

 **WIPPEER**

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**PAPER**

***GENETIC GENIE***

**The Premature Commercial Release  
of Genetically Engineered Bacteria**

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## ABOUT PEER

Public Employees for Environmental Responsibility (PEER) is an association of resource managers, scientists, biologists and other government professionals committed to upholding the public trust through responsible management of the nation's environment and natural resources. PEER advocates sustainable and responsible management of public resources and seeks to be a catalyst for supporting professional integrity and promoting environmental ethics in government agencies.

PEER provides agency managers, scientists, and other resource professionals committed to ecologically responsible management with credible voice for expressing their concerns.

PEER's objectives are to:

1) **Organize** a broad base of support among employees within local, state

and federal resource management agencies;

2) **Inform** the administration, Congress, state officials, the media, and the public about substantive issues of concern to PEER members;

3) **Defend** and strengthen the legal rights of public employees who speak out about issues of environmental management;

4) **Monitor** land and resource management agencies.

PEER recognizes the valuable role that government employees play as defenders of the environment and stewards of our natural resources. PEER supports resource professionals whose want to advocate for environmental protection in a responsible, professional manner.

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## ABOUT THIS REPORT

This document was painstakingly prepared by a number of current Environmental Protection Agency employees from both the agency's regional and headquarters offices. They are biological, ecological and human health scientists whose expertise ranges from agricultural marketing to pharmacology.

Their report has been reviewed by faculty members from major American universities. The principal reviewers are a public health entomologist and a professor of ecology and evolutionary biology.

The authors of this white paper are committed professionals who merely want to communicate that more research needs to be done before genetically engineered microorganisms are introduced on a commercial scale into the environment. They believe that EPA is currently unprepared to perform its statutorily mandated task of performing

competent risk analysis prior to approving the release of new life forms.

The authors remain anonymous in order to avoid the inevitable retaliation that would be taken against them by their supervisors in EPA. Among federal agencies, the EPA is one of the least tolerant of internal dissent, even on purely scientific matters. The U.S. Department of Labor and the Merit Systems Protection Board has repeatedly found EPA in violation of whistleblower protection statutes for professional reprisals against its own scientists. Current management of the EPA has not signaled an interest in hearing the candid views of its own professionals.

Public Employees for Environmental Responsibility is proud to serve the conscientious few in the EPA who produced this report by being the intermediary in its distribution.

*Jeff Ruch, Executive Director*



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**Public Employee for Environmental Responsibility**

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October 13, 1997

Dr. Susan Gottesman  
National Institutes of Health Bldg. 37, Room 2E18  
9000 Rockville Pike  
Bethesda, MD 20892-4255

Dear Dr. Gottesman:

Enclosed is the Consent Order by which the Environmental Protection Agency (EPA) has approved the manufacture and commercial release of *Sinorhizobium meliloti* RMBPC-2, and the final risk assessment describing EPA's rationale for permitting widespread environmental release of this genetically engineered (GE) microorganism.

Public Employees for Environmental Responsibility (PEER) is sending these documents to you and to members of the Biotechnology Science Advisory Committee's Subcommittee on Premanufacture Notification Review of Nitrogen Fixing *Rhizobium meliloti*, which advised EPA on its draft risk assessment for release of this organism.

PEER wishes to ensure that you are aware of how EPA has used the BSAC deliberations to support its decision. We believe that in its enthusiasm to pass an application through the regulatory system, EPA ignored BSAC Subcommittee recommendations to collect additional data. It stated in several cases that it does not understand BSAC's rationale in requesting additional data, yet if did not bother to seek clarification from either the Subcommittee or individual members. It dismissed concerns and interpreted the diversity of opinion and the scientific uncertainty expressed by BSAC Subcommittee members as lack of concern about widespread release. It failed to address additional issues raised by the BSAC (e.g., eradication, monitoring). And most importantly, it failed to specifically solicit Subcommittee opinion about the quality and adequacy of the overall risk assessment to support a widespread commercial release. The result is a premature decision based more on speculation and illogical conclusion than on hard data and thoughtful consideration of the complexities of assessing the risks of this new technology.

PEER understands that EPA has the authority to make these decisions and that the BSAC acts in an advisory capacity. However, this first widespread environmental release of a GE microorganism under the Toxic Substances Control Act sets an important precedent for risk assessment of GE organisms. Certainly scientific opinion used to support such a release should be treated with respect. On the contrary, PEER understands that one member of the BSAC Subcommittee actually resigned over the contents of the Subcommittee report, which was in fact drafted by EPA employees.

PEER respectfully suggests that you consider reconvening the Subcommittee to discuss whether the references to Subcommittee deliberations contained in the Consent Order and final risk assessment honor the intent and substance of those deliberations. If you agree that they do not, we would call upon you to bring this to the attention of the EPA Administrator to request that the Consent Order be suspended pending further review.

PEER is an association of resource managers, scientists, biologists and other government professionals committed to upholding the public trust through responsible management of the nation's environment and natural resources. PEER advocates sustainable and responsible public management of public resources and seeks to be a catalyst for supporting professional integrity and promoting environmental ethics in government agencies.

In the fall of 1995, PEER issued a critique of EPA's process for assessing risks associated with the commercial release of genetically engineered microorganisms. A copy of this critique, entitled *Genetic Genie: The Premature Release of Genetically Engineered Bacteria*, is enclosed for your perusal. The criticisms leveled in this 1995 PEER white paper, in our view, remain valid today.

If PEER can be of any assistance in communicating your concerns to the EPA, please do not hesitate to let me know.

Sincerely,

Jeffrey Ruch  
Executive Director

Cc. BSAC Subcommittee members

## EXECUTIVE SUMMARY

The EPA is preparing to approve the first commercial release of genetically engineered bacteria for use in agriculture. EPA scientists charge that the agency has not adequately assessed the environmental or human health risks of releasing this new life form into the environment.

The newly created "super bacteria" contains genes drawn from a number of bacteria, including the pathogen *Shigella flexneri*, which causes dysentery and infantile gastroenteritis in humans. The gene from *S. flexneri* carries with it resistance to commonly used antibiotics. The function of other introduced, but "uncharacterized," genetic material is unknown.

Overeager to promote biotechnology, EPA has either deliberately ignored or actively suppressed concerns raised by staff and independent scientists. This report contains a scathing critique of the internal agency approval process, the shoddiness of which prompted one prominent scientist to resign from the advisory panel charged with assuring scientific thoroughness and integrity.

EPA's own scientists charge the agency with failing to assess the risks associated with the massive release — hundreds of thousands of pounds over several million acres — of a

new living organism that cannot be contained or eradicated. Human health risks may include the

- Transfer of antibiotic resistance genes to pathogens thus creating drug-resistant diseases in humans, livestock and wildlife and/or the
- Potential toxicity to humans who touch, eat or inhale the micro-organism.

Significant environmental dangers for which there are inadequate data include:

- "The Frankenstein Effect" whereby wild plants colonized by the new bacteria could become serious problems (The new bacteria could facilitate kudzu-style invasion by legumes such as clovers and vetches into new areas where they would compete with and possibly displace native plants.);
- Loss of endangered plant species and reduced biological diversity in affected areas as a result of increased competition from weedy species; and
- Irreversible changes in soil ecology and fertility.

Ironically, the effectiveness of the new bacteria, which would be used as a seed inoculant to increase yields in alfalfa and other legumes, is questionable. If the new bacteria is not effective, it could permanently reduce crop yields from the land where it is applied and, if it spread, from adjoining tracts.

The efficacy of the new bacteria is not even considered in the EPA's risk-benefit analysis, which only looked at the potential market for the product in calculating the economic ramifications of approval. If the new bacteria is not effective, EPA scientists ask, then why should *any* risks be countenanced, particularly when the environmental and health effects could be irreversible?

One overarching concern expressed by EPA scientists is that the antibiotic resistance gene that has been inserted into the organism serves no function other than acting as a "marker gene" to distinguish this bacterium from others. There are other effective methods to mark bacteria that do not confer antibiotic resistance to the new species. In fact, other countries, most notably the United Kingdom, discourage antibiotic resistance markers to avoid the hazards associated with antibiotic resistance.

EPA is poised to approve the commercial release of this bacteria. Moreover, EPA has

proposed to abandon its authority to regulate whole classes of genetically engineered creatures, including this new bacteria, through a pending rule change that is still subject to public comment.

The report recommends that EPA

- ✓ Develop rigorous risk assessment guidance and field test requirements for the introduction of genetically engineered organisms into the environment;
- ✓ Declare a moratorium on the release of new organisms until appropriate risk assessment practices are in place;
- ✓ Withdraw its proposed waiver of authority to regulate *Rhizobia* and *Bradyrhizobia* genera and state as its official science policy that there is *no* genus whose organisms can always assumed to be safe for introduction;
- ✓ Strengthen independent scientific peer review on issues relating to the introduction of new species and genetically engineered products; and
- ✓ Coordinate with the European Union to achieve common safeguards concerning the transport, containment, eradication and gene transfer to, or from, genetically engineered organisms.



## ABSTRACT

### THE IMMINENT AND PREMATURE COMMERCIAL RELEASE OF GENETICALLY ENGINEERED BACTERIA

*The Environmental Protection Agency (EPA) has approved for commercial release into the environment a genetically engineered strain of corn; the Agency is now reviewing over two thousand applications for unlimited release of other genetically engineered organisms.<sup>a</sup> EPA currently lacks a sound process to assess the risks these organisms may pose to human health and North American ecosystems, yet the Agency proposes to abandon its authority to regulate whole classes of genetically engineered creatures.<sup>b</sup> The case of *Rhizobium meliloti* RMBPC-2 illustrates EPA's lack of preparedness for the biotechnology revolution.*

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<sup>a</sup>On March 30, 1995, EPA granted registration to CIBA Seeds, Mycogen Plant Sciences, and Monsanto to plant 60,000 acres with pesticidal corn, potato and cotton plants. Full scale commercialization has been approved for corn, and could come as early as 1996 for potatoes and cotton. October 1994 Status Report: Biotechnology Premanufacturing Notifications (PMNs), U.S. EPA Office of Prevention, Pesticides and Toxic Substances, Chemical Control Division, Program Management Branch.

<sup>b</sup>Regulation of all genetically engineered forms of the *Rhizobia* and *Bradyrhizobia* bacteria would be waived under the Proposed TSCA Test Rule, 59 Federal Register 45526, September 1, 1994. Under this rule, genes from any species, including those conferring pathogenicity, could be added to any *Rhizobia* or *Bradyrhizobia* bacteria for any purpose.

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## CHAPTER ONE

# BIOTECHNOLOGY TODAY

In May 1994, EPA received a request from Research Seeds, Inc., of St. Joseph, Missouri, to market a genetically engineered bacterium, *Rhizobium meliloti* RMBPC-2, as a seed inoculant for commercial use on alfalfa seed.<sup>1</sup> If approved, this permit will be EPA's first authorization of the widespread release of a genetically engineered micro-organism into the environment.<sup>2</sup> Because it is the first of its kind, it will set the precedent for how, and to what extent, EPA will assess and regulate the commercial use of genetically engineered organisms.

Genetic engineering — the transfer of individual genes from one organism to another — is an emerging and potent technology used to create plants, animals, microbes and medicines that could never be produced through conventional breeding or chemistry. Using genetic engineering techniques, scientists have inserted a bacterial gene into corn, potato and cotton plants that enables them to produce a toxic protein, *B.t.* toxin,

derived from the bacteria *Bacillus thuringiensis*. Caterpillars, which are common agricultural and forest pests, are highly susceptible to the *B.t.* toxin.

In another example, scientists have inserted a gene from the arctic winter flounder, whose liver proteins lower the freezing temperature of blood, into Atlantic salmon, thus enabling the salmon to survive in frigid waters they would not otherwise inhabit. The gene for human growth hormone has been inserted into turnips, and human growth hormone has been extracted from this vegetable.<sup>3</sup> Human genes have also been inserted into bacteria so that they now produce human insulin and growth hormone in quantity for pharmaceutical applications.<sup>4</sup> There is now a method to insert human genes into cattle and sheep and to harvest human proteins from their milk.<sup>5</sup>

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<sup>1</sup>May 26, 1994 letter from Research Seeds, Inc., to EPA's TSCA Biotechnology Section, Office of Prevention, Pesticides and Toxic Substances (OPPTS).

<sup>2</sup>Some modified organisms, such as those that make enzymes used in detergents, which are not widely released, have already been approved.

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<sup>3</sup>Lee, T.F., Gene Future: Promise and Peril of the New Biology, Plenum Press, New York, 1993.

<sup>4</sup>Zuagg, R.H., and Swarz, J.R., Assessment of future environmental trends and problems: Industrial use of applied genetics and biotechnologies. Teknekron Research, Inc., McLean, VA, for Office of Research and Development, U.S. EPA, Washington, D.C., EPA-600/8/81-020, 1981.

<sup>5</sup>Wright, G., et al., High level expression of active human alpha-1-antitrypsin in milk of transgenic sheep.

The potential societal and economic benefits generated by this emerging industry provide powerful incentives to expedite the development of new products and technologies. But there is a dark side. The biotechnology revolution poses hazards to human and environmental health.<sup>6</sup> How is society to predict and assess the potential hazards to ensure that they are not realized?

Time and hindsight have demonstrated that new technologies frequently result in unforeseen problems. While the promise and potential of new chemicals is easily recognized, their potential adverse effects may not be anticipated.<sup>7</sup> We now know that synthetic chemicals can work their way up the food chain into human body tissue. PCBs, for example, were useful and efficient in electrical transformers, but their carcinogenic properties were discovered after they had been in use many years. Similarly, we now know that the consequences of altering ecosystems,

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<sup>5</sup>(...continued)

*Bio/Technol.* 2: 830-834 (1991); Glanz, J., Herman, the pharmaceutical industry's next star?, *R&D Magazine*, May, 1992.

<sup>6</sup>Hazard, is the potential adverse effects of chemical, biological or radiation exposure; risk is the probability that the identified hazards will come to pass under specific conditions of exposure. Although it is not in itself a science, risk assessment requires scientific evaluation of data from toxicological, epidemiological, or exposure studies. Regulatory agencies must balance the projected and perceived risks with benefits, costs, political pressures, legal obligations, and other factors to make decisions about whether a proposed exposure is "acceptable."

<sup>7</sup>Ehrenfeld, D.W., *The Arrogance of Humanism*. Oxford University Press, 1978, 286 pp.

ecosystem components and the interactions among them can be profound.

