BT BRINJAL Event EE1

The Scope and Adequacy of the GEAC Toxicological Risk Assessment

Review of Oral Toxicity Studies in Rats

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SUMMARY

This evaluation of Bt brinjal studies is based on requirements for a rigorous evaluation of food safety for the people of India and their health. Departures from Indian and international published standards for the 14-day and 90-day studies are a cause for concern[1].

The current food safety studies for Bt brinjal were not conducted in accordance with published standards, did not accurately summarize results, and ignored toxic endpoints for rats fed Bt brinjal: in particular, rats fed Bt brinjal for 78 out of 90 days (only one dose level) experienced:

- organ and system damage: ovaries at half their normal weight, enlarged spleens with white blood cell counts at 35 to 40 percent higher than normal with elevated eosinophils, indicating immune function changes.
- toxic effects to the liver as demonstrated by elevated bilirubin and elevated plasma acetylcholinesterase.

Major health problems among test animals were ignored in these reports. The single test dose used was lower than recommended by the Indian protocols. Release of Bt brinjal for human consumption cannot be recommended given the current evidence of toxicity to rats in just 90 days and the studies' serious departures from normal scientific standards.

Unanswered concerns regarding the safety assessment of Bt Brinjal

Neurological function, behavioral effects, reproductive performance and biological resilience of test animals were not evaluated in these studies. Further research based on properly conducted and supervised studies is needed to resolve indications that Bt brinjal may have adverse effects on these clinical endpoints.

Dietary equivalence of dried brinjal, dried Bt brinjal and control diets was not addressed. Concentrations of the new insecticide protein Cry1A(c) were not measured in dried brinjal powder. It is important to know how much of this new protein was actually in the dried samples fed to the rats, especially since there is data to suggest that Cry1A(c) is at least partially destroyed in laboratory heating conditions. That omission makes it impossible to compare the test diet with insecticide concentrations expected in cooked human food.

[1] The Indian toxicology standards for 14-day and 90-day feeding trials published by the Department of Biotechnology (DBT) in 1998 and in 2008 fall short of the international standards (OECD 1998 and Codex Alimentarius 2003a-c), allowing a significant loss of scientific rigour. Therefore, although this critique is based on the Indian DBT protocol, meaningful departures and omissions from international standards are noted. It is important to clarify that 14 and 90 day exposures to rodents are insufficient periods of time on which to base food safety decisions for humans.

The use of laboratory animals to test food safety, although widely accepted as a toxicological tool, is only an indication of effects that might be expected from human exposure[2]. It is essential that deviations from standard protocols be evaluated carefully, as these changes can have a profound impact on results. Yet every departure made by INTOX (the laboratory contracted to do the research) from the Indian Department of Biotechnology protocol (1998) has resulted in lower standards being used, with less power to detect changes in rats eating Bt brinjal. These include: skipping important endpoints such as IgE measurement to test for allergenicity, testing only one dose that was lower than human consumption is likely to be, ignorance of toxicological equivalence, lost data, lack of Good Laboratory Practice standards, inadequate observation of animals, a 29% decrease in exposure days in one study (doses were administered 5 days per week instead of 7), etc.

Consequently, the studies submitted by Mahyco are woefully inadequate to determine the safety of Bt brinjal for long-term human consumption.

Table 1. Summary of statistically significant findings in rats fed Bt brinjal in 90 day study with implications for human health

Indicator	What it might indicate	Significant potential adverse effect
Elevated white blood counts from chronic exposure	Inflammation, allergy, tissue injury	√
Higher aspartate aminotransferase in blood from acute exposure	Liver damage	√
Elevated bilirubin in blood		
Altered plasma acetylcholinesterase		√
Smaller ovaries	Reproductive toxicity	√
Enlarged spleens	Chronic infections or blood cancer	√

[2] Significant genetic and phenotypic variation between humans makes it statistically impractical to conduct food safety trials on humans. As a result, the use of animals to test food safety cannot completely resolve all potential health implications because it also introduces uncertainty in risk assessment. In other words, the lingering uncertainties in animal feeding studies require that they be interpreted cautiously and conservatively. Any indication of adverse effect (even if statistically small) should be followed up.

METHODS

The current assessment is of three toxicology studies conducted by commercial toxicology laboratory INTOX PVT LTD on behalf of Maharashtra Seed Company, also known as Mahyco. Study details and raw data have recently been made available to the public through the internet link http://www.envfor.nic.in/divisions/csurv/geac/bt_brinjal.html.

All three studies tested the genetically modified food brinjal (herein referred to as Bt brinjal)[3] containing the insecticide protein Cry1A(c) and other novel genetic components, as it is proposed for sale of seed in India;

- a 14-Day Dose Range Finding Study
- an Acute Oral Toxicity study of Transgenic Bt brinjal containing Cry1A(c) gene in rat (Study No. 218301)
- a 90-day Subchronic Oral Toxicity Study of Transgenic Bt brinjal (Study No. 218304).

These studies are herein referred to as the 'dose-range finding', the '14-day' and the '90-day' studies, respectively.

The dose-range finding study

The first of these published studies is a 14 day dose-range finding study of Bt brinjal in rats. Although this study was submitted by INTOX PVT LTD via Mahyco, it is misleading to do so as it occurs within a report for another study. Only limited information about the study is provided, and then only in summary form on pages 12 and 13 of the 90-day study report, as well as some raw data in Appendix D. This study is disregarded from further consideration for the following reasons:

- Only three animals were tested per dose group, which is insufficient to make any valid conclusions. According to the Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds and Plant Parts (DBT 1998), a minimum of ten animals per dose group is necessary. This is considerably less than the OECD standard of ten animals per sex and dose group.
- The problem with using fewer than the recommended number of animals is an increased chance of Type II errors – that is, failing to observe a treatment-related difference when in truth there is one.

In addition to using too few animals to provide confidence in the findings, there were other arbitrary and unjustified methodological practices:

- The rationale for using doses of dried brinjal powder at 500 and 1000 mg/kg was not provided.

[3] Brinjal is also known as eggplant or aubergine

- The study guidelines and laboratory standards for this study were not provided. Statements about following good laboratory practice (GLP) or having GLP certification are also absent. Lack of stated adherence to laboratory standards or protocol puts the quality of the research conducted into question.
- The dates of the study and the names, titles and signatures of the people conducting the study were not provided.

The 14-day and 90-day studies

The 14-day and the 90-day studies are stated to have been conducted according to "Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds and Plant Parts"[4] as outlined by the Department of Biotechnology (DBT) in India in 1998, and in compliance with the principles of Good Laboratory Practice as established by the OECD in 1998.

Statements of compliance with Good Laboratory Practices and Quality Assurance (pages 3 and 4 of both study reports) are not signed. This omission does not inspire confidence in the published results.

Inclusion of extra control groups

Only one control group was required according to the Indian protocol (DBT 1998, page 61) and international protocols (OECD 1998, Item 14 page3)(Codex Alimentarius 2003b). INTOX PVT LTD on behalf of Mahyco used three control groups for each single dose test group:

- G I (14 day test) and G1 (90 day test): Controls receiving vegetable oil only (vehicle control)
- G II (14 day test) and G2 (90 day test): Vegetable controls receiving non-transgenic brinjal powder in oil
- G III (14 day test) and G3 (90 day test): Vegetable controls receiving commercially available non-transgenic brinjal powder in oil.

Was the non-transgenic brinjal group included under the assumption that the studies would find no toxicity at the doses used [5000 mg/kg-day in the 14-day study and 1000 mg/kg-day in the 90-day study], and therefore suffice as the 'limit tests' described on pages 54/55 and 62 of the protocol (DBT 1998)? The second and third control groups listed above were not required for the 14-day or the 90-day studies.

[4] Herein referred to as 'the protocol'

The inclusion of extraneous control groups is not scientifically or methodologically valid. Increasing the number of control groups in this manner decreases the chances that true and relevant differences will be consistently observed between the Bt brinjal group and others.

