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**GENETICALLY ENGINEERED BACTERIA:
US LETS BAD GENE OUT OF THE BOTTLE**

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GENETICALLY ENGINEERED BACTERIA: US LETS BAD GENE OUT OF THE BOTTLE

Ignoring the advice of its own scientists, the US government has approved the genetically engineered *Rhizobium* bacterium

In September 1997, the US Environmental Protection Agency (EPA) approved a bacterium containing genes from five different species for agricultural use in the USA. The bacterium was genetically modified to increase its ability to serve as a fertilizer on alfalfa plants. Despite obvious risks to human health and the environment, the EPA ignored warnings of some of its staff and an independent scientific panel that had been set up by the EPA to review the risk assessment. One scientist resigned from the review committee during the process because he felt that the committee's final report was "*little more than a long argument to support an affirmative decision already made before the subcommittee met.*"

The product

The artificial microbe, which was dubbed RMBPC-2, is a strain of the soil bacterium *Rhizobium meliloti*ⁱⁱ. It is produced by Urbana Laboratories, a small company in St. Joseph, Missouri, which began selling the bacterium to alfalfa farmers in 1998 under the label "Dormal plus"ⁱⁱⁱ. The bacterium was genetically altered to enhance its ability to provide nitrogen to alfalfa plants on farmland^{iv}. A mixture of several genes plus regulatory elements were introduced into the bacterium. In addition to genes intended to increase nitrogen fixation and nutrition of the bacteria, one gene conferring resistance to antibiotics was also engineered into the bacterium. In full, the following genetic elements were artificially introduced:

- A regulatory element^v intended to enhance expression of the genes necessary for nitrogen fixation.
- Three genes^{vi} that are intended to enhance nutrition uptake of the bacterium, with the aim of enhancing its performance as a nitrogen supply for the alfalfa plant.
- A gene conferring resistance to the antibiotics streptomycin and spectinomycin^{vii}.
- A regulatory element^{viii}, and some uncharacterized sequences^{ix} - a cause for particular concern. Complete characterization of any introduced sequence is usually a cornerstone of a proper risk assessment, and this organism is probably the only one ever approved for commercial release without full knowledge of the genetic elements introduced.

Urbana Labs has a 60% share of the market for alfalfa inoculant sales in the US^x. The majority of alfalfa growers in the US use inoculants on their crop. In 1999, alfalfa was harvested in the USA on a total of 24 million acres, with nearly half in the northern States along the Canadian border^{xi}. It is unknown whether or not batches of "Dormal plus" or seeds coated with "Dormal plus" have been moved across the border to Canada.

The risks

A micro-organism is particularly difficult to control. Once released into the environment, it can never be recalled or exterminated if environmental or health problems become evident. Micro-organisms are easily spread to adjacent fields by wind, by farmers and through farm machinery, a fact that was repeatedly

pointed out by EPA scientists^{xii}. A commercial release of genetically engineered bacteria across millions of acres might easily end up in a global distribution of this bacterium.

Environmental risk: creating superweeds

Scientists submitted evidence that *Rhizobium meliloti* can inoculate not only alfalfa, but also other legumes such as sweet clover or mesquite^{xiii}, which are both known as weeds in US agriculture. Mesquite is a brushy plant that is an important weed in the South-western states of the USA, where about 670,000 acres of alfalfa are grown. Even small populations of mesquite markedly reduce grass forage production^{xiv}. Mesquite is also common in the desert areas of North-western Mexico^{xv}. If RMBPC-2 were able to inoculate weedy mesquite and enhance its nitrogen supply, this may lead to the weed thriving even better on arid soils and to potential devastation of forage production in the American South-west. Although this risk is high, and with potentially significant impacts, it was never addressed experimentally during the approval process of RMBPC-2. No experiments were conducted to evaluate the ability of the newly designed bacterium to enhance the weediness of mesquite. Since 670,000 acres of alfalfa are grown commercially in the American South-west^{xvi}, there is a strong probability of mesquite plants coming into contact with the new microbe. In addition, the adjoining areas in Mexico could become affected in time.

Health risk: Transfer of antibiotic resistance to human pathogens

The antibiotic resistance gene that was engineered into RMBPC-2 could be transferred to other bacteria and potential pathogens. The medical world is already experiencing problems with antibiotics that are increasingly failing because pathogens are developing resistance. This danger is increased through the use of antibiotic resistance marker genes in genetically modified organisms^{xvii}.

