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EFFECTS ON HEALTH AND ENVIRONMENT

OF TRANSGENIC (OR GM) BT BRINJAL

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CRIIGEN

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SUMMARY

The dossiers submitted by Mahyco in support of their application for commercialisation of genetically modified (GM) Bt brinjal raise serious concerns. Most of these are not signed by researchers that have performed the tests on pages where they should be (signature frames empty), and could be considered as non valid. Bt brinjal has been modified to produce an unknown chimeric insecticide toxin containing Cry1Ab and Cry1Ac modified sequences. This chimeric toxin has not been used in several toxicity tests commented below, but instead an improper Cry1Ac toxin was replacing it because this control was easier. This could also make these tests not valid. Moreover, Bt brinjal produces into the vegetable cells a protein inducing resistance towards at least kanamycin, a well known antibiotic. This is typical of the first generation of GMOs which have been made without consideration of the problem. Antibiotic resistance is recognized to be a major health problem because of the growing development in the environment and bodies of antibiotic resistance genes. It is very inappropriate to consider commercialising a food containing an antibiotic resistance gene since several modern biotechnology companies have already developed transgenic plants without this kind of marker genes. It is possible that Mahyco has bought an old unused GMO technology to Monsanto Company. Bt brinjal has not been properly tested at a safety or an environmental point of view. However in feeding trials, numerous significant differences were noted compared to the best corresponding non-Bt controls: Bt brinjal appears to contain 15% less kcal/100 g, have a different alkaloïd content, and 16-17 mg/kg Bt insecticide toxin poorly characterized for side effects, and produced by the plant genetically modified for this. Parameters affected in animals fed with this GMO are in blood cells or chemistry, but in different manners according to the period of measurement during the study or the sex: in goats prothrombin time is modified, and biochemical parameters such as total bilirubin and alkaline phosphatase are also changed, as well as feed consumption and weight gain. For rabbits less consumption was noted and also prothrombin time modification, higher bilirubin in some instances, albumin, lactose dehydrogenase and the hepatic markers alanine and aspartate aminotransferases. Sodium levels were also modified, as well as glucose, platelet count, mean corpuscular haemoglobin concentration and haematocrit value. In cows milk production and composition were 10-14% changed. There was more milk and more roughage dry matter intake like if the animals were treated by a hormone. Rats GM-fed had diarrhoea, higher water consumption, liver weight decrease as well as relative liver to body weight ratio decrease. Feed intake was modified in broiler chickens as well as glucose in some instances. Average feed conversion and efficiency ratios are changed in GM-fed fishes. All that makes a very coherent picture of Bt brinjal that is potentially unsafe for human consumption. It will be also potentially unsafe to eat animals with these problems, having eaten GMOs. These differences are most often not reported in the summaries of the different experiments but are in the raw data. These differences were, when discussed, disregarded, often on the grounds that they were within the range of a wide "reference" group (really larger than the real closest control group). This reference group represents a wide range of brinjal types and is not a strict comparison. Other reasons for disregarding the differences were that they did not show linear dose response or time response, or that they were only present in either males or females, but not both. Such declarations that the differences seen are not of biological relevance are not substantiated by the data presented from the feeding trials. Clear significant differences were seen that raise food safety concerns and warrant further investigation. The GM Bt brinjal cannot be considered as safe as its non GM counterpart. Indeed, it should be considered as unsuitable for human and animal consumption. In addition, the longest

toxicity tests which are for only 90 days do not assess long-term effects like the development of tumours or cancers.

It is almost impossible through measurements of toxicity to a few species of non-target organisms to get a sufficient view of possible harm to complicated ecosystems, which, moreover vary substantially from place to place in India. The experiments on the potential toxicity of GM Bt brinjal to non target organisms (such as butterflies and moths), to beneficial insects and to long-term soil health are woefully inadequate and give no assurances for the environmental safety of growing GM Bt brinjal. Indeed, in many cases the experiments were considered irrelevant (e.g. do not take indirect effects, such as effects up the food chain into account). The gene flow studies assess but not extensively and not in an adequate manner the possibility of GM contaminations, in particular to neighbouring brinjal crops. This neglects other routes of contamination (e.g. by mixing seeds).

Based on these tests, Bt brinjal cannot be considered as safe. It is known anyway that natural Bt toxins have never been authorized as such for mammalian consumption. Artificial ones should not be either, before a more serious assessment. Significant effects in comparison to controls are also noticed with other GMOs tolerant to Roundup, and in total with at least four GMOs for which these kinds of tests have been done. These resemble classical side effects of pesticides in toxicology; and these have also been observed for MON810 maize producing a related insecticide which is present in part in the Bt brinjal, Cry1Ab.

Brinjal is known to have existed in India for 4000 years. Given that India is also a functional Centre of Origin of brinjal, any release of Bt Brinjal into the environment, poses a significant risk of contamination to sexually compatible wild species and consequent harm to the environment in addition to the contamination of Non-GM varieties. The commercialisation of Bt Brinjal will exacerbate that risk. The release of Bt brinjal for these reasons as well would be a problem.

The agreement for Bt brinjal release into the environment, for food, feed or cultures, may present a serious risk for human and animal health and the release should be forbidden.