The salient analysis of toxicology results is between equal numbers of individuals from the Bt brinjal group and a single control group. Since it is unnecessary to produce more than one control group and since we have no information to confirm that the commercially-available brinjal did not contain the Cry1A(c) protein, or other agricultural chemicals that may adversely affect the health of animals eating it, commercially available brinjal dose groups (G III and G3) are not considered further in this analysis.

Presence or absence of Cry1A(c) protein in brinjal powder

Were fruit powders received from Mahyco verified for the presence or absence of transgenic material just prior to conducting toxicity tests? The only evidence we have that testing was conducted to confirm the presence of Cry1A(c) protein in Bt brinjal and non-Bt brinjal in these studies is a single page that was produced twice: at the end of the 14-day study report (no page number) and also as Appendix E on page 106 of the 90-day study report. Since there is no date on this page and these two studies were conducted more than one year apart, it is impossible to know which study it was produced for.

Evidence of testing for the Cry1A(c) protein in animal feed is *either* misrepresented in one or both of these reports *or* both studies used the same stored batches of dried brinjal powder. The possibility that transgenic proteins degraded during drying or after storage cannot be ruled out, representing a significant potential loss of potency of the test article. Furthermore, there is no indication of the concentrations of Cry1A(c) protein in dried brinjal powder either before or after several months of storage. In turn, this would be a further loss in representation of laboratory tests at a dose that consumers are likely to be exposed to.

New statistical analyses

Raw data from the published reports were used to calculate statistically significant differences between test groups using a student's t-test for two independent samples with unequal variance using Microsoft Office Excel 2007. The raw data selected were variables noted from visual inspection of the summary tables for each report. This included concentrations of acetylcholinesterase (a neurotransmitter enzyme) from plasma and red blood cells, bilirubin (increases indicate liver complications from infection or chemical exposure), total white blood cells (increased in response to infection) and aspartate aminotransferase (increases are used to diagnose liver or heart damage) in blood. In the 90-day study, organ weights for ovaries (which give an indication of reproductive health), spleen (this organ purifies the blood) and kidneys (which excrete waste products from the body) were also analysed.

Direct statistical comparisons in both studies are made between the main test group (G IV and G4, receiving Bt brinjal powder in peanut oil) and the group receiving peanut oil only (G I and G1).

Comparisons between the Bt brinjal test groups and the control group receiving non-transgenic brinjal in peanut oil (G II and G2) are described in the text and noted in Appendix B of this report.

This report addresses the following questions:

1. Do the two studies meet the stated 1998 protocol standards[5] for India and the OECD standard?
2. Have the studies been accurately summarized to be consistent with the raw data results? Were statistical assumptions valid and adequately described?
3. Would an impartial technical reviewer derive the same conclusion as the laboratory contracted by Mahyco (and accepted by the second Expert Committee or EC II)?

The larger question of whether or not these results are sufficient to draw conclusions of food safety is addressed in the Discussion section of this report.

[5] The DBT protocol was updated in 2008. Since this research was conducted prior to 2008, the 1998 protocol was relevant at the time. Neither of these protocols adhere to international standards.

RESULTS

Table 2 below summarizes the compliance of the 14 day acute toxicity test and the 90 day feeding study with the stated guidelines (DBT 1998).

Table 2. Summary of study characteristics in compliance with protocol guidelines (1998)[6]

Protocol Requirement, Department of Biotechnology 1998	14 day study	90 day study
Sufficient number of animals tested per dose group; 10 for 14 day study and 20 for 90-day study	Yes	Yes
Animals housed singly or in pairs (not a protocol requirement for 90-day study)	Yes	No
Test doses selected according to protocol	Yes	No
Daily (twice daily required for 14-day study) observations of animals to look for signs of toxicity including tremors, convulsions, salivation, diarrhea, lethargy and sleep, dyspnea, coma, nasal bleeding, etc[7].	Undetermined	No
Daily observations of behavioral abnormalities	No	No
Statistical methods described	No	Yes
Statistical methods used	No	No
Statistical results reported	No	No
Significant differences discussed in terms of biological significance and impact on food safety	No	No
Study summary reflects results	No	No

[6] Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds and Plant Parts. Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998. Public Printing Service (Delhi) 96 pp.

[7] Updated protocol for 2008 emphasizes the importance of behavioural signs of toxicity not limited to hunched posture, lethargy or persistent recumbancy, labored breathing, any condition interfering with eating or drinking (e.g., difficulty moving), or excessive or prolonged hyperthermia or hypothermia.

The 14-day acute oral toxicity of transgenic Bt brinjal containing Cry1A(c) gene in rat

An acute oral toxicity study (a limit test) was performed on rats fed 5 grams of dried brinjal powder per kg of body weight in peanut oil. Doses were administered over 24 hours and rats were observed for 14 days following dosing.

As shown in Table 2, the 14 day study was conducted with several deviations from the 1998 DBT protocol: the report lacks a description of statistical methods used, study results were not compared using a statistical analysis, and important variations in health endpoint outcomes were not discussed in terms of biological significance. These are critical and unjustifiable omissions by the researchers. Consequently, while the study has been used by Mahyco to provide evidence that Bt brinjal is safe to eat, this conclusion cannot be substantiated.

New Statistical Comparisons for the 14- day study

For the purposes of verifying the conclusions reported in the 14 day study, the following statistical comparisons have been made on endpoints of interest[8] from the following sources in the INTOX report:

- Appendix B1 of INTOX report: Individual animal hematology data
- Appendix B2 of INTOX report: individual animal clinical chemistry data

[8] Endpoints of interest were selected from quick visual inspection of data summary tables. This is not an exhaustive analysis of all raw data from the 14-day report.

Table 3. Results of statistical analysis of raw data from the 14-day study

Toxicological endpoint	Arithmetic mean values for females/males/total	
	Vehicle control group (G I)	Bt brinjal group (G IV)
Total white blood cells ($x10^3$/cmm) females/males/total	8.6/9.0/8.8	7.7/8.2/8.0
Aspartate aminotransferase (IU/L) females/males/total	164.2/154.0/159.1	251.8**/244.8*/248.3**
Plasma acetylcholinesterase (IU/L) females/males/total	641.8/656.2/649.0	534.0/529.3/531.7**
Red blood cell acetylcholinesterase (IU/L) females/males/total	407.6/398.8/403.2	351.9/324.9/338.4
Bilirubin (mg/dl) females/males/total	1.1/0.9/1.0	1.1/1.2*/1.2*

*Statistically significant difference from G IV at $p \leq 0.05$

**Statistically significant difference from G IV at $p \leq 0.01$

Toxicological implications of the results in Table 3 from the 14 day study

Total white blood cell counts were found to be 9 to 12% lower among the rats fed Bt brinjal compared to controls. The toxicological implications of *decreased* white blood cell count following an acute exposure include a possible recent infection or impaired immunological function.

Increases in aspartate aminotransferase (AST) among Bt brinjal-fed rats were 54 to 60% higher than controls. Increased AST indicates damage to the liver or heart. In this case, coupled with elevated bilirubin (another measure of liver dysfunction also noted in this table), damage to the liver from short-term exposure at the dose of 5000 mg/kg-day is indicated.

Plasma acetylcholinesterase was 22% lower among rats fed Bt brinjal than that observed for controls. Significant changes in plasma acetylcholinesterase (a neurotransmitter enzyme) concentrations could be further evidence of liver damage in rats fed Bt brinjal.

Inconsistencies in the 14-day study report

Page 6 of the INTOX report is a summary of the 14-day study. In this summary there are only three study groups mentioned as follows: "…the test article was administered orally to a group of 5 male and 5 female rats as an acute dose at the limit dose of 5000 mg/kg body weight, suspended in peanut oil, as a vehicle. One concurrent control group of 5 male and 5 female rats was similarly gavaged with nontransgenic brinjal powder in peanut oil, while a third group of 5 male and 5 female rats was gavaged with normal powdered rodent diet in peanut oil only, and served as an untreated control." The study summary appears to be at odds with the data reporting results for four study groups, not three.

