Analyses of controversial cases

The Dr. Puzstai case

Research results at the Rowett Research Institute in Scotland leaded by Dr. Arpad Puzstai were reported to show that genetically modified potatoes expressing the snowdrop (Galanthus nivalis) bulb lectin gene (GNA) were harmful to laboratory rats fed with lectin-containing potatoes. Dr. Puzstai concluded publicly that genetically engineered potatoes would be harmful to humans because engineered potatoes affected the immune systems of lab rats, and the size of the rats’ brains shrank. By measuring the thickness of the stomach and the crypt length of the intestine it was shown that proliferation in the gastric mucosa was in part caused by GNA gene product. However, the growth-promoting stimulus on the small intestine of diets containing GM potatoes was putatively not a GNA effect. There were a slight binding of GNA to the small intestinal epithelium and GNA is not a mitotic lectin and therefore did not induce hyperplastic growth in the tissues.

In the Audit report conducted by the Rowett Research Institute in August of 1998, the group of experts concluded "The data do not support any suggestion that the consumption by rats of transgenic potatoes has an effect on growth, organ development or the immune function". According to this report, all plants have lectins as one of the proteins in their seeds, where they play a defense role against insects and nematodes. Lectins are also known to be toxic to mammals, including humans, but eliminated in our diet through cooking. The report, as well as scientists involved in related research, feel strongly that Puzstai's results have yet to be repeated using much higher scientific rigor, and validated by established statistical methods. Dr. Puzstai was later sanctioned by its former institute for having behaved in a non-scientific way and having damaged the image of the institute towards the public.

For more information

Archive Information on Arpad Pusztai GMO Research


Although there is only one case of putative risk involving a transgenic potato (Dr. Puzstai case), we provide here an analysis on all cases, (only 9 after 10 years of GE-crop cultivation) independently of the crop. We have also included one non crop plant case. The reason for this is that these are used as arguments by non-specialists and, in particular, the press, to speculate on potential risks of genetically engineered organisms.

The "contaminated" mexican maize biodiversity (2001)

In the issue of November 29, 2001 of the journal Nature, two researchers at the University of California at Berkeley reported the presence of transgenic DNA constructs in native maize landraces grown in remote mountains in Oaxaca, Mexico. In addition, the authors reported that it occurred as multiple introgression events the transgenic DNA constructs seemed to have become re-assorted and introduced into different genomic backgrounds, possibly during transformation or recombination the transgenic DNA constructs are probably maintained in the population from one generation to the next.

This event of introgression of transgenes, their instability in the genome, and maintenance through generations, immediately raised the interest and concern of the entire scientific community, the environmental protection organizations, and the general press. The transgenic maize was soon tagged as threat to existing maize biodiversity and to germplasm collection maintained ex-situ at CIMMYT.

However, the results reported were soon questioned by scientists skilled in the art from the technical standpoint (biotechnologists, maize breeders) as well as by scientists involved in biodiversity conservation on the conclusions drawn from such introgression. The auth
ors recognized later major weaknesses in the technique used but failed to repeat their results using unambiguous techniques.

Today, as we write these words, neither the authors nor other scientists have published unambiguous evidence of introgression of transgenes into the native maize landraces grown in remote mountain areas in Oaxaca, Mexico.

The case of the presence of transgenic maize in the region of Oaxaca in Mexico was discussed through three presentations at the 7th International meeting on the biosafety of genetically modified organisms, October 2002, Beijing, China.

It was reported that criollo maize sampling in this region has revealed the presence of transgenes in several, not all, of the sites tested. Unequivocal molecular analyses were conducted. The official report has unfortunately not yet been released but it is now confirmed that the transgenes are present in this region. Two key questions remained, however, unanswered. What is the extent of this presence? What does the transgene-positive maize material look like? This presence was expected because farmers receive US corn (mixture of varieties including transgenic ones) as pig feed, as food aid from the government, and from emigrants. This finding demonstrates that a moratorium on transgenic maize planting in Mexico will not prevent transgene flow in center of origin of maize.