DOCUMENTS USED FOR THIS REPORT

For this report, we have compared and compiled four kinds of documents:

1) Background documents in the public domain for general and specific considerations (like EFSA or AFSSA reports).
2) Scientific peer-reviewed literature from various international journals. This literature is cited on *www.ncbi.nlm.nih.gov/pubmed* and mostly in « Ces OGM qui changent le monde ».
3) Files from Mahyco made available publicly at the website *http://www.envfor.nic.in/divisions/csurv/geac/bt_brinjal.html* (November 2008). According to European law (Dir CEE 2001/18, Art 25, these should be public as admitted by a judgement from the Appeal Court in Germany, 2005. Some of the experimental reports are not signed by the researchers. There are however being included in this critique.
4) Reports obtained via CRIIGEN (*www.criigen.org*) non covered by confidential agreements and communicated as such by the French government after request; they are considered as public data. The government has given that to CRIIGEN after order from CADA (Commission of Access to Administrative Documents). These documents are written by Monsanto and different State Members asking relevant questions about toxicity of various commercialized GM and particularly Bt plants, and part of it were made public initially in April 2004 (Le Monde, 23/4/04, p. 10). The paper has been translated in English by various organizations.

INTRODUCTION

Background information. All GMOs commercially available and cultivated in the world are known. In the decade or so since their commercialisation, soy and maize, account for 80% of GM crops, with cotton and rape accounting for the rest.. Bt brinjal is a very important vegetable and fruit in India and widely consumed.

The genetic modification of a major vegetable/fruit crop like Bt brinjal, which is of national importance in India, if commercialized, will constitute a major advancement for GM crop expansion strategy, with the authorization of the National Regulator, the GEAC. It is a vegetable that is widely and frequently consumed by all income groups in India. There are many varieties of brinjals in India, including wild species, and therefore there are important implications about Bt brinjal being introduced in India, a functional Centre of origin of brinjal. This will be the first time anywhere in the world that such a major item of food will be genetically modified with the Bt gene, and that in a Centre of origin for a crop.

All of GMOs worldwide have been reported to present only two kinds of characters:

1) either they *tolerate a pesticide (in this case, the pesticide is an herbicide)*, that means that they can absorb it without dying, while the herbicide (Roundup) in most cases – is largely used on GM fields to kill all undesirable weeds (this is the case for maize NK603 or Roundup Ready Soya); or:
2) they *produce a pesticide (in this case, the pesticide is an insecticide)* like in maize MON 863 or MON 810 – this insecticide is artificially derived from bacteria called Bt. Bt Brinjal is included in this second category. This kind of GM crop may be called insect-resistant but this is an inappropriate term, because first they are still sensitive to several kinds of insects, and secondly because the desired "resistance" to some particular insects is in fact only an objective of the company, not always reached. This is because the GMO only could become resistant, or because the GM character is not functioning well or enough, like for the first GM maize authorized and then removed in Europe called Bt 176. In this case, the secondary

effects are even worse because they are insidious. The reality is that in this case the plant is GM designed only to make in its cells a new kind of non natural insecticide (always modified from natural Bt toxins found in soil bacteria), thus it is an "insecticide producing plant" that could be also called a "pesticide plant" (pesticides include herbicides, insecticides, fungicides...).

From 1998, a *new second generation* of GM plants has mixed both characters: herbicide tolerance and insecticide production, or they produce two different insecticides and tolerate one or two herbicides; they all are again pesticide plants.

The use, absorption in the food chain, or production in the food chain of new pesticides residues is thus clearly linked to all commercialized GM plants, constituting the first easily identified risk, the second one being linked to the no predictable metabolic actions of the genetic modification itself. The latter risk is called "insertional mutagenesis" and/or "metabolic unintended effects" by all scientists, due either to the insertion of the artificial man-constructed DNA sequence (by chance and with poor characterization in a mostly unknown genome, of Brinjal in this case), or to a new metabolic unpredicted effect induced by the technique (the new protein synthesis interferes with a metabolic plant pathway creating undesirable effects).

The goals of all environmental and health studies with GMOs are to evaluate the potential consequences of both sort of risks, and thus it is of particular interest to analyse how this is accomplished in commercial files, and this is what we are going to do for Bt brinjal in this document.

Different strains of Bt bacteria may produce up to 100 or more different natural toxins that are mostly crystal natural insecticides, several of those have been genetically modified and associated in their compositions to become stronger or more stable, and to be produced in a soluble active form in different GM maizes or cottons, or in this brinjal. This artificial insecticide production by the Bt brinjal reaches generally around 16-17 mg/kg; these different insecticides are produced in about 20% of GMOs. The second generation of GMOs (8% of total) developed from 1998 make then both: producing and tolerating a pesticide. Then virtually all GMOs commercialized in agriculture have been designed to contain pesticides that they absorb and / or produce (all the remaining characters are less than 1%). The third and fourth generations are anticipated from the actual experiments in fields to produce two insecticides and to tolerate one or two herbicides. **The toxicity of GMOs is thus known to be associated, or at least partly due to, to the toxicity of the pesticides that they are designed to absorb and/or produce.**

Description of the GMO: Bt brinjal. Bt brinjal is claimed by the company to be phenotypically (externally) equivalent to its non transgenic counterpart, in terms of agronomic traits (phenotypical) as well as for its gross chemical composition, and also it is claimed to be equivalent for two specific diseases, little leaf and the wilt. In most of the cases in GM plants, the genetic modification has inserted an artificial genetic construction, called the transgene, by particle bombardment by chance in the genome from immature cells. Here it is with a modified vector from bacteria called *Agrobacteria tumefaciens*. These cells have then regenerated new transformed plants, becoming the parents of a long lineage of plants so called GMOs. It is generally known in molecular biology that this may have created insertional mutagenesis effects that are not visible by the compositional analysis; this kind of analysis by « substantial equivalence » can by definition only be the very beginning of chemical assessment since all the toxic residues are not known and thus they cannot be chemically measured. From a reductionist point of view, the hypothesis taken is that an artificial genetic modification does not create more risk than unknown genetic effects possibly visible after

classical hybridization. This hypothesis has not been demonstrated yet, but has been used to avoid labelling and long-term feeding studies with GMOs in America.