## The Lessons of DDT

Fifty years ago, the Department of Agriculture was charged with determining the conditions under which DDT and other promising chlorinated hydrocarbon pesticides could be applied. The power and promise of these chemicals was impressive; only decades later did the scientific community begin to comprehend the long-term, far-reaching and unanticipated effects of DDT.

Scientists in the 1940's could not have known that in less than 40 years 93 percent of all American men, women, and children would harbor DDT metabolites in their body fat;<sup>8</sup> nor could they know that these carcinogenic and estrogenic chemicals would persist in the environment for years, bringing species like the peregrine falcon and bald eagle to the brink of extinction.

DDT looked remarkably safe; after all, GIs were literally dusted with it to kill lice — without apparent effects. Chlorinated hydrocarbons became the archetype of "Better Things for Better Living Through Chemistry." The risks were inconceivable, and the benefits, compelling. At that time, scientists could not

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<sup>8</sup>DDT metabolites have been found in fat tissues in all age groups and in all areas of the country, at concentrations up to 6.8 parts per million, U.S. EPA, Broad Spectrum Analysis of the FY82 National Human Adipose Tissue Survey Specimens, EPA 560/5-86-035, December, 1986.

## THE PREMATURE COMMERCIAL RELEASE OF GENETICALLY ENGINEERED BACTERIA

have asked the pertinent questions. Environmental persistence was not a known concept. Risk assessment, the process used to evaluate hazards, was not yet born.

EPA regulators are now in the same position as their predecessors in the Department of Agriculture fifty years ago. The power and promise of new technology is impressive; also like their predecessors,

today's regulators face uncertainty about the risks involved in applying this technology. Because regulatory agencies do not want to be perceived as roadblocks to "progress," it is convenient that the potential benefits of this new technology are more easily identified than are its risks.



## CHAPTER TWO

# THE FIRST COMMERCIALY AVAILABLE GENETICALLY ENGINEERED MICRO-ORGANISM

*Rhizobium meliloti* is a naturally occurring bacterium that lives in soil. *R. meliloti* colonizes alfalfa, fenugreek and sweet clover, and legumes, which are important as food crops for farm animals and as rotation and cover crops used to increase soil fertility. Since plants cannot produce their own nitrogen, nitrogen is the nutrient that often limits plant growth. In a process called nitrogen-fixation, the natural or "wild" variety of *R. meliloti* absorbs nitrogen gas from the air and converts it to water-soluble forms that are readily used by plants.

Plants in the legume family — peanuts, soybeans, alfalfa, clovers, vetches, locusts and mesquites — have developed a symbiotic relationship with *Rhizobia* bacteria. The bacteria colonize the host legume by entering its roots and forming nodules; in return for providing shelter and nutrients to the bacteria, the plants benefit from the *Rhizobium*'s ability to convert nitrogen into an accessible form.<sup>9</sup> It

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<sup>9</sup>Burton, J.C., (1975) In: *Symbiotic Nitrogen Fixation in Plants*. P.S. Numan, ed., IBP Synth. ~~1975~~ (1975)

is not known, however, how many of the thousands of wild legumes found in North America *R. meliloti* might also colonize.<sup>10</sup> This missing information has important implications for how well the risks of distributing a genetically manipulated form of *R. meliloti* throughout the environment can be assessed.

For close to a century, farmers have inoculated alfalfa and soybean seeds with wild strains of *Rhizobia* and *Bradyrhizobia* bacteria, respectively. An inoculant industry has developed to provide farmers with pre-coated seed and with packaged inoculant with which farmers can do their own inoculation.

Today, virtually all alfalfa seed is inoculated with the wild strain of *Rhizobium meliloti*. Each acre planted with alfalfa receives between 5 and 80 billion bacteria<sup>11</sup>,

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<sup>10</sup>(...continued)

Cambridge Univ. Press, London and New York.

<sup>10</sup>*id.*

<sup>11</sup>The manufacturer, Research Seeds, Inc., estimates each alfalfa seed will be coated with from 2,000 to 20,000 (continued)



which multiply in the soil and plant roots. Wind disperses seed and inoculant during planting. Interestingly, farmers and the agricultural seed industry widely regard the wild *Rhizobia* as only marginally effective in boosting alfalfa yield; not unlike supplemental dietary vitamins, the wild *Rhizobia* is a kind of "insurance" to guarantee that soil will have plenty of nitrogen-fixing bacteria.<sup>12</sup>

Typically, commercial *R. meliloti* is grown in a fermentation process in closed vessels — to prevent contamination of the culture from other bacteria or viruses. At this stage of their production, the bacteria are contained; but once the culture vessel is opened, distribution through air, soil and water takes place. The cultured bacteria are typically sprayed onto a finely powdered clay or peat carrier. Coating results in each seed being covered with thousands of bacteria. The coating, called an inoculum, is either sold directly to farmers or is applied to crop seed before sale.

Like its wild counterpart, RMBPC-2 is expected to multiply in soil and alfalfa roots and survive there indefinitely. The manufacturer expects that 250,000 pounds of RMBPC-2, enough to treat several million

acres, will be used in the first three years alone.<sup>13</sup>

## The Genetically Engineered *Rhizobium*

The engineered strain of *R. meliloti*, named RMBPC-2, contains genetic information from six "donor" bacteria from five genera:<sup>14</sup> *Rhizobia meliloti*; *Bradyrhizobium japonicum*; *Rhizobia leguminosarum*; *Shigella flexneri*; *Escherichia coli*; and *Klebsiella pneumoniae*.<sup>15</sup>

One gene comes from a wild strain of *Rhizobia meliloti*. This is a duplicate of the gene that normally codes for the enzyme that fixes nitrogen. Thus, the engineered RMBPC-2 has two nitrogen-fixing genes rather than one. The extra gene theoretically increases the nitrogen-fixing capacity of the engineered bacterium.

Another gene comes from *Bradyrhizobium japonicum*, a bacterium that colonizes soybeans and that "promotes" or regulates nitrogen fixation. From *Rhizobia*

<sup>11</sup> (continued)

genetically engineered bacteria. Each pound of alfalfa contains 200,000 seeds, and 50 to 60 pounds of seed are used for every 3-4 acres. Thus, each acre planted with alfalfa will receive at a minimum  $5 \times 10^9$  (5 billion) and up to  $8 \times 10^{10}$  (80 billion) bacteria.

<sup>12</sup> Bronsten, D., Soil inoculants: dubious data, fuzzy future, *Agrichemical Age*, January, 1987, p. 17-20.

<sup>13</sup> May 26, 1994 letter from Research Seeds, Inc., to EPA's Biotechnology Section requesting authorization to commercialize inoculant containing RMBPC-2.

<sup>14</sup> Genera is the plural of genus. A genus is a taxonomic grouping of closely related species. For example, humans, classified as *Homo sapiens*, are the species *sapiens* in the genus *Homo*.

<sup>15</sup> U.S. EPA, Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994.

*leguminosarum*, a *Rhizobium* variety that colonizes peas and vetch,<sup>16</sup> come three genes intended to enhance RMBPC-2's ability to absorb nutrients.

From *Shigella flexneri*, a bacterium that causes dysentery and infantile gastroenteritis (inflammation of the stomach and intestine) in humans, comes a gene that confers resistance to the antibiotics streptomycin and spectinomycin. This is a "marker gene" whose sole purpose is to allow the engineered bacteria to be identified in the laboratory or in soil. For example, if either of these antibiotics is applied to a soil sample containing wild strains of *R. meliloti* and the RMBPC-2 strain, any wild strains that are not antibiotic-resistant will die; any surviving strains are assumed to be the genetically engineered RMBPC-2. The utility of this marker is questionable, however, and as will be described later, this is an important uncertainty in the assessment of RMBPC-2's risks.

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<sup>16</sup>*R. leguminosarum* makes an antibiotic, trifoliotoxin, which is toxic to a number of soil micro-organisms. Genetic engineering experiments are underway to incorporate the gene for trifoliotoxin production into *Rhizobia* species, in hopes of increasing their competitiveness in soil. What effect such a manipulation would have on soil ecology and fertility is unknown. Animal Plant Health Inspection Service Permit # 94-207-02.

Two "fragments" of DNA come from *Escherichia coli*, an intestinal bacteria, which, though normally harmless, may sometimes cause disease. These fragments signal the boundaries of the antibiotic resistance gene.

Some DNA is included from the Tn5 transposon (a movable part of DNA) from *Klebsiella pneumoniae*. *K. pneumoniae* is a gram-negative bacteria found in soil, water, grain and in the respiratory, intestinal and urogenital tracts of humans. It can cause urinary tract infections in humans and uterine infections in horses. This DNA originated in a larger piece of DNA used to construct the genetic material placed in RMBPC-2 (the "omega fragment"). It was not entirely deleted before the omega fragment was placed in RMBPC-2. In addition to being a pathogen, the donor *K. pneumoniae* is a clinical isolate resistant to the antibiotics bleomycin, kanamycin and neomycin.

The genes from *E. coli*, *B. japonicum*, *S. flexneri*, and *K. pneumoniae* make the engineered RMBPC-2 "intergeneric" and thus subject to regulation under the Toxic Substance Control Act (TSCA) because they were obtained, not from *Rhizobia*, but from donors in genera different from *Rhizobia*.<sup>17</sup>

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<sup>17</sup>59 Federal Register 45526, September 1, 1994; Dr. Conrad Istock, a member of the BSAC stated at the Subcommittee meeting on January 4, 1995, that RMBPC-2 is "a chimera of five species and four genera."



## CHAPTER THREE

# EPA'S RISK ASSESSMENT: THE PROBLEM OF PANDORA'S BOX

### Regulatory Framework

Along with the National Institutes of Health, the Food and Drug Administration and the Department of Agriculture, the EPA regulates the biotechnology industry to determine the conditions under which its products may be used.<sup>18</sup> Regulators must assess what risks will be incurred by releasing genetically engineered organisms into the environment, and then make hard decisions about which releases are acceptable.

The possibility of release of RMBPC-2 into the natural environment raises many questions new to agency and non-agency regulators alike.<sup>19</sup> EPA's assessment of the

human, animal and ecological risks of the release of *Rhizobium meliloti* RMBPC-2<sup>20</sup> demonstrates that it is prepared to allow the introduction of genetically engineered micro-organisms into the environment without having in place agencywide, peer-reviewed methods for adequately assessing the risks associated with such a widespread release of these new organisms.

EPA regulates substances under the Toxic Substances Control Act (TSCA)<sup>21</sup> and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).<sup>22</sup> TSCA covers the release of micro-organisms; FIFRA covers genetically

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<sup>18</sup>NIH regulates Public Health Service laboratory research; EPA, controlled introductions of micro-organisms; USDA, controlled introduction of plants and plant pests; FDA, food, drug and non-food products destined for human use and registration of veterinary products. Coordinated Framework for Regulation of Biotechnology, §1 Federal Register, June 26, 1986.

<sup>19</sup>Gabriel, W., Technologically modified genes in natural populations: some skeptical remarks on risk assessment from the view of population genetics; and Mieth, D., The release of micro-organisms - ethical criteria, in: Transgenic Organisms, K. Wohmann and J. Tomiuk (eds, Berlin)

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<sup>19</sup>(...continued)

hauser, Basel, Switzerland, 1993; The Release of Genetically Engineered Micro-organisms, M. Sussman, C.H. Collins, F.A. Skinner, D.E. Stewart-Tull (eds) (1988); Hynes, H.P., Biotechnology in agriculture: An analysis of selected technologies and policy in the United States, Reproductive and Genetic Engineering 2: 39-49 (1989).

<sup>20</sup>U.S. EPA, Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994.

<sup>21</sup>15 U.S.C.A. §§ 2601-2654

<sup>22</sup>7 U.S.C.A. §§ 136-136.

engineered organisms intended for pesticidal purposes. Because TSCA was not originally intended to regulate living creatures, EPA considers genetically engineered microorganisms that contain genes from a genera different from that of the parent organism subject to review as "new chemicals."

No laws or regulations prevent the release of the new organism, RMBPC-2, before the completion of 1995 field trials. Further, EPA will have no control over RMBPC-2 if EPA's recently proposed Biotechnology Test Rule, designed to give the agency authority to require biotechnology data under TSCA, is finalized.<sup>23</sup> In fact, there will be no limits whatsoever to which genes may be added to any bacteria in the *Rhizobium* or *Bradyrhizobia* genera, even if the resulting new organisms were known to have detrimental environmental or health effects and would be likely to be released. The case of *Rhizobium meliloti* RMBPC-2 illustrates these problems. The *Rhizobium* case raises questions about whether the Agency can carry out its regulatory responsibilities under the enormous dual pressures to promote products perceived as "green" and to facilitate the rapid development of the biotechnology industry.<sup>24</sup>

<sup>23</sup>Microbial Products of Biotechnology; Proposed Regulation under the Toxic Substances Control Act, 59 Federal Register 45526, September 1, 1994. The test rule was signed by the EPA Administrator August 19, 1994 and awaits the blessing of the Office of Management and Budget for finalization.

<sup>24</sup>"A central purpose of any governmental effort in this area must be to encourage and facilitate the growth of biotechnology research, development and

In February 1987 Biotechnica International submitted a Premanufacturing Notification (PMN) to EPA requesting permission to test several intergeneric strains of the bacterium *Rhizobium meliloti* in a field in Wisconsin.<sup>25</sup> In February 1991, Biotechnica sold the research project to Research Seeds, Inc., a subsidiary of Cenex/Land O'Lakes. The following year, Research Seeds submitted five additional PMN requests for field tests of the modified bacteria to EPA.

These PMNs were approved and in 1993 modified to permit field trials in Wisconsin, Minnesota and Missouri. In 1993, EPA allowed Research Seeds to test the marketability of the product at 13 sites in Wisconsin, Minnesota, Iowa, Nebraska, South Dakota, North Dakota, Montana and California, although the company did not actually carry out the tests until 1994.

<sup>24</sup>(...continued)

implementation," says Senator Albert Gore, Jr.": See Governmental regulation of biotechnology. In: Biotechnology, S. Olson, National Academy Press, Washington, D.C., 1986; Science 268, 5/12/95, Letter to the Editor, from Lynne Goldman, Assistant Administrator for EPA's Office of Prevention, Pesticides and Toxic Substances, in response to criticism in the December 16, 1994 Science by Henry Miller that "EPA's approach ... to biotechnology regulation is likely to exert a profoundly negative effect on ... the commercialization of biological pest management." Goldman replied: "In general, EPA wishes to *promote* (emphasis added) development of environmentally safer products and technology. EPA's accomplishments in the biotechnology area show that it is achieving this goal." (None of EPA's regulations require any demonstration that proposed new products of biotechnology are safer than older technologies).