Page 9 of the report states: The total number of animals tested per sex is 20 (five per dose group using four dose groups) but the table lists only 15 animals per sex. These may only be typographical errors. However, they may also indicate that data for the third test group (G III, not mentioned in the study summary) was added at a later date, as suggested by the study summary.

The results given in Table 3.3 (individual animal fate and pathology findings) of Appendix B (of the INTOX report) for group G III are identical to the results in the next table for Bt brinjal-fed rats among both males and females. This additional suspected formatting/typographical error carries other implications: where are the missing data for these animals and what information is contained in those missing data?

The protocol requires twice daily observation of animals for signs of toxicity since the test article is given in a single acute dose. Tables A1.1 through A1.4 report no clinical abnormalities over 14 days of observation. It is unlikely that clinical observations would not pick up a single abnormality among 40 rats over 14 days, if observations had been conducted by trained researchers.

Statistically significant differences between the Bt brinjal and control groups for toxicological endpoints were not noted or discussed in the INTOX report.

The 90-day Subchronic Oral Toxicity Study of Transgenic Bt brinjal

Animal Husbandry

Caging groups of 5 animals together is considered to be extreme crowding unless unusually large cages were used. Instead, they should have been housed singly or in pairs. Group caging also has the effect of "washing out" individual differences in amounts of food and water consumed over the course of the study. If one animal in five has an abnormal eating or drinking pattern (as was the case among goats fed Bt brinjal) this is unlikely to be observed in a group measurement, even though a health outcome for 20 percent of the population (one in five animals) is of interest. It is noted that this is not strictly a deviation from either the 1998 protocol or the OECD 408 protocol which states that animals may be housed in small groups of the same sex in the 90-day study (OECD 1998).
Were all test group animals placed in the same room to minimize differences in temperature, humidity and air changes that will impact on the overall health of the animals? This needs to be specified but was not.

Were all test group animals obtained from the same source, at the same age, previously unexposed to Bt brinjal and nulliparous at the start of the study? Were animals randomly assigned to dose groups at the start of the study? All of these variables need to be reported as they have the potential to affect health outcomes measured in this study, but none of them were.

Dose groups

The 90-day toxicity study is meant to include 3 doses of Bt brinjal. According to the Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds and Plant Parts;

> "The selection of the dose is made on the basis of acute toxicity studies of the test chemical. At least 3 dose levels, one maximum, one minimum and one intermediate are used. Consideration is given that the highest dose may result in toxic effects without causing excessive lethality and the lowest dose may not produce any toxic effects. A group of vehicle controls is also used." –(DBT 1998).

The 90-day study was conducted using a single dose level for which there is no demonstrable toxicology information prior to conducting the study. Without evidence to support the assumption that 1000 mg/kg-day will result in toxic responses in a 90-day study, this particular dose does not make sense scientifically.

One possible outcome of using a dose for which there is no evidence of toxicity would be a false finding of safety because the dose was too small to observe toxic effects in rodents over 90 days. This increases the chance of failing to observe a treatment-related toxic endpoint when in truth there may be one.

Reasons for using the dose of 1000 mg/kg were tacitly given by stating that a dose range finding study had been conducted. Since this test used a total of three animals per test group, and for other reasons listed above, this cannot be considered justification for selecting the 1000 mg/kg-day dose over 90 days.

Moreover, there are justifications for believing that one gram of brinjal per kg body weight is inadequate to determine the health effects of this crop on the Indian people. Brinjal is a crop that is widely consumed in significant amounts in India. The dose used in this study is equivalent to only 40g (about 2 tablespoons) of Bt brinjal/day for a slightly-built woman and 70g/day (about 4 tablespoons) for a reasonably-sized man. Notably, Codex Alimentarius 2003b recommends:

"Information about the known patterns of use and consumption of a food, and its derivatives should be used to estimate the likely intake of the food derived from the recombinant-DNA plant. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage." paragraph 49, Section 1.

This recommendation is emphasized in all guidance for conducting the 90-day subchronic toxicity study, including the 1998 DBT protocol: "expected human exposure may indicate the need for a higher dose level."

Other omissions in the 90-day study

IgE was not measured in this study, even though the report states on page 16 that IgE was analysed. Clinical chemistry data in Appendix B2 report IgE results as <1.00 IU/ml for every observation. Since IgE concentrations vary widely between individual rats (Abadie and Prouvost-Danon 1980) and expected values in rats are greater than 200 IU/ml[9], it is likely that:

- The IgE measurement method used by the researchers using the "Erba Smartlab Random Access Batch Analyser" (page 16 of the report) was not sensitive enough to accurately measure IgE in rats.

- Blood samples were incorrectly stored prior to chemical analysis leading to serious errors in the results.

The lack of IgE data is unfortunate as IgE is especially important as a measure of allergic reactivity. Quantitative evaluation of IgE is required in the protocol on page 62 of DBT 1998 and emphasized in an expert consultation from FAO/WHO (2001). This is a considerable omission and protocol deviation that has not been addressed. Without IgE data, there is a lack of important information about the possible effects of the Cry1A(c) protein on the mammalian gut resulting in possible hypersensitivity/allergic reactions, observed as increased concentrations of IgE compared to controls (Karlsson et al 1979). On the other hand, decreased concentrations of IgE in Bt brinjal rats would be consistent with diseases such as hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, and hepatitis. It is important to know whether the new brinjal may simply act as an irritant that produces allergic responses in the gut, or as an endotoxin conferring damage to the liver with ingestion.

Raw data in Appendix B1 of the INTOX report for differential white blood cells are reported in whole numbers without decimal places, preventing analysis of eosinophil concentrations. The summary table for these results (Table 8 on pages 38-39 of the INTOX report), however, reports concentrations at two decimal places (two more significant digits than the raw data support). Is this is a lack of precision in reporting individual raw data, or is it over-precision in aggregate data? It is impossible to tell if the aggregate data actually reflect the raw data in this case. The overall effect of leaving out these important raw data is to prevent independent analysis of differentiated white blood cell counts in rats fed Bt brinjal.

[9] Background concentrations of Total IgE in Sprague-Dawley rats are 0.6 ug/ml (Abadie and Prouvost-Danon, 1980); this is equivalent to 250 IU/ml. Thus, it is extremely unlikely to measure less than one IU/ml of IgE in any rat, and even more impossible in 80 rats.

New statistical comparisons for the 90-day study

In order to verify the conclusions listed in the 90-day report, statistical comparisons on endpoints of interest[10] have been made using the following sources in the report:

- Appendix A3: Individual animal organ weight absolute values
- Appendix A4: Individual animal organ weight relative values
- Appendix B1: Individual animal hematology
- Appendix B2: Individual animal clinical chemistry

Table 4. Results of statistical analysis of raw data from the 90-day study

Toxicological endpoint	Test group mean values females/males/total	
	Vehicle control group (G1)	Bt brinjal group (G4)
Organ weight – ovaries (g) females only	0.11	0.06**
Organ weight – spleen (g) females/males/total	0.86/1.34/1.10	1.02/1.19/1.11
Organ weight – kidneys (g) females/males/total	1.42/1.34/1.38	1.48/1.19/1.34
Total white blood cells ($x10^3$/cmm) females/males/total	9.3/11.1/10.2	14.0*/12.6/13.3*
Aspartate aminotransferase (AST) females/males/total	134.5/189.5/162.0	151.7/156.5/154.1
Plasma acetylcholinesterase (IU/L) females/males/total	591.6/604.0/597.8	875.0/902.6**/888.8**
RBC acetylcholinesterase (IU/L) females/males/total	299.9/388.3/344.1	265.7/335.6/300.6
Total acetylcholinesterase (IU/L) females/males/total	891.4/992.4/941.9	1140.7/1238.2/1189.4*
Bilirubin (mg/dl) females/males/total	.58/.51/.54	. 81**/.52/.66*

[10] Endpoints of interest were selected from quick visual check of data summary tables. This is not an exhaustive analysis of all raw data from the 90-day report.