The three presentations addressed the key question, so what. One presentation focused on population genetics of these land races in this region. Main conclusions were:

1. Land races cross over long range and hence population scale is far bigger than single farm; 2) a meta-population is proposed to describe the land race population dynamic as opposed to a collection of isolated local populations; (3) 30-40 years of presence of hybrid maize in the region have not changed the genetic diversity of the overall land race population; (4) land races are constantly intercrossed and this is likely needed for their survival; (5) there is no evidence that an additional gene could alter the existing genetic diversity of this meta population; (6) Teosinte as weed needs careful monitoring to avoid enhanced invasiveness due to transgene or endogenes introgression.

The general discussion did not resulted in any particular concern for the presence of the existing transgene from corn in the land race gene pool of Oaxaca.

It is generally believed that these transgenes will not be under selection and will remain essentially without any effect on the gene pool. Such assumption needs however to be carefully verified through monitoring of the evolution of these land races. A last, the general comment was that transgenic varieties are not a threat to land races any more than hybrid corn and that if a threat exists it comes actually from modern agriculture. Transgenics are being used and abused as the symbol of modern agriculture.

**Main references**


Christou, P. (2002). No credible evidence is presented to support claims that transgenic DNA was introgressed into traditional maize landraces in Oaxaca, Mexico. Transgenic research 11:iii-v

[http://www.cimmyt.org/whatscimmyt/Transgenic/Iwanaga_051202.htm](http://www.cimmyt.org/whatscimmyt/Transgenic/Iwanaga_051202.htm)
The soybean case

In the middle of 2001, a group of scientists from The Agricultural Research Institute in Belgium published their findings of a DNA fragment inserted next to the inserted gene in Monsanto's Roundup Ready Soybeans. Such discovery immediately stigmatized negative opinions from some media and environmental groups against the genetic engineering technology. This discovery follows a detailed molecular characterization of the Roundup Ready soybean variety by Monsanto scientists published 2 years earlier. In this study, an extra copy of 72 bp of the transgene was found at a co-segregating locus and its flanking DNA sequence characterized.

At the site of integration of the fully functional copy of the transgene, DNA rearrangement had occurred. An extra piece of 254 bp of the transgene was found to be present. The Belgian group found in addition to this 254 bp fragment an extra piece of DNA with no DNA sequence homology to any published DNA sequence available in the gene bank databases.

By analyzing this locus in untransformed soybean, PCR analyzes did not reveal the presence of this extra 534 bp fragment. This finding led the authors of the publication to make a scientifically correct statement that would however be interpreted by the non-specialists as an alarming fact. They claimed the presence of unknown DNA in this variety.

Greenpeace alerted immediately the press by claiming that this variety was unsafe because alien or mystery DNA was found in this transformed plant. Scientists at Monsanto had actually found this extra DNA sequence and assumed it to be rearranged DNA for soybean genome. This is a common feature of insertion of DNA into plant genome and is not limited to genetic transformation. Therefore, it is not a mystery or alien DNA as tagged by Greenpeace but a rearranged soybean DNA with no sequence homology because it is a genomic DNA devoid of function or genes. Since only genes and very few genomic DNA of soybean have been sequenced, it was expected to find no DNA sequence match.