<sup>25</sup>p87-568/69/70, Biotechnica, International, Inc., 52 Federal Register 5333, February 20, 1987.

## Risk Assessment

In its review, the Environmental Protection Agency failed to follow its own mandatory guidance on characterizing the uncertainties of its assessment or consult with its own experts on how best to assess the risks. Release of RMBPC-2 without adequate evaluation would establish a dangerous and far-reaching precedent regarding the future release of genetically engineered organisms into the environment.

Regulatory agencies must balance the projected and perceived risks with benefits, costs, political pressures, legal obligations and other factors to make decisions about whether a proposed exposure is "acceptable." Although it is not in itself a science, risk assessment requires scientific evaluation of data from toxicological, epidemiological or exposure studies. Risk is the probability that the identified hazards will come to pass under specific conditions of exposure. Hazard is the potential adverse effects of chemical, biological or radiation exposure.

For over ten years, EPA has used the National Academy of Science risk assessment paradigm for evaluating risks from *chemical* contamination.<sup>26</sup> While the Agency has developed guidance for its own scientists in using that paradigm, it does not have in place

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<sup>26</sup>National Research Council, Risk Assessment in the Federal Government: Managing the Process, National Academy of Sciences Press (1983).

similar guidelines, protocol, or policies<sup>27</sup> for evaluating risks of introducing *organisms* into the environment. EPA has systematically applied this clearly defined approach in assessing its air, water, hazardous waste and pesticide programs.

EPA scientists have published articles on risk assessment<sup>28</sup> and the Agency has commissioned additional studies of biotechnology risk assessment methods. In addition, EPA has discussed the regulation of biotechnology with other federal agencies for

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<sup>27</sup>When scientific knowledge is limited, EPA adopts guidance for its risk assessors. These "science policies" may take the form of default positions. For example, unless there are data to the contrary, EPA considers infants and the elderly to be more sensitive to the toxic effects of chemicals than adults. For Risk Assessment Guidelines for Cancer, Developmental Toxicity and Exposure Assessment, see also, 51 Federal Register 3391 (9/24/86); 56 Federal Register 63798 (12/5/91); and 57 Federal Register 22888 (5/29/92).

<sup>28</sup>Seidler, R.J., Walter, M.V., Hern, S., Fieland, V., Schmedding, D., and Lindow, S.E., Measuring the dispersal and reentrainment of recombinant *Pseudomonas syringae* at California test sites, Microb. Releases 2: 209-216 (1994); Seidler, R., Evaluation of methods for detecting ecological effects from genetically engineered micro-organisms and microbial pest control agents in terrestrial systems, Biotech Adv. Vol. 10: 149-178 (1992); Walter, M., Porteous, A., and Seidler, R.J., Measuring genetic stability in bacteria of potential use in genetic engineering, Appl. and Environ. Microbiol. 53: 105-109, 1987. Milewski, E.A., Field testing of micro-organisms modified by recombinant DNA techniques: applications, issues and development of "points to consider" document, Recomb. DNA Tech. Bull. 8: 103 (1986) (Dr. Milewski works in EPA's TSCA Biotechnology Section and was in charge of the BSAC review of RMBPC-2).

more than a decade.<sup>29,30</sup> Nonetheless, EPA has failed to establish a risk assessment process for the release of living creatures — particularly genetically engineered organisms — into the environment, and it has not carefully analyzed the risks posed by the impending commercial releases of genetically engineered micro-organisms. On the contrary, EPA appears to have adopted a promotional stance for biotechnology.<sup>31</sup>

EPA's risk assessment of RMBPC-2 is characterized by an overall assumption of safety as a "rebuttable presumption." That is, the Agency concluded that because the parent organism is not known to cause any problems, the genetically engineered version would also be safe in every way. The practical result is that data demonstrating safety were not considered necessary. Judgment and opinion on the likelihood of safety were accepted as sufficient. At the same time, valid concerns about possible hazards were dismissed. Data demonstrating hazards were required before

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<sup>29</sup>G. Stotzky, Methods to measure the influence of genetically engineered bacteria on ecological processes in soil, EPA/600/3-90/001, J. Armstrong, Project Officer.

<sup>30</sup>51 Federal Register, June 26, 1986.

<sup>31</sup>At the 1995 meeting of the International Society of Biotechnology in San Francisco, David Giamporcaro, chief of EPA's TSCA Biotechnology Section, which is charged with regulating the new organism, spoke to a group of biotechnology entrepreneurs. He stated that he believes the federal government has an appropriate role in business development, and that "It consists of two Phases. Phase one is identifying and putting in place incentives for businesses to develop. Phase two is putting in place regulatory programs that are in compliance with laws and are incentives for development."

the Agency would consider seriously the possibility that release would carry risk. (*See Appendix 2.*) In 1986, the federal agencies regulating biotechnology stated their assumption that considerable research would have been completed on a genetically engineered organism prior to a manufacturer's application for commercial release:

By the time a genetically engineered product is ready for commercialization, it will have undergone substantial review and testing during the research phase, and thus, information regarding its safety should be available.<sup>32</sup>

This was due in part to:

The Agency's concern that micro-organisms formed with genetic material from different genera warrant regulatory review because of the inherent uncertainty about the characteristics and behavior of such micro-organisms."<sup>33</sup>

EPA stated in the Framework: "Under Section 5(d)(1)(b), submitters must provide all test data related to the health and environmental effects of the new chemical substance in their possession or control."<sup>34</sup>

This was to include:

(b) Risk assessment information ...

(D) Containment and mitigation measures (e.g., procedures in event of accidental release, for emergency termination and to reduce dispersal beyond the site).

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<sup>32</sup>51 Federal Register June 26, 1989, Coordinated Framework for Regulation of Biotechnology; Announcement of Policy and Notice for Public Comment, Page 23326.

<sup>33</sup>51 Federal Register, June 26, 1986, p. 23304.

<sup>34</sup>*id.*, p. 23327.

## THE PREMATURE COMMERCIAL RELEASE OF GENETICALLY ENGINEERED BACTERIA

For field test and other environmental releases, data on environmental fate and effects will be *essential* (emphasis added) ... Therefore EPA will expect manufacturers to provide test and other data demonstrating the micro-organism's safety. These data should include:

- (i) ... potential for genetic exchange in nature.
- (ii) Test data on the new micro-organism itself, indicating its potential for survival, replication, dissemination and genetic exchange with other organisms.<sup>35</sup>

EPA either did not require that data such as these be submitted for the RMBPC-2 assessment or, for unknown reasons, decided they were not necessary, since they are not in evidence anywhere in the RMBPC-2 assessment. Since these data were expressly identified by EPA as critical to reviewing a potential commercial release, the assessment, by EPA's own standards, appears to be incomplete.

In 1990, the Office of Science and Technology Policy (OSTP) proposed principles for federal oversight of biotechnology,<sup>36</sup> which included criteria for evaluating risk:

Agencies may evaluate the risk or safety of the introductions by considering relevant risk factors, which may include: For the organism: fitness ... toxicity; host range; ... environmental limits to growth ... susceptibility to control ... For the environment: selection pressure for the introduced trait, presence of wild, weedy or feral relatives within dispersal capability of the

organism or its genes; presence of vectors or agents of dissemination or dispersal (e.g. ... rodents ... humans, machines, wind, water); direct involvement in basic ecosystem process (e.g., nutrient cycling); ... range of environments for testing or use in light of potential geographic range, ... mitigation plans.

EPA has not required Research Seeds, Inc., to provide any of these data, and the risk assessment for the release of RMBPC-2 that the Agency produced contains no actual data on any of these elements. The review would therefore appear to be inadequate in light of OSTP's recommendations.

It is hard to imagine how the Agency might come to the conclusion that data on host range, environmental limits to growth, procedures for control, or presence of wild relatives would not be necessary for this precedent-setting risk assessment. Even as recommendations, they seem essential. If a determination was actually made that they were not needed, it is reasonable to expect a delineation of the rationale for that determination in the risk assessment.

The evaluation of the proposed release of RMBPC-2 focused on why the organism would *not* pose a problem, rather than an objective review of what is and is not known about the hazards it presents. When the Agency had an opportunity to seek important advice on fundamental issues related to this precedent-setting risk assessment from its Biotechnology Science Advisory Committee, it restricted the Committee's participation to narrow questions that skirted the considerable uncertainties in this assessment.

<sup>35</sup> *id.*

<sup>36</sup> 55 Federal Register 31118, July 31, 1990.



## Failure to Characterize Uncertainties

All significant EPA risk assessments are required to contain a risk characterization section.<sup>37</sup> This is a description of the full range of uncertainties in the assessment, designed to help risk managers understand the reliability of the information upon which they make decisions. Its utility has been recognized for a decade by scientists familiar with the issues raised by biotechnology:

The type and quality of information and data that are available at the time an assessment is made will be reflected in the reliability and utility of predictions. Therefore the identification of the most relevant inputs to a risk assessment has been critical in the development of assessment processes themselves.<sup>38</sup>

When there are quantitative data, risk characterizations may use statistical techniques like sensitivity analyses to evaluate the relative size of the variability of parameters used in risk models. When probabilistic models are not available, central tendency and high-end exposures can be compared with doses identified as having significant effects.

At this time, EPA has no methodology to quantitatively express the risks (*i.e.*, probability of adverse effects occurring) posed by genetically engineered organisms and has no risk assessment methods or guidance on the subject. However, qualitative methods to express uncertainties, such as listing or ordering the uncertainties, or identifying research necessary to resolve uncertainties, could help decisionmakers evaluate the "state of the science" when considering a proposed release.

While EPA clearly believes there is little or no risk to releasing RMBPC-2, the assessment did not express the degree of confidence in its conclusions in any organized way. There is no listing of the major uncertainties in evaluating this new technology and no discussion of which data or statistical methodologies could be used to form a quantitative evaluation of the uncertainties.<sup>39</sup> The number of glaring data gaps in the assessment and the uncertainties expressed by many scientists about the new

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<sup>37</sup>February 26, 1992, memorandum from Deputy Administrator Habicht "Guidance on Risk Characterization for Risk Managers and Risk Assessors;" March 21, 1995, memorandum from Administrator Browner, "EPA Risk Characterization Program" and March 1995 Policy for Risk Characterization.

<sup>38</sup>Gillett, J.W., Stern, A.R., Levin, S.A., Harwell, M.A., and Andow, D.A. and Alexander, M., Potential impacts of environmental release of biotechnology products: Assessment, regulation and research needs, Ecosystems Research Center, Cornell Univ., 1985.

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<sup>39</sup>Despite these deficiencies, David Giamporcaro, Chief of EPA's Biotechnology Section, stated in an October 13, 1994, letter to Citizens for Accountable Genetic Engineering (CAGE), that the agency was denying CAGE's request for a moratorium on release of genetically engineered organisms in part because: "The process the Agency follows involves the preparation of a *thorough* (emphasis added) risk assessment."

technology<sup>40</sup> make the highly confident tone of the assessment all the more disturbing.

## Quantitative Uncertainties

In the case of RMBPC-2 there are several important areas where quantitative data or science policies are needed to reduce uncertainty about the interpretation of the risks that RMBPC-2 poses. A major issue is what constitutes a "significant" introduction of a genetically engineered micro-organism. The risk assessment cited the fact that there are already in soil naturally occurring organisms with antibiotic resistance as support for EPA's conclusion that the number of antibiotic resistance genes added with RMBPC-2 would be "insignificant." There was no quantitative estimate of naturally occurring organisms with the specific gene conferring resistance to streptomycin and spectinomycin by adenylation.<sup>41</sup> What would constitute a "significant" increase in soil micro-organisms with antibiotic resistance was not defined.

There may be information that would allow a reasonable estimate of that number, or EPA might need a policy to define a default number.

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<sup>40</sup>See, for example: December 19, 1994, Chemical and Engineering News, p. 30-31; Letter to the Editor, New York Times; March 29, 1994; Gabriel, W., Technologically modified genes in natural populations: some skeptical remarks on risk assessment from the view of population genetics, IN: Transgenic Organisms, K. Wöhrmann and J. Tomiuk (eds), Birkhauser, Verlag Basel, Switzerland

<sup>41</sup>The bacteria inactivate these antibiotics by adding to their chemical structure an "adenyl" group.

The Agency could then present its rationale for defining a specific number as "significant." Neither was presented. Further, the risk assessment does not show the number of RMBPC-2 that actually are applied in a typical application, how many actually survive in soil or how many with a similar antibiotic resistance gene actually exist. A similar line of reasoning was used for the relative importance of human exposure to the organism. The assessment stated that the number of micro-organisms on human skin and in the lungs is so great that additional exposure to farmers and seed treaters from RMBPC-2 would be "insignificant." Yet no data showing actual survival in human lung or gut or in animal models were presented, and there was no indication of how a determination of "significance" was made.

Another important quantitative issue that the risk assessment did not address, is the effects that *multiple* plantings of RMBPC-2-coated seed will have on: the bacteria's survival in soil; its dispersion; gene transfer potential; and human, livestock, and crop exposures. The tests done with RMBPC-2 have been limited to single applications at each site, but alfalfa, when used as a rotational crop, is replanted every three to four years. Analysis of the effects of repeated applications will only be possible if long-term research with the new organism is done, but it is impractical to conduct field studies for many years.<sup>42</sup>

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<sup>42</sup>Of course, if proper experiments had been designed and instituted early in the PMN process, much more information on RMBPC-2's behavior would have been

Thus, the long-term effects of RMBPC-2 can only be understood after release has taken place. This raises a troubling issue for the Agency: under what circumstances can a regulated organism be released if data about the effects of that release cannot be collected except by observation after the release has taken place? In other words, how large an experiment can be performed on the public and the environment with a regulated organism?