The EPA's decision documents stress that streptomycin is an important drug to treat illnesses like brucellosis^{xviii} or nontuberculosis mycobacterial infections that affect tens of thousands AIDS patients annually^{xix}. Both antibiotics are also crucial in the treatment of *Neisseria gonorrhoeae*, and spectinomycin is used to treat certain gonococcal infections during pregnancy^{xx}. Indeed, the use of the streptomycin resistance gene in genetically engineered plants has recently been deemed as potentially harmful by a British scientific advisory body^{xxi}.

The use of antibiotic resistance marker genes becomes even more disturbing when one considers that such genes are completely useless in terms of the function of the final genetically engineered product. Since genetic engineering is a rather random process, only some cells amongst millions are successfully altered in any one experiment. To identify the few cells that have been modified, genes conferring antibiotic resistance are used as markers. Thereafter, these genes fulfil no function in the final product. It is possible to remove the genes later on in the process of product development, or one can use other marker genes that have potentially less dangerous impacts.

In essence, the use of antibiotic resistance genes as markers is an old-fashioned technology, posing a completely unnecessary threat to human health. This has been increasingly acknowledged by regulatory authorities around the world. Norway banned all genetically engineered plants that contain antibiotic resistance genes. Austria and Luxemburg banned Novartis' transgenic maize line in February 1997 citing the threat to the sustainable use of antibiotics. In April 1999, Switzerland did not allow experimental field trials of genetically engineered potato that contained an antibiotic resistance marker gene and the European Parliament voted in February 1999 in favour of a general ban on crops containing antibiotic resistance marker genes.

The approval process

In approving RMBPC-2, even the basic standards of risk assessment were ignored. Decisions were made on estimations and beliefs, with no scientific data available to develop any scientifically sound opinion about the possible risks. Statements by expert scientists were overruled, and questions raised by EPA scientists were responded to with misleading and false statements.

Lack of scientific data and useless field trials

Extremely limited scientific data on RMBPC-2 was submitted by the applicant company. Further, the few experiments that had been done by the applicant were performed in many cases with genetically engineered bacteria other than RMBPC-2. This approach runs counter to the basic principle of GMO regulation in the USA - the „case by case“ rule – which should have required individual testing of RMBPC-2. This fact was heavily criticized by the EPA’s own scientific advisors in the Biotechnology Science Advisory Committee (BSAC) Subcommittee: „(...) *the lack of having PC-2 as one of the test strains in many of these studies is a serious problem and I would say, (...), that the available data is not strong enough to give a positive answer in this case*“^{xxvii}.

Some of the data provided by the applicant were considered useless by the BSAC („*The dissemination tests by wind were really inadequate.*“^{xxviii}), and other experiments were considered as laboratory errors^{xxiv}.

In the absence of hard data, the EPA based its risk assessment on analogies, beliefs and positive thinking. This was one of the major reasons for Professor Istock resigning from the BSAC: „*Repeatedly the report [the BASC Subcommittee’s report on RMBPC-2] argues that there are no potential problems with massive release of RMBPC-2 based on the absence of evidence. It is not a matter of an absence of positive or negative evidence, it is the absence of evidence altogether.*“^{xxv}

BSAC subcommittee against approval without further testing

On January 4, 1995, a subcommittee of the Biotechnology Science Advisory Committee met to review the application and EPA’s draft risk assessment. During the meeting, the subcommittee identified several key areas where data was missing. At the end of the meeting, the subcommittee members were polled as to whether they would approve RMBPC-2 for commercial use based on the risk assessment. Only one scientist (Dr. R. Maier) indicated his approval. Four scientists would not give their approval^{xxvi} and one abstained^{xxvii} from the vote. The four scientists who voted against commercial use specifically asked for further testing before any approval. This was also reflected in the report from the BSAC subcommittee meeting, which specified the following two experiments as critical before further approval:

- Greenhouse experiments to address the possible effect of RMBPC-2 on mesquite or sweet clover;
- Assessment of RMBPC-2 persistence, dissemination and competitiveness through reseeded experiments on former experimental plots^{xxviii}.

However, the EPA ignored its own scientific panel and ruled that no additional testing was necessary.

Concerns voiced by EPA scientists were not properly or not at all addressed

Scientists from an EPA office in Denver asked the EPA to determine whether the new *Rhizobium* could survive in a sewer system. The EPA answered^{xxix} that only an insignificant amount of bacteria would be released beyond the production facility, but did not support this statement using any scientific or other evidence. However, it became evident that the opposite was in fact the case. When the EPA prepared an

Exposure Assessment one year later, it became clear that several million of billions of bacteria were being discharged to a local waste water plant several times a year^{xxx}.