The Belgium team headed by Dr. Marc De Loose said after the burst of alarmist news in the international press that there were no evidence that suggest potential health risks, dismissing assertions made by the environmental group Greenpeace. "There is 0 (zero) scientific data to support this idea because we checked this sequence in different generations that were on the market and we didn't see any differences. This means that the sequence is stable and all the data concerning safety are still valid in my opinion, and he continues, "There is no evidence that the sequence causes any expression, so we did not demonstrate that the sequence is expressed... there is no indication that (soy) might cause any allergy"

A spokeswoman from The European Commission said also that there was no scientific evidence that the DNA fragment found in the Soybean made the product unsafe. She also refers that "the existence of this DNA does not mean it is a consequence of modification in the plant. It could have been generated by a spontaneous mutation, which often happen in plants"

Since the publication of the story, many environmentalist groups started to campaign against biotechnology without any scientific evidence supporting their critics. The result of such harassment was just to produce more confusion among consumers and farmers about the biotech soybean varieties. This is again a case of poor attitude from fundamentalists radically opposed to biotech crops and unfortunately a press prompt to report alarmist news provided it attacks or criticizes multinational companies.

What lessons should be learned from this new critics cases

Firstly, it is our responsibility as scientist to think about the possible misinterpretation of our scientific results and the way we express it. Whether this sentence 534 bp for which no sequence homology could be detecteds was written without thinking this could lead to alarmist interpretations by the general public or intentional to emphasize the novelty of their findings, a negative perception has developed against this technology in the absence of scientific
arguments and it reinforced the apprehension of the public towards biotechnology and scientists in general. Hence it is extremely important that scientists be more responsible of their writing considering possible misinterpretation by non-scientists of their publications.

The second lesson to be learned is the need to better characterize the transformed events and disclose all findings susceptible to question the changes that occurred as a consequence of the transformation event. In this case, the producer of the RR soybean 40-3-2 event, Monsanto, has been particularly slow to provide the molecular information. Indeed, these were disclosed a couple of years after the deregulated release of the new variety. In addition, the full characterization of these insertion events is still incomplete.

The homologous untransformed locus was not characterized either for the functional insert or for the 72 bp co-segregating locus. This should be done in all cases because it would provide information whether a DNA rearrangement had or not taken place and whether or not some functions or genes were lost during this process. Even if the demonstration of the safety of the transgenic soybean has been achieved and represents a tremendous amount of evidence that it is equivalent to conventional product, a full characterization of the transformation event would be helpful.

The StarLink™ case

StarLink™ controversy started in the middle of September 2000, when traces of the Aventis CropScience Bt-corn also known as StarLink™, that was approved by the EPA for animal and industrial applications, was identified in Taco shells manufactured by Kraft foods and distributed by the Taco-Bell chain.

StarLink™ corn was modified to contain the Cry9C protein, isolated from Bacillus thuringiensis subsp. tolworthi to resist the European corn borer (Ostrinia nubilalis). The US regulatory agency in charge of regulating pesticides, the EPA, approved in 1998 a restricted registration of StarLink™ corn to be used in animal feed, industrial non-food use and seed increase. The product wasn?t allowed for human consumption due to the slower digestibility of the Cry9c protein and to the lack of conclusive data on the potential allergenicity of the foreign protein in absence of data on human dietary exposure to Cry9C protein. EPA also stated that Aventis has the responsibility of ensuring that the product would not be sold for food or export channels and that the users would take care on planting the seed to prevent cross-pollination with other relatives. However, the product somehow slipped into the human food production chain at an estimated contamination level of 1%. A campaign orchestrated by anti-genetic engineering technology activists was then orchestrated when Tacos were found to contain traces of StarLink™ corn. Putative allergic reaction to ingestion of food from Taco-Bell was reported immediately after and given a large publicity.

On October 2000, Aventis and EPA arrived at an agreement to cancel the StarLink™ registration. This decision at the company?s request, followed the EPA report which could not provide conclusive evidence demonstrating the CRY9C protein was or was not a potential allergen. According to the agreement no corn can be planted for agricultural purposes. Aventis has begun to buy back all StarLink™ corn from farmers as well as corn used for human consumption when evidence of a mixture was demonstrated by a 10-minute immunoassay tests specific to StarLink™ proteins. This test could not be conducted with food already processed, and this made it difficult to estimate how much corn in human food channel has been mixed with StarLink™. It is actually an indirect proof of the safety of StarLink™ corn to note that apart from the original campaign, no new massive food allergy was ever reported although there has probably been a wider contamination than only the Taco at Taco-Bell.