### Inability to Control or Eradicate

The risk assessment contained no discussion of methods to contain or control RMBPC-2. Although techniques are being developed to genetically engineer genes into microbes that would cause the microbes' own destruction ("suicide genes"),<sup>43</sup> RMBPC-2 does not contain such controls. Their desirability in this particular bacteria was not discussed in EPA's risk assessment, and EPA has developed no guidance on this topic. Joseph Carra, deputy director of the EPA Office of Pollution Prevention and Toxics, outlined that Office's position on the subject in an October 13, 1994, letter to Citizens for Accountable Genetic Engineering, in denying its request for a moratorium on the release of genetically

engineered organisms until methods for their control could be developed:

The need to control the movement of any particular genetically modified micro-organism in the environment is, by necessity, contingent on the intrinsic hazard posed by that micro-organism ... In this case, a determination of low risk means that further control and eradication are not necessary to protect human health and the environment ... EPA therefore does not agree that further research field trials of these micro-organisms must be prohibited "until means of control and eradication have been established and shown to be effective."

In other words, if EPA concludes that an organism is safe, means to control it are unnecessary. But what if the Agency's conclusion is based on an incomplete risk assessment? Would it not be better policy to require methods to control *all* released organisms as an assurance of safety in case the risk assessment is flawed?

Because alfalfa roots range from 10 to 30 feet deep,<sup>44</sup> there is no reliable method to remove RMBPC-2 from soil once it is applied. Soil sterilant pesticides or other treatments such as changing the pH of the soil cannot penetrate to such depth. Research shows that burning, tillage and biocides are not effective in controlling populations of bacteria applied to crop foliage; in fact, biocide treatment can even stimulate bacterial growth.<sup>45</sup>

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<sup>42</sup>(...continued)

available. That advanced planning can only occur if the appropriate questions are asked early in the process.

<sup>43</sup>Ramos, J.L., Andersson, P., Jensen, L.B., Ramos, C., Ronchel, M.C., Diaz, E., Timmis, K.N. and Molin, S., Suicide microbes on the loose, Bio/technology 13: 35-37, 1995.

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<sup>44</sup>Bolton, J.L. (1962) Alfalfa: Botany, Cultivation and Utilization. Interscience Publishers, Inc., New York.

<sup>45</sup>Donegan, K., Fieland, V., Fowles, N., Ganio, L., and Scidler, R., Efficacy of burning, tillage, and biocides in controlling bacteria released at field sites and effects on

Lack of control methods converts short-term or one-time hazards into permanent concerns, and should be accorded a place of great significance in the risk assessment, particularly since "commercial release" means that there will be not one release but thousands of releases of the new organisms on thousands of acres *every year in the foreseeable future*. Without controls, release of RMBPC-2 constitutes an experiment of a magnitude not seen since the introduction of chlorinated hydrocarbon insecticides.

### Dynamics of Dispersal

The degree to which *R. meliloti* can be transported by water, wind, soil erosion, rodents or insects is not known.<sup>46</sup> Nor is it clear if, once the bacteria is in the alfalfa roots, it can enter the plant foliage as well. (Although alfalfa is used primarily as livestock forage, alfalfa seed, sprouts and leaves are used for human consumption.)

The coated seed is packaged and sold to distributors or directly to farmers. Because the natural bacteria are not known to cause

toxicity or disease in humans, farmers handle treated seed without any regard for skin contact, inhalation, ingestion, contact with farm animals or environmental distribution. Farmers plant the alfalfa seed using standard planters used for other crops, and they occasionally spill treated seed along roadways or into irrigation ditches and surface water.

Seed-processing plant workers freely inhale and handle the seed inoculant, and they inadvertently carry it home on their clothing. It is standard practice for seed processors to wash coating and mixing vessels with water and dispose of the rinsate into sewer or septic systems. Standard agricultural practices result in the treated seed and bacteria-containing carrier reaching surface water and irrigation ditches. Similar processing and use practices will be used with the genetically engineered bacteria.

The risk assessment's discussion of dispersal pathways by which the new organism could escape from its intended site of application was inadequate. There were no experiments in which RMBPC-2 was actually measured in soil near a test planting, and there are no measurements of the numbers of RMBPC-2 in water or air resulting from its use. Studies done with other strains of *Rhizobia* were minimal. For example, in some cases the top two inches of soil were monitored, six inches from the edge of test plots.<sup>47</sup> Assessing the elements of an exposure

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<sup>45</sup>(...continued)

indigenous bacteria and fungi, *Appl. Environ. Microbiol.* **58**, 1207 (1992).

<sup>46</sup>Research Seeds conducted a test in which soil six feet outside test fields was monitored for *R. meliloti* carrying the antibiotic resistance marker. None were found, but the ability of laboratory tests to detect the bacteria bearing the gene has been questioned (January 3, 1995, letter from Environmental Defense Fund to the Biotechnology Science Advisory Committee).

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<sup>47</sup>U.S. EPA, Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* (RMBPC-2) (continued...)

assessment is critical to understanding the effect that the release of a new organism might have.

Disposal of large numbers of RMBPC-2, and therefore large numbers of antibiotic resistance genes, into sewer systems where human pathogens thrive will occur because rinse water containing RMBPC-2 will be dumped without control from production and seed treatment plants. This will provide ample opportunity for pathogens that are not yet resistant to streptomycin and spectinomycin to acquire these traits, even if RMBPC-2 did not survive. The possibility of genetic exchange among viruses, plasmids, transposons and bacterial mixes observed in the Chesapeake Bay at sewer outfalls, for example, has been described as "very great."<sup>48</sup>

Clearly, aerial dispersal of RMBPC-2 will occur during planting. The risk assessment does not deal with this issue, despite the fact that particulates are known to travel many miles from their source by wind dispersal. Given that RMBPC-2 has the potential to affect yield in legume crops, its inadvertent dispersal in agricultural areas should be of serious concern.

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<sup>47</sup>(...continued)

Exposure Assessment for the Commercialization of a Recombinant Strain of *Rhizobium meliloti* RMBPC-2, December 1994.

<sup>48</sup>Colwell, R.R., Kaper, J., Joseph, S.W., *Vibrio cholerae*, *Vibrio parahaemolyticus* and other vibrios: occurrence and distribution in Chesapeake Bay, Science **198**: 394-396, 1977.

The aerial dispersal issue also raises concerns about public confidence that EPA has faced before and would logically want to address. The Agency came under severe criticism from both the public and apple producers because it had not considered the cancer risks to children when permitting the use of the carcinogenic pesticide Alar (daminozide) on apples. As a result of publicity about the pesticide's presence in fruit, apple sales fell dramatically. What environmental data could EPA produce to assure producers and consumers that the genetically engineered bacteria would not contaminate apples or other produce as a result of its use near orchards?

Models designed to estimate the probability of plasmid transfer in the environment and among organisms in the environment are available, including one produced expressly for EPA's use.<sup>49</sup> None of these were used in the RMBPC-2 assessment, perhaps because such models require data and no data are available for RMBPC-2 on such parameters as gene transfer and air transport.

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<sup>49</sup>Freter, R., Freter, R.R., and Brickner, H. (1983) Experimental and mathematical models of *Escherichia coli* plasmid transfer *in vitro* and *in vivo*, Infection and Immunity **39**: 60 (1983); Gabriel, W., Technologically modified genes in natural populations: some skeptical remarks on risk assessment from the view of population genetics, in: Transgenic Organisms, K. Wohrmann and J. Tomiuk (eds), Birkhauser, Verlag Basel, Switzerland; U.S. EPA, User's guide to MICROBE SCREEN execution in GEMS (Contract No. 68-02-4281; Russell Kinerson, Project Officer), (General Sciences Corp., Laurel, MD) 1987. This model is a screening tool for the prediction of the fate and transport of genetically engineered microorganisms released in air, surface water or soil during small-scale field tests.

The utility of such models for this risk assessment, if any, was never stated.

## Breakdown of Independent Peer Review

EPA has a peer review process whereby it hires scientific experts from outside the Agency to give advice on scientific issues and to review the scientific basis for major actions.<sup>50</sup> The Biotechnology Science Advisory Committee (BSAC) was created to provide EPA with advice, review and comment from external scientists with expertise in areas relating to biotechnology.

The BSAC has addressed issues related to release of RMBPC-2 over the years. On April 27, 1987, the BSAC discussed the difficulty of extrapolating effects from field studies to commercial release. At its January 1989 meeting, the main issue remained how to predict competitiveness of the new organism without actual large-scale release, and at that meeting, Dr. Francis Macrina of Virginia Commonwealth University recommended that research with microcosms be performed on RMBPC-2. In 1989, EPA raised to the BSAC the issue of the advisability of widespread environmental release of antibiotic resistance

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<sup>50</sup>It has only recently identified peer review as necessary for evaluating risk assessments and scientific studies that support agency decision-making. In the case of risk assessments involving biotechnology, however, there is no internal peer review among EPA's TSCA and FIFRA Programs.

genes,<sup>51</sup> just as recommendations on the subject have been solicited by government agencies in other countries.<sup>52</sup> EPA has not incorporated the discussions that the BSAC had on these subjects into any Agency guidance.

Based on an informal risk assessment, scientists in EPA's TSCA Program were convinced of the safety of the proposed release as early as 1993. However, internal and external controversy over the quality of its initial evaluation developed.<sup>53</sup> EPA rewrote the original risk assessment and convened the BSAC to review the revised assessment.<sup>54</sup>

The RMBPC-2 risk assessment was performed by the Premanufacturing Notification Subcommittee of the BSAC. It has never been reviewed by the full BSAC. In preparation for the BSAC Subcommittee

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<sup>51</sup>U.S. EPA Report, Meeting of the Antibiotic Resistance Markers Subcommittee, Biotechnology Science Advisory Committee, January 19, 1989.

<sup>52</sup>Recommendation to the Ministers of Health and Agriculture in the United Kingdom: Advisory Committee on Novel Foods and Processes, Report on the Use of Antibiotic Resistance Markers in Genetically Modified Food Organisms, Ministry of Agriculture, Fisheries, and Food, London, UK, July, 1994.

<sup>53</sup>The Gene Exchange, August, 1994; Bio/Technology, p. 660; April 29, 1994 letter from Citizens for Accountable Genetic Engineering to EPA's New Chemicals Branch, OPPTS, requesting a moratorium on release of genetically engineered organisms; Action Alert from mthom@igc.apc.org to gen.biotech@conf.igc.apc.org, June 22, 1994.

<sup>54</sup>Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994.

meeting, EPA developed specific questions relative to the genetically engineered *Rhizobium*. These questions were assigned to individual Subcommittee members. Despite the fact that the Agency has no guidance on how to evaluate products of this new technology, there were no questions about *how* EPA should assess the risks of releasing genetically engineered organisms, what critical questions should be answered, or even if the risk assessment at hand was adequate to support its conclusions.<sup>55</sup>

By contrast, in 1994, in a situation where its Dioxin Reassessment Program was under intense public scrutiny, the EPA asked its Science Advisory Board very broad

questions.<sup>56</sup> At the same time the BSAC Subcommittee was given the RMBPC-2 assessment and the list of questions to address, it was also given an EPA-written draft of the Subcommittee's own report for its approval. This approach could easily be viewed as prejudicial, and is not standard procedure for EPA staff working with the Science Advisory Board. Past Office of Pollution Prevention & Toxic Substances administrators have suggested that the BSAC be incorporated into the EPA Science Advisory Board so that inter- and intra-agency issues can be reviewed

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<sup>55</sup>For example, EPA did not ask the Subcommittee for its views on whether *R. meliloti* could transfer its genetic material to other micro-organisms, but instead asked the more leading question: "Is the means of initial transfer of the introduced DNA most likely to occur as a result of conjugative transfer of the megaplasmid containing the introduced DNA?" Rather than ask for the Subcommittee's interpretation of the data on nodule occupancy and its implications for the risk assessment, EPA asked the more leading: "Do the nodule occupancy data confirm that Strain RMBPC-2 is not more competitive than the other *Rhizobia* strains which were tested?" Rather than ask for the Subcommittee's interpretation of yield data, EPA asked the more leading: "Based on the field test data, are the alfalfa yields within the range of what is typically observed with commercial *Rhizobium meliloti* inoculants?" Rather than ask for the Subcommittee's opinion of RMBPC-2's ability to create weedy species, EPA asked the more leading: "To what degree do the alfalfa yield data indicate that Strain RMBPC-2 is unlikely to increase the fitness of plants which are potential weeds?" Questions that restate the agency's conclusions rather than ask for opinion in a neutral way place the responders in the psychologically difficult position of stating in effect, "We disagree with your position."

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<sup>56</sup>"Overarching the specific issues addressed below and not withstanding any specific finding of the Committee(s), do the available data and the analyses of these data, as presented in the report, adequately support the major conclusions of the reassessment documents?"; Are currently available models and approaches for estimating and apportioning the impacts of various sources adequate for this purpose ...?"; "Has this uncertainty, due both to the possible varied quality of the data and the limited number of samples, been adequately emphasized and characterized?"; Does the document adequately characterize the strengths and weaknesses of the data base and draw appropriate inferences for this group of compounds?"; Has the Health Assessment provided a useful summary and balanced perspective on these issues? Is the advance in knowledge of details of early cellular events in response to dioxin exposure clearly distinguished from our paucity of knowledge of the direct impact of these events on toxicity?"; Have uncertainties in the epidemiology data base been well characterized?"; "Has the significance of the human data been adequately characterized? Does the Health Assessment provide sufficient discussion of the strengths and weaknesses of the animal data on immune function to support its conclusions ...?"; Does the Board agree with the approaches to dose-response which have been used in the Health Assessment?"; "Does the Health Assessment document provide a balanced perspective regarding the uncertainties embodied in this inference?" See U.S. EPA, Happenings at the Science Advisory Board, Special Edition, April, 1994.

consistently by one body. A 1994 Agency report<sup>57</sup> recommended that there be more cooperation between the two Boards.

The BSAC Subcommittee met in January 1995 to discuss its review, and the final Subcommittee report concluded that more study of RMBPC-2 would need to be done to reduce uncertainty about:

- the stability of its genetic material;
- possible methods of transfer of genetic material to other bacteria;
- the probability of transfer;
- its persistence in soil;
- its ability to disseminate;
- its competitiveness with other strains of *Rhizobia*;
- its nitrogen-fixing efficiency;
- its potential to create serious weed pest problems;
- its potential to decrease crop yields; and
- its effects on non-target plants.

The Subcommittee also recommended the release be monitored and expressed concern about the lack of a clear benefit from the proposed product. (See Appendix 1, BSAC

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<sup>57</sup> U.S. EPA, The Science Advisory Board: What's Next? Report of the SAB Reinvention Committee, EPA-SAB-EC-95-008, October, 1994.