Certain government employees realized during the approval process that the whole procedure was flawed and voiced their concerns in an in-depth report^{xxxi} published in September 1995. However, this internal critique was also ignored by the decision makers at the EPA.

No benefit

The approval process at EPA was based on the federal law called the Toxic Substance Control Act (TSCA). TSCA is a risk-benefit balancing statute which provides for the consideration of not only the risk but also the benefit of a new substance (or micro-organism). The question of whether or not RMBPC-2 had a benefit was discussed by the BSAC subcommittee^{xxxii}, but no conclusion was recorded of any benefit other than an increase in sales for Urbana Labs, the applicant company.

It is remarkable that the EPA, in its consent order, went out of its way to distort the scientific facts, minimizing the risks to the environment while emphasizing unproven advantages. On environmental risks, EPA stated^{xxxiii} that RMBPC-2 was no different to any natural bacterium. But when it came to benefits, the EPA came to the opposite conclusion: “..strain RMBPC-2 has demonstrated a significant advantage over other commercial alfalfa seed inoculants in improving alfalfa yields under certain circumstances.”^{xxxiv}

However, the limited data available showed a statistically significant increase of alfalfa yield only at one of the several test sites. Since RMBPC-2 had only limited and equivocal yield enhancing capacity, the only benefit is to the biotech applicant company, while society will bear the risks.

EPA grants only “limited” use that is in fact unlimited

The EPA was clearly aware of the many shortcomings of their risk assessment procedure and tried to calm down their critics by granting only a “limited” use of RMBPC-2^{xxxv}. However, the maximum annual production limit granted by EPA is 500,000 pounds, an amount that is enough to inoculate 50% of all alfalfa planted each year in the US.

Conclusions

In summary, the process leading to the approval of the genetically engineered *Rhizobium* was driven by the United States’ desire to promote biotechnology at all costs and was without any scientific basis.

The EPA’s breaches of basic scientific processes in this case, although extreme, are emblematic of the US government’s failure to address scientific concerns about GMOs, while falsely claiming the mantle of “sound science”.

This inadequate approach may have consequences not only for US citizens’ health and biodiversity, but also neighbouring countries, and countries to which the US exports its approved GE products. Since simply walking across a field and then boarding a plane wearing the same shoes may result in a transboundary movement of this bacterium, there is every likelihood that there will also be unauthorised transboundary movements of genetically engineered *Rhizobium*.

The RMBPC-2 case illustrates the importance of setting international standards based on precaution for proper risk assessment procedures for micro-organisms, particularly since an array of different viruses, bacteria and fungi are currently being genetically engineered for eventual commercialisation. These include the following micro-organisms that have already been approved for field testing: Baculoviruses equipped with a lethal toxin to kill insects has been tested in the UK; soil bacteria (*Bacillus thuringiensis* and others) were modified in France and the USA to increase their insecticidal effect; viruses were released to vaccinate wildlife and cattle in several European countries; and a tobacco company in the USA started to modify a tobacco virus (TMV) for the production of pharmaceutical proteins^{xxxvi}. The US government is still encouraging development of new *Rhizobia* strains and has approved field testing at three sites in Wisconsin of a new strain which has an additional antibiotic gene designed to kill other bacteria in the soil to make the *Rhizobium* more competitive^{xxxvii}. Once approved for commercial release into the environment, all of these micro-organisms may well pose global environmental and health problems which cannot be controlled.

Greenpeace demands:

- There should be no irreversible releases of Genetically Modified Organisms (GMOs) into the environment.
- The Biosafety Protocol to the UN Convention on Biological Diversity should establish strong rules so that countries can take measures to protect against the irreversible consequences of releasing GMOs in accordance with the precautionary principle. This principle encourages thorough evaluation of potential impacts, and sets out the need to take measures to prevent ecological or human harm even where there is no full scientific certainty or scientific consensus on the potential impacts.
- The Biosafety Protocol to the Convention on Biological Diversity must apply to all GMOs, including genetically engineered micro-organisms, so that parties to the Protocol have international rights to take preventive action to protect their biodiversity. All parties should be given advance warning of any proposed shipments of genetically engineered micro-organisms coming into their country, and must have the right to refuse any transfer, handling or use of genetically engineered micro-organisms.
- The Biosafety Protocol should provide for all nations to be consulted in advance by neighbouring nations who intend to release into the environment genetically engineered micro-organisms.