Following the accident of September 2000, the FDA received 51 reports alleging allergenic reactions as a consequence of consumption of corn-based food supposedly containing Cry9c protein from StarLink™. According to the FDA, the reports were received soon after The Washington Post announced that traces of the Aventis CropScience Bt-corn were found in Kraft foods. On November, the FDA contacted the CDC in order to assess the
51 cases. From the 51, only 28 presented real symptoms of allergic reaction. CDC concluded in a final report published in June 2001 that: "Although the study participants may have experienced allergic reactions, based upon the results of this study alone, we cannot confirm that this reported illness was a food-associated allergic reaction".

One lesson learned from this food contamination incident is that it is probably impossible to control corn variety mixture in the food industry. Because the company is liable for any inconvenience such the StarLink™ case, it would be advisable to release corn for all three intended uses: human consumption, animal feeding, and industrial production. The second lesson is that there is a clear correlation between a campaign to alert about food contamination and reported cases of food poisoning. One wonders if such opportunistic public reaction should not be strongly criticized as it may create undesirable suspicions when real cases of food poisoning would happen.

For more information see:


The Monarch Butterfly case

A group of scientists at Cornell University alarmed the world by showing an unintended side effect of the pollen of genetically engineered corn on the monarch butterfly.

In May 1999, the toxic effects of Bt-corn pollen on monarch butterfly larvae were reported in the journal Nature. In this laboratory study, milkweed leaves were dusted with pollen collected from either Bt or non-Bt corn plants. Milkweed leaves without any pollen were also included in the experiment. Three-day-old monarch caterpillars were placed on these leaves and allowed to feed up to four days. The study reported that 44% of the monarch larvae fed on leaves coated with Bt-pollen died. None of the caterpillars that fed on leaves dusted with non-Bt corn pollen or without pollen were affected. In addition, larvae that fed on leaves dusted with any pollen ate significantly less and were smaller than the larvae that fed on leaves without pollen.

At Iowa State University, entomologists placed potted milkweed plants within a Bt cornfield and a non-Bt cornfield as well as at various distances from the fields' edges. As expected, the highest concentration of pollen was found on plants within the fields. Pollen density decreased between 70% and by 90% at a distance of only three meters from the edges of the field. Leaf samples were taken from the milkweeds within and adjacent to the fields and used to assess the mortality of newly hatched monarch butterfly larvae. Within 48 hours, 19% of the larvae eating leaves with pollen from the Bt-corn died compared with 0% on non-Bt pollentreatment, and 3% in the control, which had no pollen.
Both studies demonstrated that monarch caterpillars are killed between 20 to 44% when feeding on a Bt pollen diet under laboratory conditions, where the larvae are given no choice but eating Bt pollen coated leaves.

This undesirable effect of Bt-corn on the Monarch butterfly was perceived as a real threat considering that in the US the Corn Belt is largely a monoculture of very few maize varieties and that the use of a unique Bt-corn variety would endanger the monarch butterfly population. The central question here was whether this potential negative effect was really meaningful in the natural environment. Data and analysis made public since this burst of concern on Bt-corn revealed that the impact would be at the most marginal. Indeed, monarch butterfly is under threat from the fragility of their forest sanctuary and the use of pesticides in agricultural fields. Studies have even shown that Bt-corn, by reducing pesticide use, have already contributed to the increase of monarch butterfly and other insect populations. Several factors argue against the natural occurrence of high pollen exposure levels. Maize pollen was shown to drop in density down 75-90% at a 3m distance from the field.

A high dose (the only one capable to kill larvae) was found only within a 1m distance. Pollination occurs in a very short time period ranging between 5-10 days. Corn pollen shed was reported to be completed before monarch caterpillars were first observed feeding on milkweed. Pollen is washed out from leaves by rain and irrigation. The larvae were also shown to have preference for milkweed far from cornfield and devoid of pollen. Milkweed is a weed farmers tend to eliminate from their fields and is mainly found along roadsides, hence far from the reach of pollen from corn fields.