*Statements Indicating Inadequate Data for RMBPC-2.)*<sup>58</sup>

Before the final BSAC report was released, Subcommittee member Dr. Conrad Istock, professor of Ecology and Evolutionary Biology at the University of Arizona, resigned in protest over what he viewed as irregularities in the BSAC process and the questionable scientific integrity of the Subcommittee's final report.<sup>59</sup> Dr. Istock was concerned that the report drew conclusions in the absence of adequate supporting data or analysis. For example, concerns about the potential of the new *Rhizobium* to promote the growth of weed species like mesquite were brushed aside.

Evidence that RMBPC-2 is able to engage in genetic exchange by several mechanisms was not fully evaluated. Dr. Istock raised issues of scale and rare events. If RMBPC-2 were released on sufficient scale, its exchange of genetic material with other microbes, a rare laboratory event, would be virtually certain to occur in soil, since RMBPC-2 would live there permanently.

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<sup>58</sup> Biotechnology Science Advisory Committee, Subcommittee on Premanufacture Notification; Review of Nitrogen-Fixing *Rhizobium meliloti*. A review of the risk assessment prepared by the EPA for the commercialization of the nitrogen-fixing *Rhizobium meliloti*, RMBPC-2, March 6, 1995.

<sup>59</sup> "Science advisory panel split over need for more microbe testing; member resigns," Bureau of National Affairs, Inc., Washington, March 9, 1993; "Experts question approval of altered bacterium", *New Scientist*, January 4, 1995.



Adequate discussion of the complex genetic makeup of the new strain was removed from the final report. Other information did not appear in the final Committee report, including statements and documents submitted by scientists during the meeting and Committee members' statements submitted both before and after the meeting. Dr. Istock thought EPA seemed willing to permit the release the organism without anything approaching data sufficient to demonstrate its efficacy.

In April 1995, EPA began planning small-scale field trials of the new organism, this time in conjunction with genetically engineered alfalfa plants.<sup>60</sup> These trials will be conducted

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<sup>60</sup>Modification of TSCA Section 5(e) Consent Order for Premanufacture Notices P88-1118 and P92-403, April 6, 1995.

in Wisconsin and at EPA's research lab in Corvallis, Oregon, and the trials are expected to last two to three years. EPA has never made a statement about what additional data, if any, it believes will be necessary to approve the release of this organism. It is not clear if approval for unlimited release is pending the results of 1995 field tests, the three-year tests or the tests the BSAC Subcommittee suggested be performed.<sup>61</sup>

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<sup>61</sup>Some studies may not be possible. During a discussion regarding monitoring to evaluate RMBPC-2's persistence and ability to cross inoculate other organisms, at the January 4, 1995, BSAC meeting, Dr. Eric Triplett stated that he did not know if he could relocate the original test plots to perform monitoring studies.

## CHAPTER FOUR

# HAZARDS ON THE HORIZON: THE FRANKENSTEIN EFFECT

The difference between introducing chemicals and living organisms into the environment is that, unlike even the most persistent of pesticides, living releases have the potential to multiply. If they cannot be eradicated or at least controlled, they can literally take over an environment, wiping out native species and radically altering ecological niches and interactions that have evolved over millions of years. There are many examples of ecological communities devastated by exotic species.<sup>62</sup> The adverse environmental impacts of introducing zebra mussels into the Great Lakes, non-native fish into the nation's lakes and streams, and the kudzu vine into the Southern U.S. have been described as "the Frankenstein Effect."<sup>63</sup> And the cost of trying

to contain out-of-control organisms can be enormous.<sup>64</sup>

EPA recognizes that living organisms pose hazards not possible with chemical contaminants like chlorinated hydrocarbons, dioxins or PCBs.<sup>65</sup> Unlike chemicals, which degrade over time, living organisms may increase and disseminate through reproduction. The introduction of living organisms into an environment in which they have few or no competitors can result in their

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<sup>62</sup>Pimm, S.L., The Balance of Nature? Issues in the Conservation of Species and Communities, University of Chicago Press, Chicago, 1991.

<sup>63</sup>Moyle, P.B., Fish introductions into North America: patterns and ecological impact, In Biological Invasions in North America, H. Mooney and J.A. Drake, eds., Springer Verlag, New York, 1986.

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<sup>64</sup>Harmful Non-indigenous Species in the United States, U.S. Congress Office of Technology Assessment, OTA-F-566, 1993.

<sup>65</sup>Proposed TSCA Test Rule, 59 Federal Register 45526, September 1, 1994: (unlike chemicals, micro-organisms) "May reproduce and increase beyond the number initially introduced, may establish in the environment (*i.e.*, develop a self-sustaining population), and may spread beyond the test site. Thus, what begins as a small, localized population of micro-organisms may even become a large, widespread population. Even if certain micro-organisms do not ... reproduce, increase in number, establish and spread beyond the test site, they may be capable of passing some of their traits to other micro-organisms ... These other micro-organisms may, in turn, multiply, establish, spread and subsequently pass the acquired trait to other micro-organisms. This could result in widespread propagation of the trait, and exposure of a number of different environments to novel traits."

displacing native species and altering the natural balance of plants and animals with profound consequences.

The RMBPC-2 risk assessment failed to recognize some potential hazards of RMBPC-2, but most were simply dismissed without actual experimental data to support the conclusions of safety. Hazards are the potential adverse effects that are evaluated in a risk assessment. Risk, by contrast, is the probability that any specific hazard will be realized under the conditions being considered in the assessment. What is commonly regarded as "safety" is actually "negligible" or "acceptable" risk, as defined by guidance or statute.<sup>66</sup>

The existence of the unique RMBPC-2 bacteria poses questions never before asked:

- Will widespread distribution of a "superbacteria" throughout the nation's agricultural areas be harmless? Or will it constitute an assault on biodiversity, escaping to colonize native species of legumes, transforming them into "supermesquite" or "superclovers" that take over backyards, farm fields and forests kudzu-style?

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<sup>66</sup>EPA has no definition of "negligible" or "acceptable" risk for releases of genetically engineered organisms despite the fact that early products of biotechnology, such as bovine somatotrophin in milk or the FlavrSavr™ Tomato, have engendered extensive public debate. See for example, E. Andrews, Religious leaders to fight patents on genes, New York Times, May 15, 1995.

- Will the introduction of engineered nitrogen fixers be a boon to agriculture, or will they change the ecology of soil and eventually affect the fertility of farmers' fields in unforeseen ways?
- Is this new technology a way to avoid artificial fertilizers, or could farms treated or contaminated with genetically engineered bacteria be prevented from ever qualifying for the emerging "organic" production market?
- Will the antibiotic resistance gene harbored by these bacteria be an insignificant addition to the current gene pool of disease-causing bacteria? Or will it make human, animal and plant diseases more difficult to treat, or even create new resistant pathogens?
- Most importantly, if in five, ten or twenty years the introduction of these organisms is considered a terrible mistake, can they be eradicated or their spread be controlled?

It is EPA's responsibility to answer these questions in its risk assessment process. If the concerns cannot be addressed with assurance, EPA should require the necessary information and analysis before making what will be an irreversible, and precedent-setting, decision.

## Environment: Weedy Species

A major long-term concern with RMBPC-2 is the possible ecological effects of its spreading to non-target legumes, especially wild species. If RMBPC-2 were a "super nitrogen fixer" and were sufficiently competitive to colonize wild legumes, it might transform them into "superweeds" like kudzu.

Nonetheless, the risk assessment dismissed the possibility that RMBPC-2 could colonize wild legumes and create weeds. However, it cited little or no data showing:

- that the introduced genes are non-transferrable;
- which legumes are actually colonized by *R. meliloti*;
- how RMBPC-2 is distributed during typical application in the field; or
- the amount of its dispersal from seed-coating facilities or into air and water.

The assessment did acknowledge that *R. meliloti* can nodulate species in eight plant genera (*Medicago*, *Melilotus*, *Trigonella*, *Macroptilium*, *Leucaena*, *Prosopis*, *Phaseolus* and *Pisum*), which suggests that RMBPC-2 might very well be able to nodulate at least a few of the thousands of North American species of wild legumes.<sup>67</sup>

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<sup>67</sup>Strangely, Dr. Tom Wacek, Research Seeds' Director of Research and Development, stated in a June 30, 1994, letter to Citizens for Accountable Genetic Engineering: "The host range of *Rhizobium meliloti* is just for the

Despite this fact, the assessment did not recommend any experiments that would either increase certainty about RMBPC-2's true cross-inoculation group or that would characterize the competitiveness of RMBPC-2 with *Rhizobia* known to colonize wild legumes. Surprisingly, the rationale the assessment used for lack of concern about weediness was the contention that any weeds created by escaped RMBPC-2 would be unimportant:

There are a number of weeds in the family *Leguminosae* ... However, members of the family do not appear AT THE TOP OF LISTS OF THE WORLDS MOST SERIOUS AGRICULTURAL WEEDS, and in the U.S., the *Leguminosae* family is not ONE OF THE THREE families with the most important weeds ... Even if RMBPC-2 came into contact with native leguminous plants and was capable of nodulating and effectively fixing greater amounts of nitrogen in that plant compared to the indigenous *Rhizobia*, a mere increase in the growth of that plant in a localized area would not create a SERIOUS weed problem.<sup>68</sup>

This rationale is incorrect. "Local" weeds like leafy spurge in Montana, water hyacinth in Florida and kudzu in some Southern states are very serious problems. Worse, the assessment appears to dismiss as trivial problems caused by any but the "top three" (which are

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<sup>67</sup>(...continued)

Medicago species (alfalfa and medics) and for the *Melilotus* species (sweet clover). This is well-documented in many publications."

<sup>68</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, p. 19.

undefined in the risk assessment) weed families.

## Environment: Loss of Biological Diversity

If RMBPC-2 were to successfully nodulate wild species of legumes, but *not* effectively fix nitrogen, it might prevent nodulation by bacteria that normally colonize these plants. It might also cause the loss of important legumes in natural and non-agricultural areas, including the loss of uncommon or endangered species of legumes.<sup>69</sup> The risk assessment does not provide any findings to support its conclusion that this is not a concern:

Even though RMBPC-2 **MAY** be capable of nodulating **SOME** wild legumes, and **MAY** be ineffective in nitrogen fixation in those plants, there is also little concern for decreased growth of non-crop leguminous plants resulting in decreased biological diversity. First, since movement of *Rhizobia* is **USUALLY** quite limited, there **MAY** be little contact between RMBPC-2 and native leguminous species.<sup>70</sup>

## Environment: Soil Ecology and Fertility

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<sup>69</sup>The 28 species of threatened or endangered legumes listed by the U.S. Fish & Wildlife Service are found in 25 states and 3 U.S. territories. 50 CFR 17.11 & 17.12, August 20, 1994.

<sup>70</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, p. 18.

The risk assessment contained no analysis of the effects of introducing a critical soil nitrogen-fixing soil organism, with enhanced nitrogen-fixing ability and with antibiotic resistance, on soil ecology or fertility. The assessment does not describe the role of *R. meliloti* in soil ecosystems or how the introduction of RMBPC-2 might alter that role. Given that RMBPC-2 is expected to be introduced to literally millions of acres of prime farmland across North America,<sup>71</sup> soil effects would appear to be a critical endpoint, but they were totally ignored.

Commercial, large-scale releases of a nitrogen-fixing organism may alter bio-geo-chemical cycling. For example, enhanced soil nitrogen may increase the leaching of nitrates and increase the flux of nitrogen oxides into the atmosphere.<sup>72</sup> Further, soils are "teeming with organisms that have not yet been identified, many of which might one day be provided with the proper circumstances for their emergence as human, livestock or plant pathogens."<sup>73</sup> RMBPC-2 may introduce to soils the genes from *E. coli* and from *S. flexneri* that they might not otherwise have

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<sup>71</sup>The assessment estimates (p. 27) that 40,000 farms will use the new organisms in the first three years after its approval.

<sup>72</sup>Tiedje, J.M., Colwell, R.K., Grossman, Y.L., Hodson, R.E., Lenski, R.E., Mack, R.N., and Regal, P.J., The planned introduction of genetically engineered organisms: Ecological considerations and recommendations, *Ecology* 70: 298-315 (1989).

<sup>73</sup>Garrett, L., *The Coming Plague: Newly Emerging Diseases in a World Out of Balance*. Farrar, Straus and Giroux, New York, 1994.

ever had the opportunity to acquire. Thus, understanding the role *Rhizobia* play in soil ecosystems is critical for this risk assessment.

The hazards of weediness and decreased crop yield raise another "Catch-22" for the manufacturers of RMBPC-2. If the product is a "super nitrogen fixer" as hoped, it clearly presents the threat of creating "superweeds." On the other hand, if it is a poor nitrogen fixer, it may permanently reduce the fertility of the land of anyone unfortunate enough to use the product. What is worse, it might spread from users' land to neighboring farms and reduce fertility there as well, or it might invade natural areas and decrease native plant diversity. Of course, if the product is *not* a super nitrogen fixer, but merely performs within the normal range of wild strains, as the risk assessment concludes, then there is no benefit to its use.<sup>74</sup>

This raises a dilemma for Agency decisionmakers. Under TSCA, results of the risk assessment are compared with the benefits of a new chemical's use in a "risk-benefit analysis" to determine if the risks of permitting the release are worth the benefits derived. The assessment has defended its conclusion of safety on the claim that RMBPC-2 is no different than the parent organism and that it demonstrates no difference in nitrogen-fixing

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<sup>74</sup>Dr Tom Wacek, Director of Research and Development for Research Seeds, apparently made statements to EPA officials, described in a January 9, 1993 memo from Michael Broder (Chemical Screening and Risk Assessment Division) to Ellie Clark (Chemical Control Division) that "the company did not expect to see substantial yield increase attributable to the inoculum."

ability.<sup>75</sup> If RMBPC-2 is no better than wild strains at fixing nitrogen, then it has no benefits over current products. But what degree of risk is considered acceptable for a product that has no clear benefit? Again, the Agency has no published opinion or guidance on what risks are acceptable in the face of zero benefit.

