific colleague, James sacked Pusztai on grounds that were, and still are, entirely spurious.

Have the Pusztai findings been confirmed by later research?

The answer to that question is "Yes." We should remind ourselves that the research done by the Rowett / SCRI / Durham University team was of an extremely high standard, conducted by a 20-strong team that had won through in a very stiff competitive tendering process and in which all of the study objectives and experimental protocols had been worked out in the minutest of detail. Pusztai was himself a senior academic with an impeccable research pedigree, a reputation for cautious and fastidious work in the field of nutrition, and an impressive list of peer-reviewed publications to his name. Those who accused him of bias, fraud or incompetence were in general far less qualified than he in animal feeding experiments (15). It is also generally forgotten that he and his colleagues pleaded for repeat or improved experiments which could test, verify or disprove their findings on insects and rats. These experiments have NEVER been conducted, either by his critics or anybody else. It has been clear to all impartial observers that neither the Royal Society, the FSA, DEFRA nor any other body actually wanted these studies, for fear of what they might throw up. To make matters worse, in something substantially equivalent to a medieval burning of the books, all of the raw data from the Pusztai team's experiments were destroyed, by those who like to call themselves scientists.

Within the last fifteen years there has been a flood of published research which backs up Pusztai's contention that in the crude process of genetic modification something happens within the plant (via a process called mutagenesis) which makes the plant potentially dangerous to the creatures (including humans) that might consume it. In the publication "GMO Myths and Truths", the authors Michael Antoniou, Claire Robinson and John Fagan carefully document scores of papers which demonstrate either direct measurable harm to animals, or the potential for harm (16). For example, when GM peas were fed to mice the insecticidal protein was changed by the GM process so that it behaved differently in the GM peas compared with its natural form in the non-GM beans – and the altered protein from the GM peas stimulated a potent immune response in the mice. When rabbits were fed on GM soy they showed enzyme function disturbances in kidney and heart. A review of 19 studies (including industry's own studies submitted to regulators in support of applications to commercialise GM crops) on mammals fed with commercialised GM soy and maize found consistent toxic effects on the liver and kidneys. Rats fed GM Bt maize over three generations suffered damage to liver and kidneys and alterations in blood biochemistry. Rats fed GM oilseed rape developed enlarged livers, a probable sign of toxicity. Female sheep fed Bt GM maize over three generations showed disturbances in the functioning of the digestive system, while their lambs showed cellular changes in the liver and pancreas. Old and young mice fed GM Bt maize showed a marked disturbance in immune system cells and in biochemical activity. Rats fed insecticide-producing MON863 Bt maize grew more slowly and showed higher levels of certain fats (triglycerides) in their blood than rats fed the control diet. They also suffered problems with liver and kidney function. Mice fed GM soy over their lifetime (24 months) showed more acute signs of ageing in the liver than the control group fed non-GM soy. We could go on.....

The studies cited above have received little attention from the media. Indeed, most of them are modest studies reporting on quite subtle chronic effects which do not, by any means, show that all GMOs are dangerous to human health. But they do show that what Pusztai and his colleagues discovered in 1997-98 was unexceptional, and entirely in tune with subsequent studies showing a wide range of chronic toxic effects on animals which can only be related to the genetic transformation process in plants intended for consumption by animals. This has been established through careful experimentation involving the use of control groups and isolines, as used by Pusztai and his colleagues in the Rowett Institute.

Who were the key players in the destruction of Pusztai's career?

Many senior scientists played active roles in the vilification of Arpad Pusztai and the destruction of his career and reputation. At the Royal Society: Professor Noreen Murray, Professor Brian Heap, Professor William Hill, Dr Jim Smith, Professor Michael Waterfield and Dr Rebecca Bowden. We can also cite Derek Burke, Mike Gasson, John Pickett, Sir Aaron Klug, Peter

Lachmann, Patrick Bateson, and Eric Ash, some of whom were among the co-signatories of a letter condemning Pusztai in The Daily Telegraph. Others were involved in an earlier working group that had issued the Royal Society's 1998 report supporting GM foods. At the Rowett Institute we can cite the members of the Audit Committee established by the Director Philip James in August 1998: Professor F J Bourne, Dr A Chesson (Chair), Professor H Davies, and Dr H Flint. Some of Pusztai's own colleagues failed to support him when the scandal broke, or else joined in the campaign to discredit him. One was John Gatehouse. There were others too, whose names were not prominent either in the press or in the scientific literature, but who nonetheless participated in the process of suppressing the truth. Professor Anthony Trewavas was one, and Professor Tom Sanders, Ian Gibson MP and Jack Cunningham MP were others. The most serious role in the scandal was played by Philip James himself, who is on the record as having articulated very similar concerns to those expressed by Arpad Pusztai, who supported him and congratulated him following his "World in Action" interview, and who then caved in to political and commercial pressure and metaphorically stabbed his respected colleague in the back.