For most details on Greenpeace's comprehensive demands for the Biosafety Protocol , please refer to <http://www.greenpeace.org/~geneng/>

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ⁱ Prof. Conrad Istock in a letter dated Feb. 22, 1995, to the EPA announcing his resignation from the BSAC (Biotechnology Science Advisory Committee of EPA), EPA docket number 51786, D1-16. Prof. Istock was at that time Prof. in the Dept. of Ecology and Evolutionary Biology at the University of Arizona, Tucson. Since his retirement, he now holds a courtesy position at the Cornell University.

ⁱⁱ This bacterium is also known recently as *Sinorhizobium meliloti*. However it will be referred to as *Rhizobium meliloti* in this report.

ⁱⁱⁱ www.urbana-labs.com/dormalplus.htm

^{iv} It is common agricultural practice to use naturally occurring bacteria as natural fertilizers (inoculants) on legumes. Prior to sowing, the plant seeds are coated („inoculated“) with the bacteria, which colonizes the plant root and provide nitrogen to the plant during growth. Most soils are notoriously short of nitrogen. As lack of nitrogen limits plant growth, it is usually supplemented in agriculture through artificial fertilizers or, in case of legumes, through the naturally occurring *Rhizobium* bacteria.

^v nifD promoter from *Bradyrhizobium japonicum*.

^{vi} dctABD (3 genes), for C4-transport into the cell, from *Rhizobium leguminosarum*.

^{vii} aadA from the pathogen *Shigella flexneri*.

^{viii} T1T2 terminator from *Escherichia coli*.

^{ix} In the dct-fragment a small amount of uncharacterized DNA is present according to EPA's risk assessment from September 18, 1997, page 20.

^x According to the EPA's economic analysis, quoted by: Conrad Istock in: Transcript of Proceedings of the meeting of EPA's Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing rhizobium meliloti, January 4, 1995, page 127.

^{xi} Agricultural Statistics Board, NASS, USDA, <http://usda.mannlib.cornell.edu:80/usda/>. Harvest in 1999 in some of the northern States in million acres: ID 1.15, MI 0.9, MN 1.55, MT 1.75, NY 0.55, ND 1.5, PA 0.72, WI 1.9.

^{xii} „It is known that rhizobia are easily spread to adjacent fields as a result of wind-blown soil and movement of farm machinery from plot to plot.“ Philip Sayre, EPA scientist, in a memo titled „Effects on nontarget legumes on PMN P92-339“ from December 17, 1992, TSCA-Docket number B1a-06.

^{xiii} This fact is also acknowledged by EPA in the Consent Order, issued on Sep 8, 1997 to finally approve the commercial use of RMBPC-2. page xi.

^{xiv} Russelle M, Transcript of Proceedings of the meeting of EPA's Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing *rhizobium meliloti*, January 4, 1995, page 181.

^{xv} www.desertusa.com

^{xvi} Russelle M, Transcript of Proceedings of the meeting of EPA's Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing *rhizobium meliloti*, January 4, 1995, page 181.

^{xvii} EPA scientists have also acknowledged that a transfer of antibiotic resistance genes from RMBPC-2 to other bacteria is a possibility.

^{xviii} Human Health Assessment for a recombinant of *R. meliloti* strain RMBPC-2, final draft report, September 6, 1994, prepared for EPA by Dynamac Corporation, Rockville, MD, page 12.

^{xix} See xviii above.,, pages 8, 10.

^{xx} Advice on occurrence of AAD gene in Monsanto insect-protected and Round-up ready cottonseed. UK Advisory Committee on Novel Foods and Processes, Feb. 1999

<http://www.maff.gov.uk/food/foodnov.htm> .

^{xxi} „If a gene conferring spectinomycin resistance were to arise in resistant strains of *Neisseria gonorrhoeae*, then this pathogen would effectively become untreatable. (...) In view of the very serious consequences arising if a spectinomycin resistant bacterium were to evolve, it is our opinion that it would be very unwise to allow increased opportunity for such an event to happen by the introduction of plants containing this resistance gene. It is accepted that the risk of such an event is small and cannot be quantified. The clinical consequences of such an evolutionary step would, however, be grave. It is therefore entirely appropriate in this instance to adopt a precautionary stance.“ UK Advisory Committee on Novel Foods and Processes, Feb. 1999 <http://www.maff.gov.uk/food/foodnov.htm>

^{xxii} Bauer W, Transcript of Proceedings of the meeting of EPA's Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing *rhizobium meliloti*, Jan. 4, 1995, p. 133.