Surprisingly, the "risk-benefit analysis" for RMBPC-2 will not actually be a weighing of the risks against the benefits of the proposed product *to society*. It will be a "risk-market-potential analysis,"<sup>76</sup> in which the risks to society will be weighed against Research Seeds' ability to reap sales revenue. (See Appendix 4.) Because efficacy is not considered a benefit, a product that is useless, but still sells, can still be considered "beneficial." In other words, EPA is willing to approve products from which the American public bears the risk and the manufacturer reaps the benefits. Contrast this with FDA's specific requirements that products be both safe *and* effective.<sup>77</sup> Unfortunately, EPA's final decision will not be open to public review or

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<sup>75</sup>Interestingly, the assessment also holds out the hope (page 30) that RMBPC-2 may be a "super nitrogen fixer, after all: "... the nitrogen fixed during, and left in the field after rotation with RMBPC-2 MAY (emphasis added) reduce the need for chemical fertilizer in subsequent rotational field crops ... ." There are no data to support this hypothetical benefit.

<sup>76</sup>The market potential analysis — which was submitted along with the risk assessment to the BSAC under the title, "Economic Assessment of *Rhizobium meliloti* Strain RMBPC-2" — is fatally flawed. See Appendix 4: Deficiencies in the Market Potential Evaluation.

<sup>77</sup>21 U.S.C. §§321 (p), 331(d) and 355.

comment, despite the fact that consumers have a low level of confidence in the government's ability to effectively regulate biotechnology, and the public's dissatisfaction with the lack of input permitted ordinary citizens.<sup>78</sup>

## Public Health: Antibiotic Resistance

Both streptomycin and spectinomycin are antibiotics effective against gram-negative bacteria, which as a group have become more significant causes of disease in the past two decades.

Spectinomycin is used in human medicine to treat drug-resistant gonorrhea. Farmers use it for "scouring" (bloody diarrhea) in pigs and as a preventative antibacterial treatment in cattle and poultry.

Streptomycin was once one of the most widely used antibiotics. While its use has declined in the U.S., it is still used in many medical and agricultural applications, and it is still commonly used in developing countries. Streptomycin is used for tularemia<sup>79</sup> and

<sup>78</sup>Hoban, T.J. and Kendall, P.A., Consumer attitudes about the use of biotechnology in agriculture and food production. Report to the USDA Extension Service, July, 1992

<sup>79</sup>Also known as deer fly fever or rabbit fever. Caused by the bacteria *Francisella tularensis*, and transmitted to humans from rodents through the bite of the deer fly, or through handling an infected animal's carcass. Symptoms are prolonged or intermittent fever and swollen lymph nodes. Game animals are an important reservoir of the

brucellosis,<sup>80</sup> and it remains the treatment of choice for the pneumonic, septicemic and bubonic plagues.<sup>81</sup> These are all common diseases in rural areas where the new *Rhizobia* is to be used. Streptomycin is also used in combination therapy for the treatment of the drug-resistant tuberculosis, a disease that is re-emerging among drug users and homeless persons in large cities.<sup>82</sup>

Living organisms, especially micro-organisms, have the ability to exchange genetic material with other living creatures. For example, in farms and hospitals, bacteria containing antibiotic resistance genes are known to transfer those genes to disease-causing bacteria. The widespread use of antibiotics in medicine and agriculture "selects" for the resistant strains. For example, a person with flu-like symptoms does not know if he has the flu, which is caused by a virus, or a bacterial infection. If his doctor prescribes antibiotics "just in case" their symptoms are caused by a bacteria (bacteria can be killed by antibiotics, but viruses,

<sup>79</sup>(...continued)

bacterium.

<sup>80</sup>Undulant, or Rio Grande fever. An infectious disease caused by *Bruceella abortus* and characterized by fever, sweating, weakness, aches and pains, and sometimes long-lasting disability. Transmitted to humans from diseased animals, milk or cheese.

<sup>81</sup>There are about eighteen cases of plague in the United States each year. Centers for Disease Control, Document # 351512, November 19, 1992.

<sup>82</sup>Centers for Disease Control and Prevention July 31, 1994, and November 19, 1992, Drug Guidelines, Document#s 250010 and 3531512, respectively.

including flu viruses cannot), the antibiotic kills both harmful and harmless bacteria in their intestines and lungs. If the cause of the illness was actually a virus, the antibiotic was not necessary, but it may have had an unintended effect: bacteria in the patient's lungs and gut that are resistant to the drug survive, and reproduce, conferring antibiotic resistance to successive generations of bacteria.

The patient quickly passes the resistant bacteria to family and friends by casual contact. In some relative's intestine, one of these resistant bacteria contacts a disease-causing bacteria and transfers to it the resistance gene. If that newly armed bacteria multiplies and causes an illness, antibiotics will not help. This is one example of how diseases that cannot be controlled with antibiotics get started. Until now, however, resistant diseases have started because antibiotic use promotes the growth of bacteria with the occasional, naturally occurring resistance gene. Biotechnology offers the possibility of the *deliberate* selection and reintroduction of huge numbers of antibiotic resistance genes into the environment.

Concerns about the widespread dispersal of antibiotic resistance were dismissed in the RMBPC-2 assessment in part based on the assumption that the proposed introduction of the resistance genes in RMBPC-2 would be small in comparison to the natural number of genes promoting resistance to streptomycin and spectinomycin already present in soil organisms and the human body. The risk assessment does not cite quantitative data on

the actual number of soil, commensal, or pathogenic bacteria that are resistant to streptomycin and spectinomycin, or how many are, for example, actually inhaled by farmers during planting or by workers during the seed coating. Perhaps these data are not currently available, or would be difficult to collect. However, such information would allow a comparison with the number of organisms introduced to soil, or found on skin, in lung or gut as a result of exposure to the genetically engineered bacteria. At a minimum, the risk assessment should state whether this information is in fact known, and give decisionmakers some idea of how difficult it would be to verify its conclusions.

Another question raised by the "insignificant numbers" rationale is how such quantitative relationships might be used, were they available, to identify risks from the introduction of RMBPC-2. For example, how many RMBPC-2 bacteria does it take for the risk of transference of resistance to become "significant"? EPA has no guidelines on this topic.

Another issue raised by questions about antibiotic resistance is the biological significance of the mechanism of that resistance. There are important differences between RMBPC-2's antibiotic resistance and that found in soil micro-organisms. First, the RMBPC-2 resistance gene is derived from a pathogenic bacteria, *Shigella flexneri*, isolated from a clinic, not from soil micro-organisms. Antibiotic resistance elements (e.g., the Tn5 transposon, a small, transferrable ring of DNA) commonly found in clinical isolates like



*Shigella* and engineered into RMBPC-2 are found on a transferrable plasmid, while the antibiotic resistance genes in soil microorganisms generally reside on the large, less readily transferred chromosome.<sup>83</sup> Thus, the RMBPC-2 resistance gene may be more transferrable (less stable) than those resistance genes already found in soil.

An additional reason the risk assessment dismissed the concern over the antibiotic resistance gene was because streptomycin is not a primary treatment and may be used in conjunction with other therapies:

Therapies for treatment of the other diseases (tuberculosis, *M. avium* infection, gonorrhea, chancroid, granuloma inguinale, actinomycosis, pneumonia caused by *Klebsiella*, rhinoscleroma, enterococcus urinary tract infections and streptococcal endocarditis) ... do not use streptomycin as **THE DRUG OF CHOICE** for treatment<sup>84</sup>

The very number of diseases cited illustrates that streptomycin, while not a primary treatment, is still used, and it ignores the likelihood that streptomycin may again become

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<sup>83</sup>van Elsas, J.D., Antibiotic resistance gene transfer in the environment: An overview, In: Genetic Interactions Among Micro-organisms in the Natural Environment, E.M.H. Wellington, J.D. van Elsas, eds., Pergamon Press, Oxford, 1992; Chater, K.F. et al., Genome flux in *Streptomyces coelicolor* and other streptomycetes and its possible relevance to the evolution of mobile antibiotic resistance determinants, In: Transposition, A.J. Kingsman, K.F. Chater, S.M. Kingsman, eds., Cambridge Univ. Press, Cambridge, 1988.

<sup>84</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, pp. 12-13.

important in areas where it has not been used extensively and resistance to it has declined:

In 1991, with the world facing a tuberculosis crisis, it was suddenly noted that the global supply of streptomycin was tapped out. The second-oldest antibiotic in commercial use was no longer manufactured by any company. Unpatented, cheap and needed solely in developing countries, it offered no significant profit margin to potential manufacturers. When drug-resistant TB surfaced in major U.S. cities that year, the Food and Drug Administration would find itself in a mad scramble to entice drug companies back into the streptomycin-manufacturing business.<sup>85</sup>

The same logic was used, even less effectively, for spectinomycin. The assessment stated that spectinomycin, which is used to treat drug-resistant gonorrhea, has "limited application in treatment of human diseases" but admitted that "There is **SOME** (emphasis added) interest in treating chancroid among human populations that display poor patient compliance because it shows fairly good results with a single dose."<sup>86</sup>

The implication of this rationale is that it is not of great concern to EPA that the non-compliant population (*i.e.*, prostitutes) have untreated venereal disease. The AIDS experience having made clear the value of controlling venereal disease, the value of spectinomycin and other antibiotics for human medicine is clear.

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<sup>85</sup>Garrett, L., The Coming Plague: Newly Emerging Diseases in a World Out of Balance, Farrar, Straus and Giroux, New York, 1994.

<sup>86</sup>Emphasis added. page 13.

## THE PREMATURE COMMERCIAL RELEASE OF GENETICALLY ENGINEERED BACTERIA

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Finally, even though the assessment acknowledged that both humans and farm animals are treated with spectinomycin and streptomycin, it stated that there could be no selection for resistant pathogens:

Therapies for diseases which utilize streptomycin as the drug of choice (tularemia, brucellosis and plague) are not likely to be affected by commercial use of RMBPC-2. Populations of *F. tularensis*, the cause of tularemia, do not appear to have developed resistance to streptomycin **AT THIS TIME**, possibly because the rarity of the disease and the reservoir for the etiological agent do not lead to a pattern of high selective pressure for resistant strains. That is, drug therapy for a few patients does not represent a pattern of high use of the antibiotic, and wild animals (the non-human reservoir of this disease) are not generally exposed/treated in the wild by antibiotics. It is not **ANTICIPATED** that this pattern of negligible selective pressure will change ... . It is the selective pressure exerted by antibiotic use that is the most important factor in the emergence, spread and maintenance of antibiotic resistance genes in pathogen populations.<sup>87</sup>

Thus, the assessment assumes that resistant populations of pathogens occur only as a result of the mechanism that is familiar to scientists today: selection pressure exerted by clinical exposure to antibiotics. This says nothing about the significance of the proposed use of RMBPC-2, a novel method by which huge numbers of *additional* antibiotic resistance genes would be distributed. Under the proposed use, RMBPC-2 would be introduced on millions of acres of agricultural fields nationwide, where tularemia, plague, and

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<sup>87</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, page 12.

brucellosis vectors (e.g. rodents, cattle) are endemic (cattle are fed alfalfa and are allowed to graze alfalfa fields), and it would be repeatedly<sup>88</sup> introduced in a "bolus dose." There was no consideration in the risk assessment of the possibility that large numbers of resistance genes might be transferred to pathogens in this new situation, and that repeated applications might maintain endemic resistant populations of pathogens.

Further, because there are "hot spots" of bacterial activity in soil, the occurrence of antibiotics in specific sites in soil is now more accepted as possible among scientists than in the past. Conjugational transfer has been shown to take place in these hot spots:

It is likely that the micro habitats where antibiotics are produced provide selective environments where antibiotic resistance and successful transfer are favored.<sup>89</sup>

Thus, the selection pressure that the risk assessment dismisses may in fact be possible at some sites.

Ironically, RMBPC-2 does not have to contain antibiotic resistance genes. There are other effective methods to "mark" bacteria that would eliminate the antibiotic resistance

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<sup>88</sup>A field of alfalfa can be planted in one day; the crop is typically replanted in three- or four-year cycles.

<sup>89</sup>van Elsas, J.D., Antibiotic resistance gene transfer in the environment: An overview, In: Genetic Interactions Among Organisms in the Natural Environment, E.M.H. Wellington, J.D. van Elsas, eds., Pergamon Press, Oxford, 1992.

hazard altogether.<sup>90</sup> Despite the fact that the advisability of using antibiotic resistance markers has been raised as an issue to EPA's counterparts in other countries,<sup>91</sup> and the BSAC expressed some concern about widespread distribution of antibiotic resistance genes to EPA in 1989,<sup>92</sup> EPA has no policy on the use of antibiotic resistance markers, and its risk assessment did not discuss the possibility of requiring that antibiotic resistance markers be removed from RMBPC-2.

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<sup>90</sup>See for example, Pickup, R.W. and Saunders, J.R., Detection of genetically engineered traits among bacteria in the environment, *Trends Biotechnol.* 8: 329-335 (1990); Jansson, J.K., Tracking genetically engineered micro-organisms in nature, *Curr Opin Biotechnol* 6: 275-83 (1995).

<sup>91</sup>Advisory Committee on Novel Foods and Processes, *Report on the Use of Antibiotic Resistance Markers in Genetically Modified Food Organisms*, Ministry of Agriculture, Fisheries and Food, London, UK, July, 1994.

<sup>92</sup>In its January 19, 1989, report on antibiotic resistance markers, the BSAC consensus was that "In the ideal situation, GEMs licensed for release should not contain exogenously acquired genes bearing resistance to an antimicrobial agent." When asked in January 1995 if the BSAC still held these views, it stated that, although streptomycin resistance "may be preferable to markers whose effects on the behavior of micro-organisms are unknown," that: The Subcommittee on Premanufacture Notification does not believe it appropriate to treat antibiotic resistance genes as 'housekeeping genes' and **SUGGESTS THAT THE AGENCY ROUTINELY ASK APPLICANTS WHY AN ANTIBIOTIC RESISTANCE GENE WAS SELECTED AS A MARKER;** (emphasis added) why the marker is in a commercialized product, if it is, and a description of the availability of alternatives." These questions were not asked of the manufacturer of RMBPC-2.