*Statistically significant difference from rats fed Bt brinjal at $p \leq 0.05$
**Statistically significant difference from rats fed Bt brinjal at $p \leq 0.01$

According to the DBT 2008 study protocol, toxicological implications of the results must be reported:

"The 90-day study provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time covering post-weaning maturation and growth well into adulthood. The study will provide information on the major toxic effects, including possible target organs, and the possibility of cumulative effects." –DBT, 2008

Discussion on the implications of the toxicology results for the 90-day report from INTOX has been left out.

Toxicological implications of the results in Table 4 from the 90-day study

Females who were fed Bt brinjal had smaller ovaries than controls. At just over half the expected size, ovaries of Bt brinjal-fed rats exhibit a consistent[11] and profound reproductive toxicity signal that is statistically significant at $p < 0.0001$[12] even with the small number of animals tested and the relatively short exposure time (90 days). Unfortunately, a 90-day study is not long enough to know what the long term reproductive performance outcome would be for animals fed Bt brinjal. Other research has shown that when mice were fed genetically modified food containing the Bt toxin in a multigenerational study, they had decreased reproductive performance as demonstrated by smaller litter size and lower average litter weight (Velimirov 2008)[13].

Spleen weights among Bt brinjal female rats were 19% higher than the control group (and 26% higher than the vegetable control group – see Appendix B, Table B2). Differences were statistically significant when compared to the vegetable control group (Appendix B, Table B2)[14]. These differences were not noted in the INTOX report.

Significant changes in both the ovarian and spleen weights for the female rats fed Bt brinjal were apparent from summary values listed in Table 6 of the 90-day report. However, page 21 of the report incorrectly summarized the results, saying:

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[11] Vegetable controls also had normal-size ovaries compared to Bt brinjal rats (see Appendix B, Table B2).

[12] Statistical significance remained the same using ovary weights relative to total body weight.

[13] It is worth noting that only one of two reproductive toxicology study protocols was powerful enough to observe this sensitive outcome; the Reproductive Assessment by Continuous Breeding (RACB).

[14] Statistically significant differences are dependent on sample size: doubling the observations of each group G1 and G4 results in statistically significant increases in spleen weights for females fed Bt brinjal.

> "The values of absolute and relative weights of kidneys, liver, adrenals, testes, spleen, brain and ovaries of male/female rats treated with Transgenic Bt brinjal containing Cry 1 A(c) gene, non-transgenic brinjal and nontransgenic brinjal (commercially available) at 1000 mg/kg were found to be comparable to those of the control group rats at termination of the treatment."

Page 22 of the report further incorrectly concludes:

> "No alterations in the absolute and relative organ weights of rats treated at 1000 mg/kg [were found]".

These statements clearly indicate that the authors are not familiar with the principles or procedures for evaluating their own results in the 90-day study. Only the vehicle control group (G1) was required in this study (DBT 1998, page 61 and OECD 1998, Item 14 page3). As shown in Table 4 above, significant measures of organ toxicity (resulting in lower organ weights) to female rats consuming Bt brinjal in the 90-day study were evident. Findings of statistical significance further emphasize the seriousness of these differences.

Rats fed Bt brinjal also displayed elevated white blood cell counts (up to 50 percent higher among females and 33 percent higher overall), compared to controls. This was consistent among vegetable controls (Appendix B, Table B2). In differentiated white blood cell counts, this increase includes a near doubling in the count of eosinophils (a type of white blood cell) among Bt brinjal-fed rats. Eosinophils typically increase in response to allergic disorders, infection or to epidermal inflammations (such as those caused by parasitic infections). Bt brinjal-fed female rats had nearly twice the concentration of eosinophils compared to control groups, consistent with subchronic gut irritation, possibly caused by Cry1A(c) protein in the diet. For reasons discussed above, it is impossible to compare eosinophil concentrations using the raw data presented in the 90-day report.

Bilirubin concentrations are also elevated among female rats, with a 35% increase compared to vegetable controls (Appendix B, Table B2) and a 40% increase compared to vehicle controls. Bilirubin is a measure of liver function. Increased bilirubin in the absence of harmful xenobiotics or infectious hepatitis indicates obstruction of the biliary ducts in the liver. The increases in bilirubin of females in this study are extreme enough to result in statistically significant differences for females and the total groups. It is not clear how the authors missed this result, but the differences are not described or discussed in the 90-day report.

Plasma acetylcholinesterase (a neurotransmitter enzyme) is 20-49% higher among all Bt brinjal-fed rats, both male and female, compared to control groups. Statistical significance for these increases were observed in comparison with the vehicle control group among males and total. The implications for elevated acetylcholinesterase in plasma samples could include hepatotoxicity (Garcia-Ayllon et al 2006), early onset of type II diabetes or neurological impairment (Garcia-Ayllon et al 2010).

When combining these individual signs of toxicity, a more concrete picture emerges:

- Increased white blood cell counts coincide with enlarged spleen weights observed among Bt brinjal-fed rats, further indicating immune responses to toxic exposure.
- Elevated bilirubin concentrations and elevated acetylcholinesterase concentrations are consistent with hepatotoxicity from subchronic exposure in rats fed Bt brinjal.

DISCUSSION

Shortcomings of using these data to approve Bt brinjal for human consumption

Consumers, objective scientists and government representatives need to be aware of the potential health effects of new foods proposed for sale in India so they can take part in the decision about how much uncertainty remains after feeding studies have been conducted and how that affects risk assessment. Although animal feeding studies are limited in their representation of human responses, they form an important basis from which to gauge possible toxic response to new products. Even when these limited short-term feeding trials are conducted correctly using Good Laboratory Practices and following internationally accepted protocols, there will be some exposures that are still untested; chronic (long term) exposure to humans and animals, occupational exposure to people growing Bt brinjal and inhalation exposure to those who cook or process Bt brinjal.
Adverse effects of Bt brinjal exposure may be more easily transmitted by inhalation than by ingestion. As has been shown in a study with Wistar rats, inhalation exposure caused immunomodulation in control rats housed in the same room as those fed a GM Bt rice diet (Kroghsbo et al., 2008). Human reactions to the Bt toxin via inhalation have been observed in occupational settings (Bernstein et al 1999): greenhouse workers exposed to Bt toxin in sprays developed allergic responses and elevated IgE compared to pre-exposure concentrations (Doekes et al 2004).

Multiple control groups and other methods of obscuring toxic response to GM foods

Previous publications from commercial seed producers on the toxicological research of transgenic foods have included multiple control groups (Hammond et al 2006). The use of multiple control groups has the effect of increasing the variation (wider confidence intervals) in the combined controls, which decreases the chance that a difference will be found between the test group and the controls. In some cases, as possibly indicated by group G III in the 14-day test described in this paper, the data are recorded under different circumstances than the animals consuming the test diet. In toxicology research, comparing equal numbers of individuals from two groups that receive different diets while all other variables are kept constant is the established method for investigating health effects related to diet. Establishing dose and response effects requires *at least* three test diet dose groups, as required by DBT 1998 and OECD 2003. Somehow the presentations made by commercial seed producers have allowed the opposite set of comparisons to be made: one or two test doses compared to several control groups.