^{xxiii} M. Russelle, *ibid.* page 114.

^{xxiv} „Although it is impossible to explain with certainty the unexpected results obtained in the 1994 sampling and analysis of nodule occupancy at the 1993-initiated Strain Comparison test (...), laboratory error probably seems most likely“ Gwendolyn McClung, EPA scientist, in a memo titled „addendum to

the 1994 BSAC exposure assessment for the commercialization of RMBPC-2“, undated, TSCA-Docket number B4-029.

^{xxv} Professor Conrad Istock in a letter dated Feb. 22, 1995, to EPA announcing his resignation from the BSAC.

^{xxvi} Dr. M. Russelle: *“I am not ready to agree that this is ready for commercial release, but I don’t think it is very far away“*. Dr. W. Bauer: *„We still need data on persistence. that is too much of an open question at this point and is relatively easily solved“*. Dr. Levy: *„I would like to see more data on the relative survival“*. Dr. C. Istock: *„I want to see some effort made to understand the gene exchange aspects of this“*. Transcript of Proceedings of the meeting of EPAs Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing rhizobium meliloti, January 4, 1995, page 236-237.

^{xxvii} Dr Cohen: *„I don’t think that this represents a human health hazard. I have to be concerned that the people who are ecologists are concerned.“* Transcript of Proceedings of the meeting of EPAs Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing rhizobium meliloti, January 4, 1995, page 236.

^{xxviii} Appendix of the final report of the BSAC subcommittee, March 6, 1995.

^{xxix} *„To obtain sufficient quantities of the target strain, cultures must be grown under sterile conditions, which considerably limits any significant release of the organisms to the local environment. As a result, few cells released into the production facility would survive or be released beyond the production facility. (...) good quality control requires strict cleanup which generally consists of autoclaving contaminated materials and equipment. Spent fluids often are treated additionally with concentrated solutions of sodium hypochlorite or other effective antimicrobials. This sort of treatment is merely good laboratory practice for any microbial laboratory or manufacturing facility.“* H.Kay Austin, EPA scientist, in a memo titled *„Response to region 8 questions regarding research seeds test market exemption/5(e) consent order modification“*, dated Sept. 20, 1993 (it is assumed that this means 1993), TSCA-Docket number E1a-4.

^{xxx} *„The primary source of aqueous discharge of the bacteria is the discharge from the centrifuge operation. (...) this waste stream is untreated. (...) the number of bacteria discharged from this source is estimated to range from 1.9 to 7.6 10¹⁵ CFU/bt. This discharge may occur 4 to 8 times over a 2 month period. This waste is discharged to a POTW:“* Gregory Macek, Chemical engineering branch, EPA: Engineering Report. Exposure and release assessment commercialization of P-92-403, December 6, 1994. TSCA Docket number B1a-10.

^{xxxi} The premature commercial release of genetically engineered bacteria. A white paper prepared by PEER - public employees for environmental responsibility, September 21, 1995. PEER, 810 1st St., NE, Washington DC 20002.

^{xxxii} Clark E, Transcript of Proceedings of the meeting of EPA’s Biotechnology Science Advisory Committee Subcommittee on premanufacture notification review of nitrogen fixing rhizobium meliloti, January 4, 1995, p. 13.

^{xxxiii} *„However, the yield increases realized are modest, and not outside the range of yields encountered in commercial alfalfa production using naturally occurring rhizobial inoculants...Overall, strain RMBPC-2 was shown to perform within the normal range expected of naturally occurring commercial inoculants.“* Consent order, issued by EPA on Sep 8, 1997 to finally approve the commercial use of RMBPC-2. pp. x, xiv.

^{xxxiv} Consent order, issued by EPA on Sep 8, 1997 to finally approve the commercial use of RMBPC-2. page xiv.

^{xxxv} *„...questions raised by the BSAC Subcommittee (...) lead EPA to conclude (...) that the information currently available to the Agency is insufficient to permit a reasoned evaluation of the environmental effects of the PMN substance in the event of unlimited commercialization.,, Consent order, issued by EPA on Sep 8, 1997 to finally approve the commercial use of RMBPC-2. page xiv.*

^{xxxvi} Worldwide information on field trials with genetically modified organisms is available at www.olis.oecd.org/biotrack.nsf

^{xxxvii} USDA permit No 97-071-01r; see background information at www.nbiap.vt.edu.

^{xxxviii} Greenpeace International would also like to thank Peter Morris for his initial research on this subject.