## Public Health: Unknown Pathogenicity or Toxicity

The risk assessment completely dismissed the possibility that RMBPC-2 could be toxic to humans because the parent *Rhizobium* is not known to be toxic. This rationale is not universally held by microbiologists. As stated by Gillett et al.:

The extent to which an engineered organism differs from its parent wild type is not adequate for distinguishing problematic from non-problematic organisms.<sup>93</sup>

Under typical commercial use, tens of thousands of farmers, distributors and seed processing plant workers<sup>94</sup> would handle RMBPC-2 exactly as the wild strains have been handled, resulting in inhalation, skin contact and ingestion of the bacteria and in transport on clothing to their homes and families. Because no toxic effects to humans have been reported with the wild strain under these conditions, the risk assessment concludes that the bacteria is not toxic and that there will be no adverse effects, including allergic res-

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<sup>93</sup>Gillett, J.W., Stern, A.R., Levin, S.A., Harwell, M.A., and Andow, D.A. and Alexander, M., Potential impacts of environmental release of biotechnology products: Assessment, regulation and research needs, Ecosystems Research Center, Cornell Univ., 1985.

<sup>94</sup>80,000 farmers would be exposed during the first three years of use alone, U.S. EPA, Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti*, December 1994.

ponses.<sup>95</sup> It did not address the potential for opportunistic infections in immunocompromised individuals, effects in persons being treated with antibiotics, or other direct effects. Certainly new toxic and pathologic effects of well-characterized chemicals (e.g., estrogenic effects of nonylphenols<sup>96</sup>) and microbes (e.g., *Helicobacter pylori*'s role in gastrointestinal ulcers<sup>97</sup>) continue to be discovered. Thus it is entirely conceivable that *R. meliloti* is the cause of some previously unrecognized toxicity or pathogenicity.

The assessment supported its conclusion of safety with the astounding rationale that *R. meliloti* cannot have toxic properties that have never been recognized or investigated, because negative studies (i.e., those that show no effect of an agent) do not get published. The implication is that studies exonerating *R. meliloti* have actually been done, but they have not been published because they were negative:

Since findings of no effects are rarely considered acceptable for publication, the absence of publications on *R. meliloti* and human health effects cannot be dismissed simply as studies

never having been done. Rather, the absence of such information, together with the absence in *Rhizobium meliloti* of characteristics that are recognized as factors necessary for colonization or infection of humans, lead to the conclusion that no human health hazards are associated with *R. meliloti* ... The above analysis ... provides a wealth of information **BY INFERENCE** on any potential human health hazards of RMBPC-2."<sup>98</sup>

While it may be true that both *R. meliloti* and RMBPC-2 are not pathogenic, the risk assessment presents no experimental results to support that conclusion. It is generally accepted among scientists that it is impossible to do enough studies to actually *prove* that a chemical or biological agent has no adverse effect. Even well-done negative studies are regarded as less compelling than positive studies of similar quality. This raises a central question for regulators: how much negative data are necessary to reasonably support a conclusion of safety, especially when no positive data are in hand?

EPA could easily require some simple supporting data to be collected. For example, the risk assessment stated that *R. meliloti* could not be pathogenic because it is not known to colonize human or animal lung or gut. No data were presented to support this assumption or the assumption that the organism could not survive long enough in a host to transfer genes to a pathogen. Yet studies on the viability of RMBPC-2 in animal lung and gut could be easily conducted. EPA scientists in the Agency's genetic laboratory in

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<sup>95</sup>Allergic response in workers handling RMBPC-2 coated seed has in fact been reported, Biotechnica, Inc., "Field test of genetically engineered *Rhizobium meliloti*," February 6, 1987.

<sup>96</sup>Soto, A.M., Justicia, H., Wray, J.W. and Sonnenschein, C., p-Nonyl phenol: an estrogenic xenobiotic released from "modified" polystyrene, *Environ. Health Perspec.* 92: 167-173, 1991.

<sup>97</sup>Halter, F., Hurliman, S. and Inauen, W., Pathophysiology and clinical relevance of *Helicobacter pylori*, *Yale J. Biol. Med.* 65: 625-638 (1992).

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<sup>98</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, p. 9.

Research Triangle Park, North Carolina, routinely test microbial viability with animal models. Experiments to determine the potential of RMBPC-2 to transfer genes to pathogens could also be performed rather easily. A simple test to determine if RMBPC-2 could survive at human body temperature would provide at least some support for the conclusion of safety. Instead, the risk assessment cited Material Safety Data Sheets<sup>99</sup> as evidence of safety, hardly a credible source as the foundation for the first release of a genetically engineered micro-organism.

In contrast to EPA's approach to the precedent-setting release of RMBPC-2, FDA supported its review of the genetically engineered FlavrSavr<sup>TM</sup> tomato by requiring that the developer, Calgene, produce toxicity data, even though the general thought among FDA toxicologists was that there would be no toxic effects.<sup>100</sup> (*See Appendix 3, Actions by Calgene and FDA to support safety claims for the FlavrSavr<sup>TM</sup> Tomato.*) The difference between the thoroughness of FDA's and

EPA's reviews is striking in that both agencies agreed almost a decade earlier that they should "utilize scientific reviews of equal rigor."<sup>101</sup>

## Public Health: Genetic Transfer

Commercial use of genetically engineered organisms may be a way to place new genes in "vector" organisms and through those organisms, move them to humans, food animals and natural areas in virtually every part of the country. Cows turned out into fields planted with alfalfa containing the resistance genes in its roots, or fed that alfalfa will eat and inhale the genes. If they survive in farm animals, they may be found in bacteria in meat and eggs produced from these animals. Even alfalfa teas and sprouts intended for human consumption may be contaminated with the new bacteria and their antibiotic resistance genes.

The implications of this are not known, but the most obvious, worrisome scenarios should be considered more seriously than they were in EPA's risk assessment. For example, suppose that farmers in Southwestern states repeatedly plant their fields with alfalfa seed treated with RMBPC-2. This increases the number of RMBPC-2 (and the number of antibiotic resistance genes) in soils and on alfalfa. Perhaps RMBPC-2 will compete for water and nutrients more effectively than wild *Rhizobia*

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<sup>99</sup>Chemical manufacturers are required by the Occupational Safety and Health Administration (OSHA) to distribute Material Safety Data Sheets with their products. These are worker education materials that list chemical hazards, proper handling and protective gear for product chemicals. They are not reviewed by OSHA and vary in quality from well-researched, complete summaries of toxicity to the inaccurate, incomplete and misleading. The risk assessment cited the MSDS as evidence that skin protection would not be necessary for workers manufacturing the genetically engineered organism. It is highly unlikely that Research Seeds' MSDS was based on any toxicity data collected with the new organism. A copy was not included in the risk assessment.

<sup>100</sup>59 Federal Register 26700, May 23, 1994.

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<sup>101</sup>51 Federal Register 23302, June 26, 1989, Coordinated Framework for Regulation of Biotechnology; Announcement of Policy and Notice for Public Comment.

and become the dominant *Rhizobia* in the area. Now, rodents that harbor fleas carrying plague bacteria burrow in these fields<sup>102</sup> and come in contact with the alfalfa. They pick up RMBPC-2 in lung, the gut and on their fur. The rodents' fleas, and the plague-causing bacteria they carry, are exposed to RMBPC-2 and actively assimilate the antibiotic resistance gene.<sup>103</sup> What are the chances that a form of streptomycin resistant plague will become established? Theoretically, large-scale use of streptomycin would be required to "select" for a resistant form of plague, and streptomycin is not used frequently in human medicine in this country. Could RMBPC-2 displace other strains of *R. meliloti* to such an extent that plague bacteria would routinely acquire resistance? Streptomycin is important in treating advanced cases of plague, and is the drug of choice for treating the pneumonic form of that disease. Loss of this treatment would make treating advanced cases of this rare but serious disease significantly more difficult.

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<sup>102</sup>Plague occurs most commonly in Oregon, Nevada, California, New Mexico, Arizona and Colorado. Centers for Disease Control, Document # 351512, November 19, 1992

<sup>103</sup>Bacteria appear to select genes that are of adaptive value, including those conferring resistance to antibiotics (See, for example van Elsas, J.D., Antibiotic resistance gene transfer in the environment: An overview, In: Genetic Interactions Among Organisms in the Natural Environment, E.M.H. Wellington, J.D. van Elsas, eds., Pergamon Press, Oxford, 1992).

## Public Health: Uncharacterized DNA

When genes are inserted into an organism by recombinant DNA techniques ("biotechnology"), small bits of genetic material from the donor organism may be inserted inadvertently along with the intended genes. When, as in this case, the functions of the genetic material are unknown, it is termed "uncharacterized DNA." Uncharacterized DNA is also an issue for this risk assessment because it raises uncertainties about the behavior and characteristics of the engineered organism when compared with the normal parent strain.

Scientists are learning that, in microorganisms, even small amounts of DNA can code for very complicated functions. Recently, for example, it was discovered that a single gene in *Shigella flexneri* gives this pathogen the ability to use a host cell's proteins to produce a tail that *S. flexneri* needs for movement inside host cells in the intestine. That gene, when transferred to *E. coli*, a usually benign and always tailless intestinal bacteria, causes *E. coli* to grow a functional tail and become motile.<sup>104</sup>

*R. meliloti* RMBPC-2 contains DNA segments from the donor bacteria that were added unintentionally during addition of the desired genes. Some of this DNA is thought to code for *E. coli* RNA protein, some is thought

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<sup>104</sup>Theriot, J.A., and Goldberg, M.B., *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility, Proc. Nat. Acad. Sci. 92: 6572-6576 (1995).

to be "similar" to genes in another donor *Rhizobium*, *R. leguminosarum*, and some, from *K pneumoniae*, is left over from gene "cassette" construction (the piece of genetic material spliced together from the various donors and inserted into the recipient bacteria is termed a cassette). The risk assessment considers this DNA to be of "low concern" even though its functions and how it might affect the behavior of RMBPC-2 are unknown. It concludes that despite these and the intentional changes in its genetics, RMBPC-2 will express the same behavior as the wild organism.<sup>105</sup> It seems, however, that small bits of uncharacterized DNA may be more important than previously thought.

Finally, the genetic manipulations performed on RMBPC-2 have created a defect in the new creature. It cannot utilize the sugar myo-inositol because the donated genes were inserted into a region of its plasmid (the "ino" region) that would allow it to use this sugar, and upon insertion, damaged this part of its genetic instructions.<sup>106</sup> The reason that wild strains of *Rhizobia* can metabolize myo-inositol is unknown, but the risk assessment

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<sup>105</sup>Strangely, in a letter to Citizens for Accountable Genetic Engineering, from Joseph S. Carra, Deputy Director, Office of Pollution Prevention and Toxics, October 13, 1994, EPA stated that: "The Agency, through its assessment, determined that the modified strains of *Rhizobium meliloti* are well characterized, as are the inserted genetic material (i.e., **THE FUNCTION OF ALL OF THE INSERTED GENETIC SEQUENCES ARE KNOWN** - emphasis added-)."

<sup>106</sup>It is not clear if the damage is to a gene that codes for an enzyme that metabolizes myo-inositol, or if it is to a gene that controls multiple functions in the bacterium.

dismisses RMBPC-2's disability as insignificant. Myo-inositol may be provided by the host plant for nutrition of the bacteria during the winter.

### Agriculture: Antibiotic Resistance

In agriculture, streptomycin is used against bacteria causing fireblight in pears and apples, blights of rice caused by *Xanthomonas* bacteria, rose blight caused by *Agrobacterium tumefaciens*, and other diseases in tomatoes, peppers, and chrysanthemums. It is used for European Foul Brood in bees and in aquaculture it is used to treat the fish disease Ich.

It is thought that soil organisms have developed antibiotic resistance to protect themselves from antibiotics produced by other organisms living in the soil with them. The *Shigella* bacteria (and RMBPC-2) resists streptomycin and spectinomycin by a mechanism called adenylation. This is not necessarily the mechanism used by other *Rhizobia* or by pathogens resistant to streptomycin.<sup>107</sup>

The role of selection pressure in the emergence of antibiotic resistance when resistance genes are repeatedly distributed into an area in concentrated form, for example, has not been considered. Could resistance by this

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<sup>107</sup>Sundin, G.W. and Bender, C.L., Ecological and genetic analysis of copper and streptomycin resistance in *Pseudomonas syringae* pv. *syringae*, *Appl. Environ. Microbiol.* 59: 1018-1024 (1993).

mechanism allow bacteria possessing it to have an advantage over those whose resistance is through other mechanisms? This might occur if the adenylation mechanism were more effective than other conventional mechanisms. If so, it might allow soil bacteria with the new mechanism to predominate. The Agency has no guidance and apparently no information on this question.

The risk assessment did not address the significance of RMBPC-2 for the Lay Animal Health Market,<sup>108</sup> an important omission, since spectinomycin is widely used over-the-counter by farmers as a treatment for upper respiratory infections of cattle.<sup>109</sup> These animals routinely

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<sup>108</sup>Farmers and industrialized producers have learned to use pharmaceuticals in ways that may not have been approved by the FDA but which based on experience do the job. The Lay Animal Health Market is the designation that the Pharmaceutical Industry has traditionally given to the agricultural over-the-counter business in pharmaceuticals and feed additives, including products containing antibiotics. Farmers and industrialized poultry and large animal producers/feeders routinely acquire antibiotic-containing feed additives over-the-counter either from a local farm store, distributor, or directly from the pharmaceutical company. A veterinarian's prescription is often not required. Indeed, veterinarians have only an advisory role in the Lay Animal Health Market, especially with respect to the industrialized animal producers/feeders.

<sup>109</sup>Spectinomycin, in a formulation intended and FDA-approved for use in poultry drinking water, is widely used by the cattle feedlot industry as an injectable medication for prophylaxis against or treatment of upper respiratory infections. The water-soluble formulation is dissolved in a bucket of tap water and then injected into the cattle. This manner of preparing an injectable treatment from an oral formulation first originated with the large broiler chicken producers many years ago and was quickly taken up by the cattle feedlot industry. This use of a non-injectable formulation costs much less than legitimate injectables in

occur in close proximity to alfalfa fields, and cattle are routinely fed alfalfa and allowed to graze in fields planted with alfalfa. If applications of RMBPC-2 result in resistance genes in soil and on alfalfa, it is likely that animal pathogens will have an opportunity to acquire resistance genes. For example, a herd of cattle treated with antibiotics and fed alfalfa grown with RMBPC-2, might, as a result of gene transfer in the bovine lung, develop an antibiotic-resistant respiratory infection that could be spread to other herds at sale, at stock shows and during breeding loans.