Extra control groups is only one technique used by commercial operators to attempt to disregard significant differences between animals fed genetically modified foods and those on conventional diets. Other “techniques” that would be expected to disqualify research results from publication (if reviewers and publishers were blinded to the author’s interests) have been discussed previously (Seralini et al 2009). Briefly, these include:

1. A false assertion that males and females must have the same toxicological responses;

2. A false assertion that, if two doses are used, the higher dose must have a greater effect than the lower dose (a so-called dose-response observation);

3. A total omission of any data analysis enabling researchers to write conclusions in the void of data evidence;

4. A total omission of statistical results indicating significant differences in organ weights, haematology and clinical chemistry; and

5. Conclusions that ignore toxicologically significant results.

Other studies confirm toxic responses seen in Bt brinjal

The lack of scientific data on genetically modified food toxicology in the peer-reviewed literature (Domingo 2000 and 2007) indicates a scarcity of independent science in this area (Pryme and Lembke 2003). Specifically, no chronic feeding studies assessing the safety of genetically modified food containing the Cry1A(c) protein in the published, peer-reviewed literature were found at the time of this report.

Previous studies on the immunogenicity of the Cry1A(c) protein have shown that this protoxin is a potent allergen in animal models (Vásquez-Padrón et al 1999, Vásquez-Padrón et al 2000 and Moreno-Fierros et al 2000). Accordingly, this team concluded in 2000:

> "We think that previous to commercialization of food elaborated with self-insecticide transgenic plants it is necessary to perform toxicological tests to demonstrate the safety of Cry1A proteins for the mucosal tissue and for the immunological system of animals."

Although previous research has demonstrated hepatic toxicity in rats fed genetically modified foods (de Vendômois 2009 and Malatesta et al 2002, 2005), this is the first time that a food containing the Cry1A(c) protein has been tested and associated with hepatotoxicity and reproductive disorder.

Reproductive toxicity of Bt brinjal is demonstrated by the reduced ovarian weights resulting from a dose of 1000 mg/kg-day (ie 1 gram/kg body weight each day) using only ten animals, compared to two groups of controls (Appendix table B2). The likely clinical significance of decreased ovarian weights is lower fecundity, although other unintended effects may occur as well. This brings into question the possibility of hormonally-mediated toxicity that has not previously been considered for Bt brinjal but has been observed in other studies on GM foods containing proteins derived from the same *Bacillus thuringiensis* sp *kurstaki* (Bt) bacteria, Cry1Ab (Seralini et al 2007 and Velimirov et al 2008). Brasil et al (2008) found that a Bt soy diet altered ovarian and uterine morphology resulting in fewer follicles (viable eggs) and more corpus luteum (egg sacks without eggs), and a thickened uterus lining, in the second generation of female rats consuming Bt soy diets.

Fares and El-Sayed (1998) found structural damage to the ileum of mice fed Bt-potatoes over a two week period, when examining tissue with electron and light microscopes. The control group of mice fed

conventional potatoes differed significantly, with ileum tissue in its normal state. Elevated white blood cell counts experienced by female rats in the 90-day study are another indication of possible structural damage to tissues involved in processing Bt toxins such as the gut and spleen (Finnamore et al 2008). In the Finnamore study, mice eating Bt corn had increased eosinophil production and granulation, resulting in the production of specific cytokines (IL-6, IL-13, IL12p70 and MIP-1). Furthermore, it was noted that inflammatory and immunologic responses were stronger in weanling (young) mice than in older mice, because of their sensitivity to new allergens.

Compositional analysis of test article and vegetable control

The compositional analysis (reported in section 7.2 of Mahyco 2008) describes Bt brinjal as similar to non-Bt brinjal in content of protein, carbohydrate, oil, calories, ash, nitrogen, crude fibers and moisture content. These analyses were conducted by the seed company (Mahyco) at their own labs, and fall far short of required analytical parameters (Section 7.3 and Checklist 9 of Indian Council of Medical Research 2008, Sections 4.2.6 and 4.2.10 of the European Food Safety Authority 2008, Codex Alimentarius 2003b, sections 44, 45 and 49). Results are not shown in this report so it is impossible to know how large these differences might have been. Was the conventional counterpart of Bt brinjal used for the compositional analyses as recommended by section 44 of Codex Alimentarius (2003b)? The conventional brinjal parent variety was not named in the Mahyco 2008 report or the toxicology study reports. Was the conventional counterpart of Bt brinjal used in the 14-day and 90-day toxicology studies? If specific differences in vitamin, mineral, fatty acid and protein contents of the brinjal and Bt brinjal diets were not known at the time of the studies, there is some uncertainty about nutritional equivalence between test groups, and this may have impacted results.

Brinjal is an exceptional plant with many varieties. It is essential that the non-Bt brinjal comparator would have been the parent (conventional) variety of brinjal (EFSA 2008, Page S9), grown in the same location at the same time as the Bt brinjal to minimize differences in nutrients and solanine content. These important details were not described in the reports reviewed.

In particular, we have no knowledge of whether or not the Cry1A(c) and other protein concentrations in the dried brinjal powder used in this research was representative of actual cooked fresh brinjal at the point of consumption. Storage conditions of this brinjal powder are important, as we are led to believe that both the brinjal and Bt brinjal powder were received by INTOX in a single shipment from Mahyco and fed to rats over a period of years. There is no chain of custody report or acknowledgement of sample receipt, no verification of transgenic material presence and absence upon sample receipt and no documentation of proper labeling or safe storage procedures. It is likely that pesticide concentrations in the non-Bt brinjal and the Bt brinjal were measurable both prior to drying and before feeding to rats, yet we have no data on that either. According to EFSA guidance from 2006,

> "The role of the laboratory animal study is to deliver data from the basic, universal, presumably worst case situation for use in the hazard characterization. In practice, worst case will be decided on a case-by-case basis, but will most often be to test the GM food in its original raw form." -EFSA 2006

On page 105 of Mahyco's 2008 report there are descriptions of the cooking tests used to determine if the Cry1A(c) protein was stable in cooking. There are no data shown on concentrations of the protein before and after cooking trials, but the following statement was made: "Cry1A(c) protein was absent in cooked fruit. This study demonstrated that Cry1A(c) protein is completely degraded in Bt brinjal fruit upon cooking." The sensitivity values of the tests used to detect the protein were not specified, and in fact it was not clear which of these tests was actually used. However, this may indicate that at least some of the protein was lost upon heating. If that is the case, then how much of the protein was actually

in the dried fruit samples? Would this concentration be the same as that in Bt brinjal for cooked human food?

Minimum testing requirements have not been met

Commercial release of this product is not recommended prior to adequate safety testing. The minimum number of toxicity studies as recommended in the DBT 1998 protocol have not been conducted on Bt brinjal. While the OECD standard is superior to the DBT 1998, even meeting the latter would be an improvement.

The toxic effect of Bt brinjal to ovaries in female rats was completely missed by the toxicologists who wrote the report for the 90-day study, Mahyco reviewers who received the report, and the government committee who subsequently reviewed the report. If the statistical analysis had been conducted as indicated in the methods section of the report and the results of the analysis had been included in the summary tables and discussion as required by the regulatory guidance, this would have been impossible to miss.

> "Should there be structural alerts for reproductive/developmental effects or other indications from data available on a GM food and feed, then these tests [multi-generational reproductive toxicity studies] should be considered" – European Food Safety Authority GMO Panel Working Group on Animal Feeding Trials (2008)

Overall, this study fell short of the minimum testing requirements for the following reasons:

- Lack of dietary research to determine a relevant dose of brinjal in human diet, failure to analyse nutritional equivalence of test diets and identify the near isogenic parent line of brinjal, failure to perform quantitative analysis of Cry1A(c) protein before and after drying brinjal powder, failure to perform quantitative analysis of Cry1A(c) protein and pesticides in brinjal at the time of dosing test animals, no indication of proper receipt of test materials, identification or storage of test material by INTOX Lab.
- Failure to follow protocol guildelines relevant at the time the tests were conducted.
- Failure to analyse raw data, summarize results and provide discussion on the toxicological implications of results.
- Under Good Laboratory Practices, the study director is a qualified toxicologist who is responsible for the project from start to finish, and who must sign off on the final report. This did not happen.