In spite of the activities of the baying pack of hounds from the UK scientific establishment, and in spite of an absurd "gagging order" from his erstwhile employers, Pusztai was not silenced, and he went on to become a hero to many who believe that truth and honesty are more important than the commercial interests of biotechnology corporations or GM research teams who live off streams of government grants -- paid for by the taxpayer. Over the years he has given more than 200 lectures on GMOs and has made many expert submissions to GMO regulatory bodies across the globe. In 2009 he and his wife Susan Bardocz (who was also a colleague in the Rowett research project) were jointly awarded the Stuttgart Peace Prize. (17)

Fifteen years have now passed. Pusztai's observations were accurate and were carefully reported to the media, as a consequence of his own personal concerns about GM technology which flowed directly from his own experiments. His observations were unsurprising in 1998 and they appear even less surprising today in the light of scores of more recent publications. So will those who destroyed the career of a good man through their audit reports, letters and "peer reviews" (and through a wide range of despicable activities behind the scenes) now have the good grace to recognize that they were wrong and that Pusztai was right? More to the point, have any of them got the common decency to issue a straightforward apology for what they did fifteen years ago?

We look forward to seeing such an apology in print, in the near future.

GM-Free Cymru

NOTES

(1) Les Levidow (2002) IGNORANCE-BASED RISK ASSESSMENT? ScientiŽc Controversy over GM Food Safety, Science as Culture, Volume 11, Number 1, 2002

http://www.huffingtonpost.com/jeffrey-smith/anniversary-of-a-whistleb_b_675817.html

Anniversary of a Whistleblowing Hero

http://www.huffingtonpost.com/jeffrey-smith/biotech-propaganda-cooks_b_675957.html

Jeffrey Smith: Biotech Propaganda Cooks Dangers out of GM Potatoes Seeds of Deception Genetic Roulette: The Documented Health Risks of Genetically Engineered Foods

(2) **http://www.councilforresponsiblegenetics.org/genewatch/GeneWatchPage.aspx?pageId=41&archive=yes**

http://www.theguardian.com/education/2008/jan/15/academicexperts.highereducationprofile

Andrew Rowell (2003) "Don't worry, it's safe to eat: The true story of GM food, BSE and foot and mouth." Earthscan. ISBN 1-85383-932-9.

http://www.gmwatch.org/latest-listing/1-news-items/2987-seventh-anniversary-of-gm-safety-scandal

Bourne, F.J., et al (1998) Audit Report Overview (Rowett Institute) Bowden, Rebecca Six referees comments on Pusztai potato data -- Royal Society to Pusztai, 10 May 1999

(3) Ewen, S.W.B. and Pusztai, A. (1999) 'Effect of diets containing genetically modiŽfied potatoes expressing Galanthus nivalis lectin on rat small intestine', The Lancet, 354: 1353–1354**http://www.theguardian.com/environment/1999/feb/12/food.gm**

(4) Laurie Flynn and Michael Sean Gillard for The Guardian, October 31, 1999. Pro-GM scientist "threatened editor". Horton, R. (1999a) 'GM foods: "absurd" concern or welcome dialogue?', The Lancet, 354: 1314–1315. Horton, R. (1999b) 'Editor's reply', The Lancet, 354: 1729. Martin Enserink (1999). The Lancet Scolded Over Pusztai Paper. Science 22 October 1999: Vol. 286. no. 5440, p. 656 DOI 10.1126/science.286.5440.656a

(5) **https://theconversation.com/gm-crops-time-to-counter-the-scare-stories-and-relax-barriers-24678**

(6) **http://www.theguardian.com/news/1999/feb/12/food.science1**

Prior to 1995, no peer-reviewed studies had been published investigating the safety of GM food using human or animal feeding trials. In 1995 the Scottish Agriculture Environment and Fisheries Department commissioned a £1.6 million three-year research study to assess the safety of GM potatoes and to set up experimental protocols for future GM risk assessments.

Twenty-eight study proposals were presented with eight being selected for peer review by the BBSRC. From these eight the Rowett Research Institute's proposal was chosen and a combined team of academics from the Scottish Crop Research Institute, the Durham University Department of Biology and the Rowett Institute was set up with Arpad Pusztai coordinating the study. SOAEFD (Scottish Office Agriculture, Environment and Fisheries Department) flexible fund project RO818.)

(7) **http://news.bbc.co.uk/1/hi/sci/tech/291105.stm**

The Scottish Crop Research Institute has published research that found adverse effects in ladybirds when they were fed on aphids which had fed on GM potatoes. It found the female insects' lifespans were halved and their reproduction reduced. The experiments used potatoes similar to some of those used by Dr Pusztai, modified to include snowdrop lectin.

http://www.foe.co.uk/resource/press_releases/0304bugs

Archived press release: GM crops harmful to wildlife says new research 04 March 1999

(8) Hilbeck A, Moar W, Pusztai-Carey M, Filipini A, Bigler F: Toxicity of Bacillus thuringiensis Cry1Ab toxin to the predator Chrysoperla carnea (Neuroptera: Chrysopidae). Environ Entomol 1998, 27:1255-1263. Hilbeck A, Moar W, Pusztai-Carey M, Filipini A, Bigler F: Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator Chrysoperla carnea (Neuroptera: Chrysopidae). Entomol Exp Appl 1999, 91:305-316. Publisher Full Text Hilbeck A, Baumgartner M, Fried PM, Bigler F: Effects of transgenic Bt corn-fed prey on immature development of Chrysoperla carnea (Neuroptera: Chrysopidae). Environ Entomol 1998, 27:480-487.