The **conventional brinjal** is known to be cultivated since at least 4,000 years ago in India and of agronomic importance and consumed in various different ways. It is a Solanaceae. This family has more than 75 genera and 2000 species; this makes lots of possibilities for contaminations since it is almost impossible to avoid sexual compatible plants in this case. Leaves are sometimes eaten. It is both self and cross-pollinated.

The Bt brinjal has been genetically modified:

1) to produce a variant of an artificial insecticide *modified from* a chimeric (fused) Bt gene, Cry1Ab-Cry1Ac that has been inserted into the plant. This gene is switched "on" throughout and in all organs. An adapted cauliflower mosaic virus 35S promoter is used in the genetic construction to get a high level of toxin produced by all parts of the brinjal. This toxin Cry1Ab-Cry1Ac is modified from natural Bt proteins since it is chimeric and has an identified amino acid change (position 766, Leu to Ser) and several other changes from natural Cry1Ac. It is directed against lepidopteran insects like the shoot and fruit borer *Leucinodes orbonalis* (Guen.).

   The molecular mechanism of action of the toxin is not precisely known, nor is identified the receptor binding the toxin within the insect gut. The specificity of action is generally hypothesized; but no proof has been published on the action of this toxin on human cells and a controversy exists at this level. The company is not able to produce toxicity tests with the toxin extracted from the brinjal (or any other Bt plant) and put into contact with human digestive epithelia and uses a surrogate protein instead. It appears that following theoretical considerations, and preliminary data from acute toxicity experiments during a few days in a very little number of rodents, the toxin has been exempted from serious toxicity analysis. **In this context, it is scientifically possible that the toxin present in GMOs may have harmful effects on humans or animals.**

   The results of the 90 day study with rats eating this kind of GM plants is of the highest importance, because it contains data that may indicate whether there are subchronic adverse health activity in mammals, or other unexpected effects of the genetic modification. However, it would not give any indication of long-term effects like chronic toxicity.

2) To facilitate GM production, the company has used and maintained within the GM plants two unnecessary antibiotic marker genes, called NPTII (neomycin phosphotransferase II) and aad (coding resistance to streptomycin or spectinomycin) in Bt brinjal. Thus the Bt brinjal produces into the vegetable cells a protein inducing resistance towards at least kanamycin, a well known antibiotic. This is also typical of first generation of GMOs which have been made without consideration of the problem of increasing resistance of bacteria to antibiotics. Antibiotic resistance is recognized to be a major health problem in numerous countries, developed because of the growing development in the environment and bodies of antibiotic resistance genes. This is a phenomenon amplified by the common use of antibiotics according to the scientific community, which agrees in Europe to limit their use nowadays. In this context, **it is very strange to consider commercialising a food containing an antibiotic resistance**, since several modern biotechnology companies have already developed transgenic plants without this kind of marker genes. The use of antibiotic marker resistance genes should be now widely avoided in Europe and the United States and it is possible that Mahyco has bought an old

unused GMO to Monsanto Company. All the risks above considered are true even if the company says that « the antibiotic resistance has little chance to spread out from this agriculture, and that this will have if any very little effect on human and animal health ». This belief is not supported by well-designed experiments to prove it. **This could shed a very big trouble in citizens' mind on the real goals of this company on health protection**.

The chemical composition of the Bt brinjal has been compared to its non Bt counterpart. It is not stated if the controls have been insecticide-treated to be cultivated, this can change results. Bt brinjals contains on average 15% less kcal for 100 g than the control (4 replications). A sort of chemical equivalence on a limited number of parameters was found. Alkaloïdal content was measured: Bt fruit powder and roots contain less solamargine, solasonine is more elevated in Bt fruits and roots than in non Bt. These techniques are very precise but the data do not allow to calculate the statistical significance of these differences (up to 237% for instance).

Roasted, oiled fried, deep fried or steamed fruits of Bt brinjal are supposed not to contain Cry1Ac although the specificity and sensitivity of the assay is not shown in the file. Thus this cannot be accepted as proof that the Bt toxin is not present in cooked Bt brinjal. However, it is expected that cooking degrades at least in part the Bt toxin. The resulting products are unknown and the toxicity of degraded products such as insecticide metabolites is not discussed by Mahyco.

GM BRINJAL CONSUMPTION BY MAMMALS: LIMITATIONS AND SIDE EFFECTS

Introduction. A theoretical computer comparison of the new Bt toxin produced by GM brinjal is performed as usually, by Mahyco, with a data bank containing a limited number of other known toxins, but this is insufficient. Also, it is only an unidimensional homology comparison with known toxins. There is also the study of Cry1Ac by digestion in digestive fluids, with the limitations of such studies. All these are inappropriate methods as such to demonstrate the lack of toxicity, since they are largely insufficient, and the natural digestion is never fully complete. It is known anyway that natural Bt toxins have never been authorized as such for mammalian consumption. Artificial ones should not be either before a more serious assessment.