According to a report from the Canadian Food Inspection Agency, the lethal dose (LD$_{50}$) was not reached on the average at any distance from the field and during the peaks of pollen shed. Beyond a 5m distance, almost one grain pollen per cm$^2$ was found. Milkweed plant distribution was found predominantly in conservation areas compared with cultivated areas excluding roadsides.

Therefore, it appears today that the original alarmist announcement that Bt corn, and by extension Bt plants, could endanger non-target insect such as monarch is not backed up by facts in nature. It is, on the contrary, evident today that the negative impact on non-target insects and probably other organisms, is much lower than the impact by the conventional use of pesticides.

Finally, this story has affected the science largely in revealing to the general public the lack of responsibility of some scientists and journals when reporting experiments and publishing conclusions misleading for the general audience. The damage caused by the original team at Cornell University is significant, not only for the public image of science, but also by distracting the attention, evading funds from the real problems, which agricultural research needs to address: to develop more sustainable and environment-friendly agriculture technologies and practices.

For more information see:

- [Impact of Bt corn pollen on monarch butterfly populations: a risk assessment](https://doi.org/10.1073/pnas.98.14.11937)
- [GM corn poses little threat to monarch](https://doi.org/10.1038/nbt.1419)
- [Promiscuity in transgenic plants](https://doi.org/10.1038/nature00542)

In the news section of the journal Nature in 1998, (Nature 3. September 1998 p. 25) three scientists used two lines of the plant *Arabidopsis thaliana* in order to determine their out-crossing rates. In the experiment, one line was resistant to the herbicide chlorsulphuron due to the presence of a transgene. The other line was likewise resistant to the same herbicide but the resistance was due to chemical mutagenesis. Both lines were derived from the same original genotype. The out-crossing rates of both lines and the parent line
were compared by cross-pollination with wild-type line, which were planted in their vicinity. According to this study, the transgenic *Arabidopsis thaliana* line is 20 times more likely to out-cross than ordinary mutants. In other words, a wild-type *Arabidopsis thaliana* plant is more likely to be fertilized by transgenic pollen rather than a mutant one. The authors did not provide a scientific explanation for these results and added a word of caution by affirming that their results did not prove that enhanced out-crossing is due to the transgene itself. Nevertheless, this paper was published although incomplete.

This article caused a burst in the press at that time by highlighting the danger of gene flow in promiscuity of transgenic plants.

This publication has serious scientific weaknesses, which disqualify it as a publication demonstrating evidence of enhanced gene flow due to the transgenic process.

The experiment does not acknowledge that the transgenic plants could have been selected for fertility by the investigators. Then it becomes obvious that they introduce a bias in their experiment.

There is no way to determine if the results were due to increased out-crossing of the transgenic plants or decreased out-crossing of the mutant plant. Hence, no conclusions can be made about the effect of the transgene, until this information is available.

Also, the conclusions are based on two transgenic clones. It is common knowledge that after plant regeneration a lot of variations between the different regenerants are observed.

Finally, according to the data, fertility variation among the transgenic plants (significant at \( p<0.001 \)) is higher than between transgenic and mutant (significant at \( p<0.003 \)). Conclusions therefore are wrong by opposing transgenic and non-transgenic.

Since this newspaper was published, no peer-reviewed paper has come out by any of these authors, which could have provided answers to the above questions. Therefore, with the present knowledge on this study, we can certainly not conclude to any significant differences in pollination competence of transgenic crop plants.