(9) Scientific Correspondence. Nature 399, 214 (20 May 1999) | doi:10.1038/20338 Transgenic pollen harms monarch larvae, by John E. Losey, Linda S. Rayor & Maureen E. Carter

(10) Hines FA. Memorandum to Linda Kahl on the Flavr Savr tomato (Pathology Review PR–152; FDA Number FMF–000526): Pathology Branch's evaluation of rats with stomach lesions from three four-week oral (gavage) toxicity studies (IRDC Study Nos. 677–002, 677–004, and 677–005) and an Expert Panel's report. US Department of Health & Human Services. 16 June 1993. **http://www.biointegrity.org/FDAdocs/17/view1.html** Pusztai A. Witness Brief – Flavr Savr tomato study in Final Report (IIT Research Institute, Chicago, IL 60616 USA) cited by Dr Arpad Pusztai before the New Zealand Royal Commission on Genetic Modification: New Zealand Royal Commission on Genetic Modification; 2000.

http://earthopensource.org/index.php/3-health-hazards-of-gm-foods/references-to-section-3#sthash.IPDiTmn1.dpuf http://www.responsibletechnology.org/posts/throwing-biotech-lies-at-tomatoes-part-1-killer-tomatoes/ http://www.responsibletechnology.org/posts/throwing-biotech-lies-at-tomatoes-part-2-the-liars/

(11) Noteborn, H.P.J.M., Bienenmann-Ploum, M.E., van den Berg, J.H.J., Alink, G.M., Zolla, L., A.Reynerts, Pensa, M. and Kuiper, H.A. (1995). Safety assessment of the Bacillus thuringiensis insecticidal Crystal Protein CRY1A(b) expressed in transgenic tomatoes. In: Genetically modified foods. Safety issues. Eds.: K.-H. Engel, G.R. Takeola & R. Teranishi. ACS Symposium Series 605, Washington DC, pp. 134–147.

(12) Fares NH1, El-Sayed AK. (1998) Nat Toxins. 1998;6(6):219-33. Fine structural changes in the ileum of mice fed on delta-endotoxin-treated potatoes and transgenic potatoes.

(13) Vázquez-Padrón R, Moreno-Fierros L, Neri-Bazán L, de la Riva GA, López-Revilla R. (1999) Life Sci. 1999;64(21):1897-912. Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice.

(14) Prof. Philip James warns of "Frankenstein Foods" ---- Scottish Daily Record, February 3, 1998 by Ken Oxley**http://www.nlpwessex.org/docs/profjames.htm**

(15) **http://www.gmfreecymru.org.uk/pivotal_papers/ten_years_on.htm** Ten Years On -- The Pusztai Research Is Still Valid And Still Unchallenged Arpad Pusztai's Feeding experiments of GM potatoes with lectins to rats: Anatomy of a controversy 1998-2009 Klaus Ammann (open source version, 20090811)**http://fbae.org/2009/FBAE/website/images/pdf/Pusztai-Food-Safety-20090811-opensource.pdf** (This document from Ammann is typical of the vicious ad hominem attacks mounted on Pusztai by certain parts of the GMO science community.)

(16) **http://earthopensource.org/index.php/reports/58** GMO Myths and Truths Prescott VE, Campbell PM, Moore A, et al. Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. J Agric Food Chem. 16 Nov 2005; 53(23): 9023–9030. Tudisco R, Lombardi P, Bovera F, et al. Genetically modified soya bean in rabbit feeding: Detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. Animal Science. 2006; 82: 193–199. Séralini GE, Mesnage R, Clair E, Gress S, de Vendômois JS, Cellier D. Genetically modified crops safety assessments: Present limits and possible improvements. Environmental Sciences Europe. 2011; 23(10). Kilic A, Akay MT. A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. Food Chem Toxicol. Mar

2008; 46(3): 1164–1170. US Food and Drug Administration. Biotechnology consultation note to the file BNF No 00077. Office of Food Additive Safety, Center for Food Safety and Applied Nutrition. 4 September 2002. http://www.fda.gov/Food/Biotechnology/Submissions/ucm155759.htmTrabalza-Marinucci M, Brandi G, Rondini C, et al. A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livestock Science. 2008; 113(2): 178–190. Finamore A, Roselli M, Britti S, et al. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J Agric Food Chem. Dec 10 2008; 56: 11533–11539. Malatesta M, et al. A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. Histochem Cell Biol. 2008; 130: 967–977.
(17) http://www.gmwatch.org/latest-listing/1-news-items/11801-pusztai-to-receive-stuttgart-peace-prize

An extensive list of further reading is available here:
http://www.gmfreecymru.org/documents.htm