CONCLUSIONS

A review of the adequacy of current toxicology studies to address the safety of genetically modified Bt brinjal for commercial release shows that the studies were not conducted according to the published standard, did not accurately summarize results, and ignored toxic endpoints for rats fed Bt brinjal.

For a brief period of time (1998 to 2001) there appeared to be an exemption given to genetically modified foods that showed no signs of toxicity: if a food tested at a dose of 1000 mg/kg-day produced no toxic effects then further testing was not required. According to OECD 1998 (page 3, item 16) and the 1998 DBT protocol quoted here,

> "If a test at one dose of at least 1000 mg/kg body weight (but expected human exposure may indicate the need for a higher dose level) using the procedures described for this study produces no observable toxic effects, then a full study using 3 dose levels may not be necessary."

Although this apparent exemption is no longer part of GM testing protocol (WHO/FAO 2000, Codex Alimentarius 2003a-c, EFSA 2008) the 90-day toxicity study appeared to be conducted at the particular dose of 1000 mg/kg-day with the expectation of finding no evidence of toxicity.

Were the contract laboratory INTOX PVT LTD and the funder Mahyco uncomfortable with results showing evident toxicity among rats fed Bt brinjal at 1000 mg/kg-day? Did the researchers write the conclusions for the 14-day and 90-day studies themselves or did others write conclusions for them? These questions are of interest since the text does not match the data, the researchers did not sign their reports, and the cover page of the 90-day report details a completely new report number (R/2183/SOR-90) from that which may be the original, 05.0002.

Not only has the scrutiny of these data provided insight into the substandard and extremely misleading interpretation of results, but it suggests to the reviewer that urgent changes need to be made to ensure that future studies are properly conducted and interpreted.

In particular, current results from these rat feeding studies indicate that rats eating Bt brinjal experienced organ and system damage: ovaries at half their normal weight, enlarged spleens with white blood cell counts at 35 to 40 percent higher than normal (elevated eosinophils in particular) indicating immune function changes possibly due to allergen response, and toxic effects to the liver as demonstrated by elevated bilirubin along with plasma acetylcholinesterase. Further studies are required to assess the potential outcomes of these indicators of toxicity.

Unanswered concerns regarding the safety assessment of Bt brinjal

Nutritional and toxicological equivalence of dried Bt brinjal samples

Are dried brinjal samples equivalent to cooked brinjal as it is prepared for human consumption, or do dried samples differ in their concentrations of Cry1A(c) and other important proteins, carbohydrate, fat and micronutrients? Would the toxicity profile of Bt brinjal also change as a result of cooking and home processing? Notably, Codex recommends:

"The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps." paragraph 47

Dietary equivalence for brinjal-fed rats, Bt brinjal-fed rats and vehicle control rats was not addressed.

Inhalation exposure to Bt brinjal

Oral ingestion of Bt brinjal does not address the issue of inhalation exposures to people who grow Bt brinjal or live near Bt brinjal crops in the ground. Toxicological responses to proteins that reach the lining of the lungs and nasal cavity, previously found to be of concern for agricultural workers, have not been addressed.

Toxicity testing standards

The main reason for conducting the toxicology studies is to have an objective assessment of whether or not the new food is safe for humans to eat. This needs to be a careful and objective assessment since millions of people with varying nutritional status, age and biological resilience will be exposed in the event of commercial release.

Neither of the 90-day toxicity testing protocols released by the Department of Biotechnology (1998 and 2008) are as methodologically strong as accepted international standards (see Appendix 1). This makes India an "easy target" for developers since the requirements to conduct toxicology studies are less stringent those in the European Union.

The use of laboratory animals to test food safety for humans is already a significant departure from species-specific testing. Deviations and omissions from accepted protocols need to be checked. Yet every departure made by INTOX on behalf of Mahyco has resulted in lower standards with less power to detect changes in rats eating Bt brinjal. These include leaving out important endpoints such as IgE

measurement to test for allergenicity, using only one dose group that is smaller than human consumption is likely to be, ignorance of toxicological equivalence, lost data, lack of Good Laboratory Practices standards, inadequate observations of animals, a 29% decrease in exposure days (doses were administered 5 days per week instead of 7), failure to quantify Cry1A(c) concentrations in dried fruit powder, etc.

The real risk here is that potential health problems attributable to Bt brinjal will be ignored as masses of people eat the very food their government thought was safe. In the long run, it is the people of India who could pay the price for bad science!

Further research studies

The logical next steps for describing the risk profile of Bt brinjal are to:

- Meet international published standards for conducting tests with scientific rigor in independent testing, following Good Laboratory Practice described in Council Directive 2004/10/EC (EC 2004) with quality assurance checks.

- Conduct dietary equivalence tests with quantitative measurement of Cry1A(c) protein before and after processing for administration to test animals according to ILSI 2004 and 2007 and Section 4 of EFSA 2006 to ensure that: nutritional requirements of animals are met equally between dose groups and that concentrations of Cry1A(c) and other proteins at the time of administration to animals are accurately represented..

- Conduct proper exposure assessment prior to laboratory studies so that future doses of Bt brinjal reflect the maximum exposure expected in the human population: 1000 mg/kg-day is not enough for an upper limit.

- Conduct the 90-day sub-chronic feeding study according to OECD guidelines, following Codex Alimentarius (2003 a-c) recommendations. That is, use at least three dose groups with doses given on all 90 days (not 5 days per week), include IgE measurements, perform daily observations on animals and include behavioral tests on individuals, include appropriate statistical analyses comparing the Bt brinjal group with appropriate controls and report results accordingly.

- Complete chronic (2-year) rodent feeding studies and multigenerational studies as suggested by the European Food Safety Authority (2008) to assess reproductive performance, neurological function and behavioral effects.

- Reduced resilience in circumstances of infection or other adverse events needs to be addressed as a potential risk factor for Bt brinjal consumers.

The Bt brinjal EC II Report recommending the commercial approval of Bt brinjal cannot be upheld. Scientifically rigorous safety assessment is needed to dislodge a trust deficit (held by the Indian public) created by the EC II Report. Furthermore, adoption of and adherence to a stronger safety testing protocol in India than the current DBT standard from 2008 is prudent.

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Appendix A: Protocol requirements for 90-day toxicity study by various sources

	DBT 1998[15]	DBT 2008[16]	OECD 1998[17]	FDA Redbook 2003[18]
Maximum number of animals per cage	Not specified	Individually or in groups of no more than 5	Individually or in small groups of the same sex	Individually
Good Laboratory Practices	15 items	Not specified	Not specified	U.S. FDA good laboratory practice (GLP) regulations, issued under Part 58. Title 21. Code of Federal Regulations
Number of dose groups	At least 3	One or more	At least 3	At least 3 but ideally 4 or 5
Nutritionally equivalent diets required for each group	No	Yes	No	Yes
Number of animals per dose group	20	20	20	40 (20 if longer-term studies are planned)
Age of animals	Six to eight weeks old	Healthy young adult animals	As soon as possible after weaning, before they are 9	No later than six to eight weeks old

[15] Department of Biotechnology (1998) Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds and Plant Parts. Ministry of Science and Technology, Government of India. Public Printing Service (Delhi) 96 pp.