The Terminator gene case

The Terminator Technologys has brought by far the biggest and strongest criticisms against genetically engineered crops. The technology called in reality Technology Protection Systems (TPS) and lately Genetic Use Restriction Technologies (GURTs) was developed by researchers of a cotton seed company, Delta and Pine land Co. The TPS or GURT is based on the combined action of two foreign genes that can be activated to impede seed germination by using a chemical pre-treatment of the seed. This system was invented to provide both a gene expression control system and a means to block seed germination that will be useful to prevent unfair appropriation of the patented seeds or the escape into the environment of transgenic seeds.

When Monsanto acquired this technology through Delta and Pine Land Co. acquisition in May 1998, an unprecedented wave of attack came from a very large opinion sector because it was perceived as the evil technology that was going to violate traditional farmers' rights to keep seeds for the next cropping season. The generally negative public reaction was exacerbated by a long series of negative attitudes from large corporations, in particular Monsanto, towards public concerns voiced by several environmentalist organizations.

The public opinion fueled by powerful environmental organizations became so strongly against the terminator technology, by October 1999, that Monsanto announced that they will not develop and use this technology in their transgenic varieties.

Is the technology such an evil technology that it should be banned? Certainly not!
Protecting seed from farmer's reuse is neither new nor unethical. The whole seed industry is based on this strategy and is operating since half a century. Seeds are produced, selected, coated, and packaged in such a way that farmers buy these over and over while gaining in profitability. Hybrid maize seeds are other examples of seed protection. Therefore, the TPS technology would not have been essentially different than current practices and technologies of the seed industry.

The technology could be also highly beneficial to farmers as a system to activate, when appropriate, disease resistance mechanisms, thus minimizing possible resistance break down. Farmers or producers would also be able to elicit the activation of a gene to produce a desirable characteristic at the right time. Finally, it might also have been used to prevent escapes and volunteers of transgenic varieties in the environment.

Therefore, the technology in itself is not a matter of concern, it is much more who controls it and for what purpose it will be used. Public institutions such as the CGIAR center have categorically rejected the use of this technology when used solely to protect ownership of the seed.

For more information see:

www.connectotel.com/gmfood/ra291099.txt

www.netlink.de/gen/Zeitung/2000/000330c.html


The triple-resistant canola weeds in Alberta

A tri-herbicide-resistant weed has been discovered in northern Alberta (Canada) as a consequence of the use of several varieties of canola carrying transgenic resistance to one herbicide. This case is known as the ?super-weed? case and is presented as the evidence of a threat of herbicide resistant crops.

In 1997, two fields of transgenic canola were planted. The west side of a county road contained Quest, a canola tolerant to Monsanto?s Roundup herbicide. On the East Side farmers planted Innovator, a canola tolerant of Aventis?s Liberty herbicide. And the rest of the field 45A71 was planted with a SMART canola tolerant to Cyanamid?s Pursuit and Odyssey herbicides. The fields were 30 meters apart. A year after harvest, volunteer weeds resistant to Roundup and Liberty was discovered, although no canola crop had been planted. This weed had acquired a double resistance to herbicide through cross-pollination. The next year, a triple resistant weed to Roundup, Liberty and Pursuit herbicides was found. It is the first case of natural transfer of multiple genetic traits in canola since farmers started to grow five years ago these new varieties.

This event has been publicized as the emergence of super-weeds due to extensive use of genetically engineered crops. Beyond the apparent failure of an appropriate weed management procedure, one could reasonably wonder if this event could pose an additional risk due to the transgenic nature of the varieties used.

Genes can only be transferred between closely related species. In the present crop, it was known that natural gene transfer could occur between canola varieties and that canola plants behave as weeds. The transfer of an herbicide resistance trait from one plant to another will not create a super-weed because of the specificity of the herbicide resistance. The canola weed can still be eliminated by other herbicides. This cross-pollination event is not restricted to transgenic varieties, therefore it appears that a proper weed management system has not been established or the farmers did not follow it properly. Weed scientists recommend appropriate crop rotation and combination of herbicides for management and elimination of resistant volunteer canola. Among non-chemical strategies, the most recommended is tilling closely prior to seeding in order to control volunteer canola. That could be done by the use of diversified rotation of canola with cereals, pea and forage crops, and by the reduction of loss seed during harvest.
The lesson learned from this event is the need to use and enforce weed management programs even when using herbicide/resistant varieties.