Chronic Bt brinjal feeding studies with blood analyses of mammals do not exist. The consequences of Bt brinjal consumption by animals or people cannot be anticipated. Bt brinjals were given maximally three months (90 days) to adult mammals: rats, rabbits, and goats. This is a very short time-scale upon which to estimate effects on farm animals and humans (babies, elderly people, disabled or healthy adults...) and thus the so-called food and feed "safety of Bt brinjal" is non-scientifically based. Fishes (common carp, 45 days) and chicken (42 days) studies were not any longer. With lactating cross-bred cows there were only nutritional studies (42 days). They were also acute toxicity tests (5 g/kg) with rats (14 days), they were claimed negative in the abstract but in the detailed results it is obvious that female treated rats (GM eating group) consumed more (32 %) than the corresponding controls, and the hepatic marker AST was disturbed in male and female rats after 14 days only. Some tests were also performed for mice (of 7-8 days) and skin or mucous membrane irritation tests (72 h) with rabbits. Allergenicity tests (62 day-long) in young adult Brown Norway rats were performed. All these are not appropriate to assess allergenicity in humans.

Goats. There were tests with six approximately one year-old goats per sex eating Bt brinjal enriched diet during 90 days. Five hundred grams of brinjal were offered during 2 h per day before

other feed. There was significantly lower hay consumption in Bt group in week 11 in comparison to non Bt group. The authors do not conclude anything problematic from this. In the main file concerning goats (p. 323) it was stated that the feeding trial consisted of 6 males and 3 females later in the dossier! Is this serious? Where have the three additional females disappeared to? The prothrombin time as well as total bilirubin was significantly higher in the GM-fed males at termination, and alkaline phosphatase was significantly lower. Growth curve in Bt fed-males are below the others from week 7. They gain a lot less weight. The feed consumption is lot less, even 25% less (week 5) only for this transgenic fed group. This is important although not clearly reported in the summary and obviously significant after curves observation. This appears to be a sex-dependent effect like for endocrine diseases. Bt brinjal as an animal feed, or human food that it will be mostly, cannot be considered as safe with such results.

Rabbits. Young adult white rabbits (6 males and 6 females 4 months old) received GM Bt and non-GM brinjal during 90 days, and another control was added. The rabbits were offered fresh brinjal pieces during the first six hours of each day and then regular food in addition. The Bt insecticide was measured in the blood with the same problems as described below (see the cow feeding trial).

There was a reduction of consumption at week 6 in the male Bt group in comparison to non Bt, the GM fed males consumed less in general, in the female group at week 11 (due to one animal, but the groups are too limited in numbers of animals unfortunately to calculate a real statistical significance) as if the Bt brinjals were less palatable. The females consumed less Bt brinjal. There was at interim blood sampling an increase in albumin and total bilirubin in GM fed males versus adequate controls, and of total bilirubin and lactose dehydrogenase in GM fed females; at terminal blood sampling again a significant increase of total bilirubin in males and females GM fed, increases in hepatic markers alanine and aspartate aminotransferases and sodium levels in GM fed males, a decrease of glucose levels in GM fed females.

The authors of the study claim all the above differences as incidental and not treatment related, with no scientifically acceptable reasons. The platelet count was significantly reduced during the experiment as well as mean corpuscular haemoglobin concentration in the blood of Bt fed males in comparison to their controls, an increase in haematocrit value; prothrombin time was increased in females. The Bt brinjal cannot be considered as safe in these conditions, by contrast to what is claimed by the Company. If these results are examined in parallel to the significant differences in the parameters for goats, the side effects of Bt brinjal are very worrying and need further scientific investigations.

Cows. The study with lactating dairy cows consuming Bt brinjal was described. Feed intake, milk production and composition were measured and the Bt insecticide was tried to be detected in milk and blood. The experiment lasted 42 days for 8 cows and 8 controls offered 2 kg brinjal daily, either Bt or not, in addition to a regular diet. There is around 17 ppm of Bt toxin in dry matter of Bt brinjal. The treated cows ate around 2.9 mg toxin / day. The Bt toxin was claimed not detected in blood, but there was only a short description of the method of detection and its limits and efficiency as well as repeatability were not indicated. Thus once again, this assumption cannot be considered as a proof.

Cows eating GM brinjal produced significantly (14.3%) more milk, almost as if they were treated by a light hormone, in 42 days only. The ash content of the milk varied significantly for transgenic brinjal-fed cows between the second and fourth week, by the end of the experiment they had significantly more roughage dry matter intake (10.5%). It cannot be concluded from this

experiment that there are no metabolic changes after Bt brinjal consumption in lactating cows and thus this feed cannot be considered as safe.

Rats. Mahyco (or the laboratory that has performed the test sponsored by Mahyco) is among a few companies that has given GMOs by forced ingestion to mammals and that has studied carefully the effects on health in toxicity tests that means on a wide number of markers of various organ functions. Bt brinjal was given at 1 g/kg/day, 5 days per week, in powder in peanut oil (100 mg/ml in comparison to natural brinjal or oil alone). However, the experiment lasted only 90 days maximum with one dose and 10 Sprague Dawley rats per sex and group so is a very limited study upon which to base conclusions regarding food and feed safety. Thus any sign of toxicity should be taken into account within 90 days, since hundred millions of people or animals could be nourished with this GM vegetable during their entire life, which will be surely the case in India.

For Bt brinjal, the company claims that 1g/kg (corresponding to 50-100 g for a human / day, 5 days per week) is a safe dose level. But this is a fully artificial affirmation. Who can decide when to stop eating this brinjal, and if there is no mistake in this artificial calculation.