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			weeks old	
Method of administration	Dry powder added to peanut oil and administered by gavage. Oil volume not to exceed 1 ml/100 g body weight	Not specified	Gavage delivery of an aqueous solution/suspension or solution/emulsion in corn oil. Oil volume not to exceed 1 ml/100 g body weight	In the diet, dissolved in drinking water or by gavage. Oil gavage not to exceed 0.4 ml/100g body weight
Control groups required	Vehicle control	Conventional non-GM plant with similar nutritional values	Vehicle control	Vehicle control: Control diet is equivalent in caloric density and contains the same levels of nutrients (e.g., fiber, micronutrients) as the diets of the test groups
Dosing regime	5 days per week	7 days per week	7 days per week	7 days per week
Observation of animals	Daily observations of tremor, convulsion, diarrhoea, lethargy, dyspnea and nasal bleeding	Clinical signs include, but are not limited to: rapid weight loss; diarrhea (if debilitating); progressive dermatitis; rough hair coat; hunched posture; lethargy or persistent recumbency; coughing; labored breathing; nasal discharge; jaundice or anemia; neurological signs; bleeding from any orifice; self-induced trauma; any condition	Clinical observations at least once per day after dosing. Twice daily observations of morbidity and mortality. Opthalmological exams at beginning and end of trial Behavioural tests: sensory reactivity to stimuli of different types (e.g., auditory, visual and proprioceptive stimuli), assessment of grip strength and motor activity assessment[19].	Daily or twice daily. Observation of general pharmacologic and toxicologic effects but also of neurologic disorders, behavioral changes, autonomic dysfunctions, and other signs of nervous system toxicity including but not limited to changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions or

[19] Out-of-the-cage behavioural tests are conducted prior to treatment start and periodically throughout the study

		interfering with eating or drinking (e.g., difficulty moving); or excessive or prolonged hyperthermia or hypothermia		other evidence of autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern).[20]

[20] Additionally, changes in gait, posture and response to handling, as well as the presence of clonic or tonic seizures, stereotypes (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilating, walking backwards) should be recorded. Tumor development, particularly in long-term studies, should be followed: the time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor should be recorded.

Appendix B: New statistical analyses of Bt brinjal-fed rats in 14-day and 90-day feeding trials

Table B1. Results of statistical analysis of raw data from the 14 day study

	Arithmetic mean values for females/males/total		
	Vehicle control group (G I)	**Vegetable control group (G II)**	**Bt brinjal group (G IV)**
Total white blood cells ($x10^3$/cmm) females/males/total	8.6/9.0/8.8	8.7/8.4/8.6	7.7/8.2/8.0
Aspartate aminotransferase (IU/L) females/males/total	164.2**/154.0*/159.1**	165.4*/149.8*/157.6**	251.8/244.8/248.3
Plasma acetylcholinesterase (IU/L) females/males/total	641.8/656.2/649.0**	557.7/621.5/589.6	534.0/529.3/531.7
Red blood cell acetylcholinesterase (IU/L) females/males/total	407.6/398.8/403.2	303.0/369.7/336.4	351.9/324.9/338.4
Bilirubin (mg/dl) females/males/total	1.1/0.9*/1.0*	1.1/1.1/1.1	1.1/1.2/1.2

*Statistically significant difference from G IV at $p \leq 0.05$

**Statistically significant difference from G IV at $p \leq 0.01$

Table B2. Results of statistical analysis of raw data from the 14 day study

	Test group mean values females/males/total		
	Vehicle control group (G1)	**Vegetable control group (G2)**	**Bt brinjal group (G4)**
Organ weight – ovaries (g) females only	0.11**	0.10**	0.06
Organ weight – spleen (g) females/males/total	0.86/1.34/1.10	0.81*/1.20/1.00	1.02/1.19/1.11
Organ weight – kidneys (g) females/males/total	1.42/1.34/1.38	1.49/1.20/1.34	1.48/1.19/1.34
Total white blood cells ($x10^3$/cmm) females/males/total	9.3*/11.1/10.2*	9.3*/10.3/9.8*	14.0/12.6/13.3
Aspartate aminotransferase (AST) females/males/total	134.5/189.5/162.0	152.7/166.0/159.4	151.7/156.5/154.1
Plasma acetylcholinesterase (IU/L) females/males/total	591.6/604.0**/597.8**	731.0/753.2/742.1	875.0/902.6/888.8
RBC acetylcholinesterase (IU/L) females/males/total	299.9/388.3/344.1	332.1/390.1/361.1	265.7/335.6/300.6
Total acetylcholinesterase (IU/L) females/males/total	891.4/992.4/941.9*	1063.1/1143.3/1103.2	1140.7/1238.2/1189.4
Bilirubin (mg/dl) females/males/total	.58**/.51/.54*	.60**/.52/.56**	.81/.52/.66

*Statistically significant difference from rats fed Bt brinjal at $p \leq 0.05$

**Statistically significant difference from rats fed Bt brinjal at $p \leq 0.01$

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Education

2001 PhD in Epidemiology, University of Otago, Wellington, New Zealand

1998 European Educational Programme in Epidemiology, Florence, Italy

MSc, 1991 Environmental Technology, University of Washington, USA

BS, 1985 Human Nutrition and Foods, University of Vermont, USA

Work Experience

2008 - present **: Independent Contracter with the following projects:**

Applied Occupational epidemiology workshop coordinator and lecturer for clinicians studying to gain Occupational Medicine Qualification with the Australasian College of Physicians.

Food safety risk assessment in GM foods, Bt brinjal in India. Funded by GEKKO Foundation and Testbiotech, Germany.

Post-mortem toxicology of antidepressant suicides in New Zealand, collaborative project with the Institute for Environmental Science and Research, Ltd and Otago University.

Project Manager, **Bioremediation of TCDD-contaminated sediment in Whakatane,** Environment Bay of Plenty Regional Council and Te Rununga o Ngati Awa. Phytoremedation pilot project on 35 tonnes of contaminated canal sediment.

Senior Research Advisor, Policy Research and Evaluation Group, Sport and Recreation, Wellington, New Zealand (6 month contract)**.**

Literature review of historical chemical exposure to Maori in New Zealand, Te Atawhai O Te Ao

2008 **Principal Epidemiologist, Research Group at the Office of Australian Compensation Council, Department of Education, Employment and Workplace Relations in Canberra, Australia**

Strategic development of research agenda in consultation with other government agencies (federal and local), contract management, preparation of question-time briefs as needed, presentations to stakeholders.

2004 - 2008 **Environmental Epidemiologist, Institute for Environmental Science and Research, Crown Research Institute, Wellington New Zealand**
Environmental toxicology, forensic science, risk assessment and epidemiology research. Independent and group projects for a variety of government and private clients.

2005, 2006 **6-week Fellowship Appointments with Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Rockville Maryland.**
Work with Risk Assessment Group at CBER to evaluate increase in infectious unit risk to recipients of blood transfusions under current and proposed donor policies.

2001 - 2004 **Senior Research Fellow, Health Services Research Center, Victoria University, Wellington**
Study design and data analysis of multi-center, multi-discipline health services/epidemiological studies. Grant writing, publishing and public presentation of results. Advised graduate students.

1997 - 2001 **Research Fellow, University of Otago Medical School (Wellington)**
Designed studies, obtained external funding, conducted and published epidemiological research relating to occupational and environmental health.

1995 -1996 Environmental Risk Assessment Instructor (CH2MHill International) Kiev, Ukraine

1993 -1995 Risk Assessor (CH2MHill) Corvallis, Oregon, USA

1992 -1993 Risk Assessor (SAIC) Seattle, Washington, USA

1989- 1991 Research Assistant (University of Washington) Seattle, USA

1987- 1989 Environmental Fate Chemist (Springborn Life Sciences) Wareham, Massachusettes

1985- 1986 Dietetic Technician (Concord Hospital) Concord, New Hampshire

Graduate student supervision

2008 to present: PhD Supervisor for Su Mon Kyaw-Myint, National Centre for Epidemiology and Public Health, Australian National University, Canberra, ACT. Identification of Benchmark Doses for Selected Psychosocial Hazards in Relation to Mental Health Symptoms.

2002 – 2003: Master's Thesis statistics advisor for Michelle Ryder-Lewis, Victoria University of Wellington, 2002/2003. Reliability Study of the Sedation-Agitation Scale in an Intensive Care Unit, MA Nursing, completed in 2004.