For more information see:

http://www.biotech-info.net/triple_resistance.html

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**The Brasil nut allergen soybeans**

In order to create a soybean with a higher nutritional content, the company Pioneer Hi-Bred introduced the methionine-rich 2S albumin from Brazilian nut (*Bertholletia excelsa*) into soybean. Soybeans naturally have poor methionine content. Hence, the use of genetic engineering was a logical choice to transfer these well-known methionine-rich storage proteins to increase the nutritious quality of soybean-based diets for poultry feed. It is well known that nuts are commonly a source of allergen. So was the case for Brazil nuts, but there was no report of the gene(s) responsible for the allergenicity. For that reason, the Pioneer Hi-Bred company sponsored a group of scientist from the University of Nebraska in order to test their product for nutritional quality and presence of allergens. Their results suggested that the gene used to transform the soybeans is a potential allergen, and despite the findings that the nutritional quality was increased; Pioneer Hi-Bred discontinued its research in April 1993. The findings of the University of Nebraska report were published in *The New England Journal of Medicine* 11(334): 688-6920, 1996.

This case is often reported as THE example of unintentional introduction of allergens in biotech food. Actually, this example is just about the opposite. It proves that when transformation events are produced scientists have the tools to test the presence of toxic and allergenic new component. This is an option unique to the genetic engineering technology. It should be stressed here that such investigation are not possible for its conventional counterpart. Fortunately more and more regulatory agencies recognize today that by having conducted such detailed risk assessment of biotech foods, these are now probably safer than their conventional equivalent.

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**The tryptophan case**

The L-tryptophan essential amino acid can be found in high especially concentration in beef and milk products. It is also used as a mild sedative, anti-depressant, and for treatment for period pains. The tryptophan for such drugs is produced by fermentation of genetically engineered bacteria. In 1989, over a thousand people became seriously ill because of the development of a new disease called EMS syndrome (eosinophilia- myalgia-syndrome) associated with some dietary products containing the L-tryptophan amino acid. Thirty-eight of these cases resulted in the death of the patient.

This case has been often reported as one example of the deadly potential of the genetic engineering technology. What had happened is the following. The toxic compound used in these medicines is a dimer consisting of two molecules of tryptophan (monomers) linked together. Such dimers can be erroneously introduced into proteins instead of tryptophan monomers. This insertion into proteins results in conformational changes that are recognized by the immune system as foreign intruders to be eliminated. This autoimmune response is the one that occurred in EMS.

The investigation of this case concluded that the cause of the problem was an impurity in L-tryptophan production made by the chemical manufacturers in Japan, the Showa Denko KK company of Tokyo. Indeed, the production of L-tryptophan for human consumption is done by a fermentation process, which produces a number of secondary substances. A purification process is needed to remove these impurities. This includes a treatment with activated carbon and reverse osmosis.
To increase the efficiency of the system, the Showa Company had developed new techniques to increase the production of L-tryptophan by using a genetically modified *Bacillus amyloliquefaciens*. Improvement of the production was accomplished also with partial bypassing of the reverse osmosis purification procedure, and decreasing by half the amount of carbon used. The investigations on the case of this EMS disease revealed that some impurities were left after this new purification process. The disease could develop because of a residual impurity 1,1-ethyldenebis-[tryptophan] (EBT), which then broke to give 1-methyl-1,2,3,4-tetrahydro-beta-carboline-3-carboxilic acid (MTCA). Another explanation was also provided as the reaction of two or more impurities left during the purification process.

Hence, the tryptophan case was the result of a bad management of quality control during the development of L-tryptophan-based drugs for human consumption and not at all a consequence of the genetic modification of the bacteria.