A first experiment of 14 days with rats allowed to the company to test two doses of Bt brinjal, 0.5 and 1 g/kg, the highest one was selected for the following longer 90 day study. Instead of 2 doses of Bt brinjal in the 90 day study, two series of 10 rats were fed with normal brinjal (one was claimed to be commercial). This was badly designed, from a scientific point of view, increasing control animals by 2 in regard to treated rats. This was unexplained. Circling disorder and diarrhoea (3) were noticed only in the Bt brinjal group, males and females. Moreover liver weight as well as relative liver to body weight ratio decreased in the dose range study in females, by 13% apparently significantly. Bt brinjal cannot be considered safe for rats considering these results.

The longest experiments, and the most detailed, lasted 90 days on rats. For the rats fed Bt brinjal water consumption was 8-21% more than the non Bt brinjal group for some periods. The significance of this claimed to be null. However, all the scientific committees consulted agree with companies that statistical significant differences have been reported during 90 day studies between control and treated rats with different GMOs on numerous parameters, including blood composition and detoxification organs such as kidneys. These statistical significances have been however neglected quite often for reasons discussed below.

OTHER TESTS ON MAMMALS

Primary skin irritation tests are also performed on rabbits. Three rabbits only were treated with Bt brinjal on a total of 12; this is not serious at all. Patches of 0.5 g were put for 4 hours on the dorsal skin with tape and the reactions were measured. Nothing was seen.

Mucous membrane irritation tests in female rabbits are also described. Three animals on nine are treated with the GMO (0.1 g) introduced in the vagina and the irritation was measured up to 72 h, nothing was visible for the authors. Allergenicity was also measured in brown rats. Eight females (6-7 weeks old) were intradermally injected with protein extracts from Bt brinjal (0.45 mg/ml, final solutions in saline 0.3-20 microgr/50 microliter) in comparison to controls. Nothing was seen, but this kind of very limited test does not mean a lot of things. However, everything that was performed was described in order to be scientifically correct.

GM BRINJAL CONSUMPTION BY BIRDS

There was also a study on birds limited to 42 days in broiler chickens. 40 unsexed chickens received 5% Bt brinjal in their diet, 40 others, 10%, and 200 received different non GM diets. This was not a good design to detect any unintended GM effect in these conditions. In particular 10% is a too low percentage to see clearly unintended effects. There was also a very preliminary insufficient study of 7 days with 18 adult cockerels in total. However, the feed intake for GM-fed broilers (10% Bt-brinjal) was 10% lower than in the corresponding control (10% non Bt brinjal in the diet) at different weeks (21-35 days of age) and then higher, the implication of this is a differential metabolism between both groups but the experimental report did not calculate the statistical significance of this difference. The blood glucose was also significantly different in the Bt group. The authors of the experiment write that there is no global difference due to Bt brinjal consumption by chickens, but these differences lead instead to the conclusion that the Bt brinjal cannot be considered as safe according to this experiment. Moreover there is only one species of bird studied for a limited period of time.

GM BRINJAL CONSUMPTION BY FISHES

There are important insufficiencies in these tests. Growth performances of common carps were measured during 45 days of Bt brinjal consumption in comparison to controls. Analyses were performed every 15 days. There were numerous unnecessary non transgenic control groups masking the significant effects between the two closest groups, Bt and non Bt. There were fed with other kind of brinjals completely different (photographs are shown in the file). There were finally only 6 pools of 60 fishes (360) receiving Bt brinjal in the feed on a total of 24 pools, i.e. 1440 fishes, instead of having two main groups. This disproportion can mask a lot of significant effects if only a small group is compared with all the others. We will consider those first of all. Average feed conversion and efficiency ratios were significantly higher in the Bt group versus closest control, at 45% brinjal in the diet. No safety can be concluded.

LIMITED TESTS OF Bt BRINJAL ON SOME SOIL MICROFLORA

Some very limited environmental studies of Bt brinjal risks have been performed on an extremely little part of soil microflora, collembola, nematodes and earthworms. Soil fertility is driven by hundreds of species of decomposers and other insects, animals, fungi, plants and bacteria. Even if many of the actual species are unknown, still soil fertility itself should have been measured during field trials of Bt brinjals by various plant cultures assessments. It is almost impossible through a few species measurements to get a whole view of a complicated ecosystem, moreover varying a lot from place to place in India.

In addition, statistical tests that have been chosen appear to be limited, grossly inadequate as we have demonstrated in other studies (Séralini et al., Arch. Env. Contam.Tox. 52, 596-602, 2007).

There are some severe limitations to the studies performed or that can be performed: first of all the culture media used to do not allow for sure all bacteria and fungi to be measured. Secondly, not all groups of invertebrates or insects have been taken into account. Thirdly the Bt brinjal was cultivated only during 5 months before testing soil fertility, but most effects can appear after long-

term cultivations with pesticides treatments. Fourth, the new Bt insecticide present in the soil due to GM brinjal and produced by it may be partially linked to particles and be released after rain or environmental changes, this has not been assessed either. Fifth, significant differences have been observed in colembolla and earthworms populations between Bt and non Bt real control fields. Two additional controls mask the effects, by the end of the experiment (120 or 150 days), these don't have to be persistent to be of biological relevance since evolutions and reactions may exist in these complicated ecosystems that could alter in a long-term soil life and fertility. Sixth, mortality of beings is often an insufficient parameter measured, reproduction capacity or physiological parameters are more pertinent for non-acute but chronic effects.