Peer-reviewed Publications

Submitted/in press:

Heinemann J, Sherman DG, **Gallagher L**, Carman J, Prasad S (2011) Bt Brinjal: A case study in the scope and adequacy of the GEAC to protect India's farming & food security. Delhi, India. 108 pp.

Gallagher L (2010) Bt brinjal Toxicology Assessment: Review of 90-day Subchronic Oral Toxicity in Rats. *Submitted to* Standing Committee on GM Foods, Parliament of India. November 2010. 28 pp.

Published:

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

Fowles J, Noonan M, **Gallagher L**, Read D, Stevenson C, Baker V and Phillips D (2009) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Plasma Concentrations in Residents of Paritutu, New Zealand. Part I: Evidence of Historical Exposure. *Chemosphere* 75(9);1259-1265.

Anderson SA, Yang H, **Gallagher LM** et al. (2009) Quantitative Estimate of the Risks and Benefits of Possible Alternative Blood Donor Deferral Strategies for Men Who Have Had Sex with Men. *Transfusion* 49(6); 1102-1114.

Calder L, Rivers J, Hayhurst M, Brown J, Forde A, **Gallagher L** and O'Connor P. (2008) A school and community outbreak of tuberculosis in Palmerston North, New Zealand. *N Z Med J* Jul 25;121 (1278):50-61.

Gallagher L, Kliem C, Beautrais A and Stallones L. (2008) Chemical Poisoning and Other Means of Suicide by Occupation in New Zealand. *Int J Occup Environ Health.* 14(1):45-50.

Fielden J and **Gallagher L.** (2008) Building social capital in first-time parents through a group-parenting program: A questionnaire survey. *Int J Nurs Stud* 45(3):406-17. Epub 2006 Nov 9.

Gallagher L. (2007) Statistical and Toxicological Evaluation of Two Analyses on 90-day Rat Feeding Study for MON863 Transgenic Corn. Environmental Science and Research, Ltd. Wellington, New Zealand. April 2007**. http://www.nzfsa.govt.nz/consumers/gm-ge/r-gm-brief.htm**

Gallagher L, Bates M, Crane J and Fitzharris P. (2007) Occupational Respiratory Health of New Zealand Horse Trainers. *International Archives of Occup Environ Health*, 80:335-341.

Adlam B, Perera S, Lake R, **Gallagher L,** Bhattacharya A. (2007) Acute Gastrointestinal Illness (AGI) Study: Community Survey Prepared for the New Zealand Food Safety Authority by the Institute for Environmental Science and Research, Ltd. Client Report FW0711. 81 pp. http://www.nzfsa.govt.nz/science/research-projects/gastrointestinal-report/Community_Survey_Report.pdf

Gallagher L (2006). Abbreviated Risk Assessment of Human Health Impact from Whakatane Old Sawmill Site. 3 March 2006. *Prepared for Environment Bay of Plenty Regional Council on contract with Environmental Science and Research, Limited. Externally peer reviewed by Dr. Bruce Graham of Massey University.* Available at New Zealand National Library.

Panelli R, **Gallagher L**, Kearns R. (2006). Access to rural health services: Research as community action and policy critique. *Soc Sci Med* 62: 1103-1114.

Gallagher LM, Pirie R and Hales S (October 2005). Descriptive study of hospital discharges for respiratory diseases in spray zone for painted apple moth (Auckland), relative to local and national statistics 1999-2004. Report to New Zealand Ministry of Health. http://www.moh.govt.nz/moh.nsf/by+unid/EDC2D77F43DB9C33CC2570B30003B4E8?Open

Gallagher LM and Lea R (2005). The epidemiology of Multiple Sclerosis in New Zealand. *NZ Med J*, 118(1212) April 1.

Fowles, J, **Gallagher L,** Baker P, Phillips D, et al. (2005) A Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Exposures in Paritutu, New Zealand. A Report to the New Zealand Ministry of Health, Wellington New Zealand. February 2005. http://www.moh.govt.nz/moh.nsf/238fd5fb4fd051844c256669006aed57/51321c1eb1ca76fbcc256d00001233fd?OpenDocument#dioxinreport

Fielden JM, Cumming JM, Horne JG, Devane PA, Slack A and **Gallagher LM** (2005). Waiting for hip arthroplasty: Economic costs and health outcomes. *J Arthroplasty* 20(8): 990-997.

Panelli R, and **Gallagher LM**. (2003) "It's your whole way of life really": Negotiating work, health and gender. *Health and Place* **9**(2); 95-105.

Conference Publications

Gallagher L, Kliem C and Stallones L. Chemical poisoning as suicide modality in New Zealand. ACT Public Health Forum, Public Health Association of Australia. Canberra, ACT. October 2008.

Gallagher L, Adlam B, Lake R, Dyet K and Donelly T. Prioritization of chemicals for forensic science identification: Designing a model to predict laboratory capability needs. *Society for Risk Analysis Annual Meeting 2006.* Baltimore, MD USA.

Fowles J, Stevenson C, Noonan M, **Gallagher L**, Baker V, Read D, Buckland S, and Phillips D. Evidence of TCDD exposure 17 years after cessation of production of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in a New Zealand community. International Association for Environmental Epidemiology Conference, August 2006.

Gallagher L, Panting A, Theis J-C, Williams H, Gandar P. Clinical priority assessment criteria (CPAC) for orthopaedic surgery in New Zealand - what good is it doing us? Presentation to Health Services Research and Policy Conference in Melbourne, Australia, November 2003.

Gallagher L. Designing an MS Database in New Zealand. Presentation to the International Consortium of Databases in Multiple Sclerosis, San Diego, June 2003.

Gallagher L, Crane J, Fitzharris P, Bates M. Occupational risk factors for respiratory symptoms in New Zealand Horse Trainers. (2002) 16th EPICOH Congress on Epidemiology in Occupational Health. La Medicina del Lavoro 93(5):455.

Gallagher, Panelli R, Crane J, Bates M. The role of gender in the respiratory health of two New Zealand farming occupation. (2002) 16th EPICOH Congress on Epidemiology in Occupational Health. La Medicina del Lavoro 93(5):469.

Gallagher L, Panelli R, Crane J, Bates M. The role of gender in the respiratory health of two New Zealand farming occupational groups. Public Health Association of New Zealand National Conference, Dunedin, June 2002.

Panelli R, **Gallagher** L. It's your whole way of life really: Negotiating work, health and gender. Public Health Association of New Zealand National Conference, Dunedin, June 2002.

Successful grant applications

Wellington Medical Research Fund (1998)

New Zealand Lottery Health Board (1999)

The Royal Society ISAT Linkages Fund (1999)

Association for Environmental Epidemiology (2000)

Multiple Sclerosis Society of New Zealand (2002)

New Zealand Ministry for Research, Science and Technology (2003)

Victoria University of Wellington Research Fund (2003)

Health Research Council of New Zealand (2009)

Review Activities

- Reviewer of applications for health research funding with the Health Research Council of New Zealand since 2002
- Reviewer of Scientific Reports for Science Quality and Research Priorities Team, Department for Environment, Food and Rural Affairs. London since 2007.
- Journal submissions as requested

Membership in Professional Societies

Australasian Epidemiological Association since 1996

Member, Wellington Regional Ethics Committee, New Zealand Ministry of Health, April 2002 to November 2004

Society of Risk Assessment, United States

Intergovernmental Consultation in New Zealand

Chemical and microbial threat prioritisation by government agencies in New Zealand: Project to develop capability in forensic testing of chemical and biological threats to New Zealand's health and safety, economic interests and public risk perception. Project involved a group of 5 scientists and nine government agencies to develop methods for ranking hazards using old and new risk assessment frameworks: Cynefin modelling, stochastic methods and elicitation of expert opinion (2006 – 2007).

Sudden Infant Mortality proposed research coordinated with Coroners (Ministry of Justice) (2004), Police, Ministry of Health, and University researchers from three Universities: Auckland, Otago and Massey