Bt TOXICITY TESTS FOR NON TARGET INSECTS

Effects on honey bees (7 days) or larvae survival were considered non significant at 20 ppm of Bt (NOEL: chosen as the No Observable Effect Level). Ladybird beetles, or green lacewing larvae, also beneficial insects, gave similar results for the company after 30 days. Unfortunately these tests are not relevant since they have been conducted with Cry1Ac which is not the insecticide produced by the Bt brinjal at all. As anyone can see, they are also very limited in time and doses.

Field trials have been performed and the authors claim no significant impacts on non target insects. However, these field trials are an inadequate basis to assess whether there will be impacts on the agrosystem. Not only studies of long term effects are lacking, but also studies on beneficial insects (e.g. natural enemies of target pests), as well as studies of abundance of secondary pests (which would have to be sprayed with insecticides). Laboratory experiments designed to determine indirect effects (e.g. does the Bt toxin affect organisms that eat the target insect) are important in this regard.

One of the non-target groups most at risk from Bt brinjal would be lepidoptera (moths and butterflies). However, no laboratory studies have been performed to evaluate whether these are affected. If affected, this could have important repercussions to wildlife and agro-ecosystems upon which agriculture depends.

Refuges and resistance managements tested are hardly followed in any country and the past worldwide experience demonstrates that they are more theoretical than practical. They will be extremely difficult to follow by little farmers.

It is understood by ecologists that such tests are unlikely to detect many significant negative effects. For example, the harmful increase in secondary pests accompanying Bt cotton use in China and elsewhere took several years after commercialization to be manifested. Brinjal has also many insect pests (for example, sucking pests like whiteflies) that will not be controlled by this Bt toxin, and may increase over time. Thus will in turn increase chemical insecticide use compared to initial years of Bt brinjal use. This situation is difficult to predict, and would require monitoring after commercialization.

Bt TOXICITY TESTS FOR TARGET INSECTS

The toxicity of Bt toxin Cry1Ac to the larvae of a target fruit and shoot borer lepidopteran insect, *Leucinodes orbonalis* Gwen. has been evaluated by the company. The Cry1Ac was from a commercial formulation and not purified from the Bt brinjal (surrogate protein), thus modifications of the protein in amino acids, structure and post-transcriptional modifications such as potential glycosylations have not been taken into account, limiting the significance of the results. Some lyophilized transgenic fruit powder was also used in bioassays but these lasted only 7 days. There were 12-14 fold variations in the results. The Bt protein was significantly toxic in this regard; this was the goal of the GM brinjal on this insect.

POLLEN FLOW STUDIES

In order to try to study dissemination of Bt brinjal pollen, some pollen flow studies were conducted. It must be first of all very clear that cross pollination or pollen flow is a very small part of contamination possible by GM plants. Thus, such kind of study has little impact if alone on environmental risk assessment of dissemination per se.

1) First, the seeds can be contaminated during the production when bought or taken by agricultural workers,
2) the transportation and spreading of seeds for cultures is not full closed and cannot be restricted temporally to a particular designed field,
3) the cultivation can imply the sharing of workers or tools or even machines that bring contamination of pollen or seeds from one field to another,
4) the insects, birds, other animals such as rodents or mammals will bring fruits or parts of flowers or fruits from one place to another,
5) the harvest is made by tools that are shared and may mix the productions at low levels,
6) the storage is made in places that cannot be always fully dedicated to GM or non GM plants,
7) the markets or transformation factories or cookers may mix the fruits or seeds.

In the case of this file the sampling procedures are crude and limited and do not take into account the form and size of the field and the environment (wind, water): all these parameters have been demonstrated to highly influence pollen and gene flow (CGB studies, Ministry of Agriculture, France). A maximum of 50 meters from the source has been studied for dissemination, this is not significant in comparison to the well known wave's effects of pollen disseminations depending on the wind blowing and insects and this has been demonstrated for several pollens (maize, oilseed rape...). Thus the assessment was incomplete and not extensive.

However, although pollen flow rates can be low, these rates depend on a number of factors not addressed by the applicants. For example, in addition to proximity of fields, the relative size of brinjal fields can influence the rate and level of pollen contamination. Small conventional (non-GMO) brinjal fields planted near large Bt brinjal fields will have higher rates of contamination than large conventional brinjal fields in otherwise similar situations. Therefore, smaller conventional brinjal farmers may be at greater risk of higher levels of contamination than larger farmers.

Further, the applicant did not consider that levels of contamination may be additive over time if a farmer saves non-GMO brinjal seed, and if neighboring Bt brinjal farmers continue to plant Bt brinjal. If more than one brinjal crop is planted in a year, this would accelerate this trend.

The analysis of pollen flow also neglects other very important routes of contamination (e.g. by mixing seeds). Based on data from other countries on other genetically engineered crops, it seems likely that routes of contamination such as seed mixing are important. For example, in the U.S., levels (concentration) and rates (percent of the total crop) of contamination of soybean, a crop with low out-crossing rates similar to brinjal, were as high as for crops like corn that outcross at much higher rates. Since out-crossing occurs by pollen flow, these data suggest that other means of contamination are likely to be important ("A Growing Concern", Union of Concerned Scientists, 2004, http://www.ucsusa.org/assets/documents/food_and_agriculture/seedreport_fullreport.pdf). Although commodity crops like soybean grown in the U.S. are processed very differently than brinjal in India, it seems reasonably likely that small rural brinjal farmers in India may not have mechanisms in place to prevent seed mixing.

Gene flow to wild weedy relatives may result in environmental harm. This important route of possible environmental harm is widely recognized, but apparently not considered by the applicant. Gene flow from Bt brinjal in India may occur with the sexually compatible wild weedy relative *Solanum insanum*. Another sexually compatible relative, and the progenitor species of brinjal, *S. incanum*, probably also occurs in India. Gene flow from GMO crops has occurred from a large scale field trial of creeping bentgrass (*Agrostis stolonifera*) in the U.S., and from commercialized canola in Canada – in both cases involving a gene for glyphosate herbicide tolerance.

Transfer of the Cry1 gene to these wild relatives may lead to harm to Lepidoptera or other non-target organisms that feed on these wild plants, or the wild plants may become more weedy due to suppression of herbivorous insects that may help keep their growth in check. Whether these possibilities occur depends on a number of factors that have not been tested by the applicant. For example, it must be determined whether these wild species grow in areas where brinjal is cultivated, which would allow gene flow to occur. Harm from such gene flow can only be determined through appropriate tests such as determining which organisms feed on these wild species, and whether they are sensitive to the Bt toxin. It should be noted that GM crops containing a Bt gene have not been commercialized in proximity to wild relatives anywhere in the world.

Finally, gene flow to wild relatives may in some cases lead to reduce genetic diversity of the wild species. This is especially true for wild relative that grow near the crop, and occurs through the phenomenon of gene swamping when the crop is more numerous than the wild relative. It is recognized that brinjal wild relatives may provide important pest resistance genes for brinjal diseases and insects, as well as other desirable traits. The possible reduction of such diversity could have negative implications for further improvement of the brinjal crop, and should therefore be carefully considered.

GERMINATION STUDIES

Germination, aggressiveness and weediness have been measured with Bt brinjal. Similar limitations as above can be drawn from the file. Moreover, studies have been performed with 50 seeds at a time and during two weeks only; no scientific conclusion can be envisaged.

LIMITED FIELD TRIALS

These evaluations were performed and limited according to their authors themselves (in their title). Thus they cannot be conclusive as such. The necessity of such field trials is heavily questionable. The Bt plants were claimed to be efficient insecticide plants. Performance of Bt brinjal hybrids to incorporate the pesticide producing gene was also measured. Strong efficacy, no detrimental effects on beneficial insects for instance: this kind of claim cannot be made! The significant effects will not be detailed here.

The quantification of the insecticide was performed in the plants in different experiments. It is produced with a lot of variation from 1.86 ppm (root) to 45.4 ppm (flower), and 32.7 in the fruit. Only 0.059 ppm are sufficient to control the insects according to the authors of the experimental report. The insecticide produced in the flowers or the fruits even increased in other experiments to 58.7 and 51.3 ppm, respectively.

Resistance management strategies for Bt brinjal are also presented. Bt refuges are proposed that will be very hard to manage for Indian farmers.

DISCUSSION

Interpretations of above data. Most of these significant differences were judged as « not biologically meaningful » by Mahyco and it could be the case consecutively by some scientific committees. The results were considered as relevant but their interpretation may be the cause of disagreements.

The interpretations explaining that the significant differences should not be taken into account before commercial release, were:

1. The comparison with several unnecessary "reference" groups of animals that ate a different type of brinjal to that which had been genetically modified (i.e. not a sister line but different lines of brinjal). By contrast, the called "control" group in this report was fed with a plant genetically very close to the treated group with the GMO, the difference in the diet was considered in this case to be the transgene, its protein expression and its consequences alone. The total reference group was also in some cases 6 times bigger than the GMO treated group (in some instances the historical data of the laboratory conducting the experiment served also as references in some files). For some significant effects, the differential effects between males and females were interpreted as that the differences were probably not linked to the GMO. (Doull et al., Food and Chem. Tox., 2007, 45, 2073-2085). However, this interpretation could mask noticeable differences between sexes that may be important for food and feed safety of the Bt brinjal.

2. For some significant effects, their observations only during some weeks of the experiment served to eliminate those from biological significance! For instance Mahyco suggests that the differences should last during all the experiment to be interesting for them, as if the settling of chronic diseases such as cancer would no be by waves.

3. For some significant effects, the absence of linear correlation with the dose ingested by the rats was a cause to avoid linking them to the GMO.

By contrast, numerous international experts (among Joël Spiroux de Vendômois 2, Dominique Cellier 2,3, Charles Sultan 2,4, Marcello Buiatti 2,5, Lou Gallagher 6, Michael Antoniou 7, Krishna R. Dronamraju 8.

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8 Foundation for Genetic Research, Houston, USA)

after consultation consider that:

1) The statistical analysis of GMOs data may have encountered problems in the choice of methodology or unexpected bias and should be done again. This is proposed by CRIIGEN after communication of all crude data. The improper or poor statistical analysis has been admitted in some cases.

2) The differential effects should be considered primarily with the closest control and not with series of other unrelated controls if the control groups are more represented in animals in total than the treated group. The controls should not be historical data either, nor a wide range of US data. Sometimes there was unrelated commercial brinjal from several markets plus the non Bt corresponding control in the experiment, masking the true results.

3) The differential effects of a treatment by a toxic compound on males and females is observed quite often, this may be due to enzymatic and hormonal differences between the two sexes in regard to detoxification.

4) The transient effects after chemical or biological intoxications are also numerous and do not mean that the compound is safe on a long-term. Several chronic diseases settle in the organism by irregular steps, such as cancers.

5) The dose-dependent effects are not the only ones to be taken in consideration in toxicology. For instance, most of endocrine effects are not for sure directly proportional to the dose, but may present biphasic or feedback effects, and also depend on the time of administration.

Thus the interpretation of results sponsored by Mahyco is not scientifically acceptable, these show as indicated by Mahyco significant differences after Bt brinjal consumption by animals in comparison to the closest non Bt brinjal consumption. They should not be said as not biologically relevant.

Significant effects in comparison to controls are also noticed with other GMOs tolerant to Roundup, and in total with at least 4 GMOs, for which this kind of tests has been done, resembling classical side-effects of pesticides in toxicology. This has also been observed for MON 810 maize producing a related insecticide present in part in the Bt brinjal, Cry1Ab : the European food safety authority EFSA writes: « For rats fed 33% MON 810 maize, a statistically significantly lower albumin/globulin count was observed compared with control and overall reference lines at study

termination ». This is in part why a French moratorium was recently (2007) decided and a new evaluation of all GMOs is on the way in Europe.

On the other hand, public CGB discussions (the French committee of GMOs evaluation) have reported inflammation and regeneration abnormalities in male kidneys fed with MON 863, significant increase of glycaemia in treated females. Scientific committees in Austria, Italy, France, Spain, Sweden, and The Netherlands in particular have asked questions to Monsanto on toxicity and allergenicity of this maize or MON 810, or both, or MON 863 x MON 810 after the transmission of the Company data, even if the time to evaluate the documents was very short. **It has been made clear for the public and environmentalists that GMOs consumption present risks for animal and human health**, for still badly understood reasons. However, this became clearer and clearer in 2003 and overall at the beginning of 2004 when the data were made public after the actions in courts of several associations.

CONCLUSION

It can be concluded that all the experiments for Bt brinjal assessment of toxicity are sponsored and interpreted by Mahyco. In addition, the interpretations of data are controversial. There was no open access to the organs from treated rats and slides of these organs. There were no further investigations after findings of significant differences after Bt brinjal consumption, but short interpretations to assume that there were not important.

The secrecy on "confidential" raw data of toxicity for GMOs claimed by companies has no scientific basis, and **creates trouble in citizens' mind** ; all scientific data have to be published or transparent are they are in the commercial request files to the state members, like it is done for public research, if the GMO is for public feeding. The directive CEE/2001/18 indicates that the risk assessment on health and environment should be public for GMOs. Mahyco appears to have claimed confidentiality for these data in a first instance.

Whatever the results are, in such a controversial case, the minimum could be, like in public research, to repeat the experiment since no clear conclusion can be drawn from these data: in order to protect animal life, it is better to analyse more animals before giving to them this kind of feed during their entire life.

If we compare GMOs with other products tested for their safety, the closest example possible is for pesticides, since as we have explained previously the genetic modifications provoke pesticide bioaccumulation (tolerance and / or production). The European legislation concerning pesticides has been for a long time directed by the directive CEE/91/414, and its successive adaptations. This legislation states that, concerning the toxicity study of pesticides in food and feed for humans and other mammals, three month tests should be done for three species (generally rat, mouse, and rabbit), and that pesticides are given in food during one year to one species (generally dog) and during two years to another one (generally rat, this approximately corresponds to its life span). **It can be concluded that for this new Bt pesticide in Brinjal, Mahyco has not followed international rules for pesticide assessment.**

The in vivo tests are the final security that should be undertaken to test unknown products that do not present in vitro negative effects. However, specific in vitro tests should be stimulated before, and one can note that there is very large room for still improvements in GMO files, i.e. more tests with the Bt artificial toxins extracted from the GM brinjals and incubated with human cells for instance. This has not been performed yet, probably for economic reasons and to avoid testing to much seeds like pharmaceutical drugs.

The 90 day toxicology studies appear to be the longest that have been performed with mammals. The tests show significant effects in comparison to control laboratory animals, and in some instances in comparison to the so called very large "reference group", the existence of which may be questioned. In all instances, it is recommended that:

1. The statistical analysis should be repeated with independent experts and the data put on calculation sheets for the scientific community (not as images as they are).

2. The experiments should be a case of rejection of the Bt brinjal commercialization if the control groups are more than the treated groups, if the significant results are diluted by comparison with numerous improper groups. This has been done in this file.

3. The experiments should be a case of rejection of the Bt brinjal commercialization if they are too short such as 90 days, and if they are supposed to assess the whole safety of a food or feed that can be eaten during an entire life. This is the case here.

4. In vitro studies should be performed with the Cry1Ab-Cry1Ac chimeric modified insecticide extracted from brinjal, and various mammalian cells including human digestive epithelia and hepatocytes.

One should also underline that today no legal obligation is given to companies concerning the exact basic number of studies they have to accomplish on mammals eating GMOs and their length. This lack of precision (Entransfood project, EFSA) is difficult for public authorities and companies. Biotechnology will not be easily accepted in such conditions. This provokes unfortunately numerous risks and problems.

After analyses of all the present results and their insufficiencies, it is concluded that in this file there are numerous side effects described after Bt brinjal consumption. Bt brinjal cannot be considered as safe. The agreement for Bt brinjal release into the environment, for food, feed or cultures, may present a serious risk for human and animal health and the release should be forbidden.

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The author, Professor SERALINI has been for nine years (1998-2007) member of two commissions for GMOs evaluation before and after commercial release (commissions of the French Ministry of Agriculture and Ministry of Ecology), and was expert for the European authorities on the first panel in the WTO conflict with the United States, concerning the GMO moratorium (2003) and expert for the committee for European reassessment of biotechnologies (2008). He develops research on the effects of pesticides on health. He has written several books on GMOs in French, and in particular « Ces OGM qui changent le monde » (Ed. Flammarion).

This critical review of Mahyco's data on Bt brinjal is commissioned by Greenpeace