The potential effects of dispersal of RMBPC-2 on plant pests was also dismissed, even though streptomycin is used on important crops such as rice and apples. The assessment dismissed concerns about antibiotic resistance in orchards because resistance is already a problem in some orchards:

"Pathogens like *Pseudomonas syringae*, *Erwinia amylovora* and *Xanthomonas campestris* might THEORETICALLY acquire resistance to streptomycin through gene transfer from strain RMBPC-2. However, streptomycin resistance in populations of these bacterial plant pathogens was already a problem in SOME orchards before the initial field releases of RMBPC-2."<sup>110</sup>

This argument does nothing to answer the question of whether the massive release of RMBPC-2 would introduce antibiotic

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<sup>109</sup>(...continued)

sterilized vials and is typical of the Lay Market

<sup>110</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, p. 21.



resistance to orchards where it is not now a problem, or whether RMBPC-2 would exacerbate the resistance problem in orchards where it already exists.

Further, the orchard pathogens inactivate these antibiotics by phosphorylation, whereas the gene in RMBPC-2 works by adenylation. The risk assessment does not consider these differences in mechanism of action, does not present experimental results showing how many RMBPC-2 are transported through the air during planting, or quantify the relative contribution of resistance from the proposed use of RMBPC-2 in agricultural settings near orchards.

The question of what constitutes a "significant" introduction of a gene into the environmental gene pool raises a Catch-22 for the manufacturers of RMBPC-2. The resistance gene is intended as a marker so that spread of the new organisms from test plots can be monitored. If RMBPC-2 results in a significant introduction of antibiotic resistant bacteria to the soil, a concern about widespread environmental distribution of this resistance gene should reasonably follow. On the other hand, if the number of resistant bacteria added in a planting of RMBPC-2 coated seed is only a minute fraction of similarly resistant bacteria resident in soil, the gene would have no utility as a marker. If this is true, then it raises serious concerns about the reliability of field tests performed with RMBPC-2, which according to the manufacturers and to the EPA risk assessment, demonstrate that the organism is not transported in soil from the site of application.

## Agriculture: Genetic Stability and Transfer

Central to the assessment's conclusion that RMBPC-2 will not become a problem is the assumption that its new genes are "stable." Stable genes would be permanently fixed in the altered bacteria, could not be transferred directly to other organisms, especially other bacteria, be carried to other bacteria by viruses or be picked up by other bacteria from dead RMBPC-2 in the soil or the lungs or gut of a farmer using the product.

The introduced genes were inserted not into RMBPC-2's chromosomes, but into a large plasmid ("megaplasmid"), a ring of DNA often found in bacteria.<sup>111</sup> The fact that plasmid DNA is more readily transferred to other bacteria than is chromosomal DNA is significant because a central conclusion of this risk assessment is that the new genetic material is not likely to be transferred to other bacteria.

It is now widely recognized that in the natural environment, genetic instability is far from rare and that genes can be transferred among bacteria by at least three mechanisms, two of which have been demonstrated in *R. meliloti*. Gene transfer is known to take place among bacteria in aquatic and terrestrial environments, on plant surfaces and in sewage treatment plants. It occurs in the gut, the

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<sup>111</sup>Strangely, as late as June 30, 1994, Dr. Tom Wacek, Research Seeds' Director of Research and Development, wrote to Citizens for Accountable Genetic Engineering, stating that "The antibiotic marker is not on a 'marker plasmid' but rather is integrated into the main chromosome of the parent bacteria." The marker is in fact on a plasmid.

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urinary bladder, and respiratory tract of birds and mammals, including humans. Genetic exchange occurs among unrelated bacteria.<sup>112</sup> Bacteria may transfer genes to other bacteria across the "gram barrier" to yeasts and even to plant cells.<sup>113</sup> Under laboratory conditions, intergeneric exchanges between gram-negative species, including *Rhizobium*, and pathogens that cause bacterial diseases in plants can occur.<sup>114</sup> Although common genetic sequences are not required for some types of gene transfer, it is of concern that *Rhizobia* are related to a variety of pathogenic bacteria, since common DNA sequences make genetic transfer more likely:

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<sup>112</sup>Garrett, L., The Coming Plague: Newly Emerging Diseases in a World Out of Balance, Farrar, Straus and Giroux, New York, 1994, p. 225-226; Lorenz, M.G. and Wackernagel, W., Bacterial gene transfer in the environment, In: Transgenic Organisms, K. Wöhrmann and J. Tomiuk (eds.), Birkhauser Verlag, Basel Switzerland (1993). Mechanisms of gene transfer include conjugation, the transfer of genes directly between mating bacteria; transduction, in which genes are transferred from one bacterium to another by a bacterial virus, and transformation, the uptake of extracellular ("naked") DNA by a bacteria.

<sup>113</sup>Miller, R.V. and Levy, S.B., Horizontal gene transfer in relation to environmental release of genetically engineered micro-organisms, In: Gene Transfer in the Environment, S.B. Levy and R.V. Miller, eds., McGraw-Hill, Inc., 1989; van Elsas, J.D., Antibiotic resistance gene transfer in the environment: An overview, In: Genetic Interactions Among Organisms in the Natural Environment, E.M.H. Wellington, J.D. van Elsas, eds., Pergamon Press, Oxford, 1992.

<sup>114</sup>December 30, 1994, letter from Dr. Lydia Watrud of EPA's Office of Research and Development to Dr. Elizabeth Milewski, Coordinator of the BSAC review of the RMBPC-2 risk assessment.

In addition to being related to *Agrobacterium*,<sup>115</sup> *Phyllobacterium*, and several photosynthetic genera, *Rhizobium* is also closely related to animal pathogens such as *Bartonella*<sup>116</sup> and *Rochalimaea*,<sup>117</sup> soilborne and aquatic diazotrophs such as *Beijerinckia*, *Blastobacter*, *Mycoplana* and *Ochrobactrum*.<sup>118</sup> Genes from *Rhizobium* can be expressed in *Brucella* species. One of the largest reservoirs of brucellosis in this country is wild populations of ruminants such as elk, deer and bison. Brucellosis outbreaks occur sporadically in cattle, despite active vaccination programs. Some believe brucellosis in cattle is spread from wild ruminants. Since alfalfa is eaten by ruminants and is certainly browsed upon by wild ruminants, there is the opportunity for *Brucella* organisms to come in contact with RMBPC-2.<sup>119</sup>

Because the conclusion of stability is central to the risk assessment's conclusions about safety, any uncertainty in the stability conclusion is a significant uncertainty. The risk assessment concluded that RMBPC-2's genetic material is stable and is "unlikely" to

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<sup>115</sup>*Agrobacterium* is a soil organism that causes galls in some plant species by introducing into them a plasmid containing genes that code for plant growth hormones. Biotechnologists use the *Agrobacterium* plasmid as a way to insert genes of their choosing into recipient organisms.

<sup>116</sup>*Bartonella* is the cause of "cat scratch fever" and bartonellosis, an acute fatal infection or chronic disease transmissible from dogs and rodents to humans

<sup>117</sup>The *Rochalimaea* genus (now part of the *Bartonella* genus) contains bacteria that cause trench fever in humans.

<sup>118</sup>*Beijerinckia*, *Blastobacter*, *Mycoplana* and *Ochrobactrum* can cause bacteremia in humans.

<sup>119</sup>December 30, 1994, letter from Dr. Lydia Watrud of EPA's Office of Research and Development to Dr. Elizabeth Milewski, Coordinator of the BSAC review of the RMBPC-2 risk assessment.

be transferred to other organisms. The assessment concludes that conjugation with other bacteria is an event of "low probability" because conjugation has been observed only once in a related *Rhizobia* (not *R. meliloti*).<sup>120</sup> The assessment states that *R. meliloti*'s plasmid might be transferred if the bacterium contained or acquired a "helper" plasmid, but dismissed this because in two experiments, transmission was not observed. It is unclear to what extent these three experiments represent the potential of *Rhizobia* to transfer genes during nationwide application.

This raises the issue of what data are necessary to validate a conclusion of genetic stability. The Agency has no science policy or guidelines on this important topic, but simple experiments to determine if genetic exchange can take place in soil could readily be done, and would go a long way toward inspiring confidence in the conclusions of the risk assessment.

The ability of micro-organisms to transfer genes has important implications for genetically engineered "products" like RMBPC-2 since commercial use can introduce them (and the new genes they carry) into new places where they would not otherwise be found. For example, should seed treatments like RMBPC-2 become available, significant amounts of bacteria and the antibiotic resistance genes they carry will be washed into

sewer and septic systems from seed treatment plants. They will be distributed into surface streams and lakes. Farmers will plant seeds bearing billions of antibiotic resistance genes across rural landscapes not just once, but every time an alfalfa crop is planted. Antibiotic resistance genes will blow in the air down country roads each spring, onto corn fields and apple orchards, and will be washed into irrigation ditches. Repeated plantings will increase their numbers in soil.

Suppose that RMBPC-2 thrives in the Southwestern climate and soils,<sup>121</sup> and nodulates mesquite, a common plant in desert ecosystems. Could a "weedy" super-mesquite result which would devastate local agriculture? Or, if RMBPC-2 nodulates mesquite, but fails to provide adequate nitrogen, could this hardy plant disappear from Southwestern deserts? EPA has been slowly developing ecological risk assessment guidelines,<sup>122</sup> but so far has no guidance on the release of non-indigenous or novel genetically engineered species.

One hazard that the assessment actually acknowledged is that if RMBPC-2 turned out to be a poor nitrogen fixer but effective in competition with wild *Rhizobia* for occupying crop roots, it could result in decreased rather than the increased crop yields. A scenario such as this has already occurred in Louisiana, where the introduction of an ineffective genetically engineered *Bradyrhizobium* into

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<sup>120</sup>U.S. EPA, Risk Assessment Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, Constructive Analysis for *Rhizobium meliloti* Strain RMBPC-2, p. 12-14., December 1994

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<sup>121</sup>RMBPC-2 was not tested in the Southwest

<sup>122</sup>U.S. EPA, Framework for Ecological Risk Assessment, EPA/630/R-92/001, February 1992.

soybean fields now makes it more difficult to introduce more effective soybean inoculants to these fields.<sup>123</sup> If RMBPC-2 were to prove highly competitive, but a poor nitrogen fixer, it could have calamitous consequences if it were to displace wild *Rhizobia* and become the dominant *Rhizobium* in large areas.

The assessment concludes, however, that RMBPC-2 is effective at nitrogen fixation, despite the fact that tests of efficacy<sup>124</sup> were few and that most increases in yield were not statistically significant:

Data from small-scale field tests confirmed that RMBPC-2 is effective in nitrogen fixation ... There were several studies that showed that RMBPC-2 significantly increased alfalfa yields compared to its parental strain, PC and only one case of significantly decreased yield. In most of the studies over the years, RMBPC-2 produced slightly increased yields compared to PC, commercial inoculants, and uninoculated plots, **BUT IN MANY CASES, THOSE INCREASES WERE NOT SHOWN TO BE STATISTICALLY SIGNIFICANT** ... Overall, RMBPC-2 was shown to perform within the normal range of naturally occurring commercial inoculants.<sup>125</sup>

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<sup>123</sup>Tiedje, J.M., Colwell, R.K., Grossman, Y.L., Hodson, R.E., Lenski, R.E., Mack, R.N., and Regal, P.J., *Ecology* 70: 298-315 (1989)

<sup>124</sup>Bosworth, A.H. et al., Alfalfa yield response to inoculation with recombinant strains of *Rhizobium meliloti* with an extra copy of *dctABD* and/or modified *nifA* expression, *Appl. and Environ. Microbiol.* 60: 3815-3832 (1994)

<sup>125</sup>Emphasis added. Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994, p. 15.

The few tests that have actually been done with RMBPC-2 are not likely to be representative of the varied climatic and soil conditions found throughout North America, where a wide range of efficacy might logically be expected. Yet the assessment does not describe the types, numbers and characteristics (e.g., acreage, soil types, habitat) of field trials and laboratory experiments necessary to reliably extrapolate the behavior of RMBPC-2 from small-scale tests to nationwide application, nor does it recommend that such tests be done. A recent analysis of the field trial data in a USDA newsletter also questions the efficacy of RMBPC-2.<sup>126</sup>

## Agriculture: Potential Loss of Organic Status

The Organic Foods Production Act<sup>127</sup> requires that all organic farmers selling their agricultural products as being organically produced must be certified as such by the Secretary of Agriculture. A condition of certification is that the organic farmers must not have exposed their products to synthetic chemicals. The genetically engineered

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<sup>126</sup>C. Hagedorn, "Field trials with genetically engineered *Rhizobium*", *NBIAP News Report*, December 1994, citing Bosworth, et al., *Applied and Environmental Microbiology* 60:3815-32, 1994.

<sup>127</sup>7 U.S.C. § 6501, *et. seq*

*Rhizobium meliloti* appears to fall within the statutory definition of a synthetic chemical.<sup>128</sup>

The organic certification of farmers whose crops were exposed to this genetically engineered inoculant would be jeopardized. Significantly, organic farmers may be subject to inadvertent crop exposure to this genetically engineered micro-organism through two methods: inadequate labeling of inoculated seeds and migration from neighboring, treated tracts.

While the Federal Seed Act<sup>129</sup> prohibits false labeling of agricultural seeds in interstate

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<sup>128</sup>7 U.S.C. § 6502(21).

<sup>129</sup>7 U.S.C. § 1551, et. seq.

commerce, there are no specific labeling requirements for inoculants such as *Rhizobium meliloti*. Further, there are no specific requirements that the fact that the seeds have been treated with a genetically engineered organism be prominently displayed on the label or official certificate. Consequently, organic alfalfa farmers who buy seeds inoculated with *Rhizobium* may not know whether they are using a genetically engineered product.

As discussed earlier, little is known about the migration of *Rhizobia*. It is, therefore, also unknown if the crops of an organic farmer whose neighbors apply RMBPC-2 to their crops will be also exposed to the organism.

## CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

Genetic engineering is a powerful technology with great promise for human benefit, but whose potential adverse effects on the environment and human health are global, significant and largely unexplored. The federal agencies charged with looking into the future to predict and to compare the risks and benefits of releasing genetically engineered organisms have a formidable task before them. This is a task for which EPA is not yet prepared, as demonstrated by its assessment of *Rhizobium meliloti* RMBPC-2. The following recommendations, if implemented, could put the Agency on a more sound and defensible path toward wisely executing its responsibilities in this area.

- ✓ EPA should regulate products of biotechnology to **protect human health and the environment, not to promote** those products.

The Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) confer on EPA the authority to regulate "products of biotechnology." Given the DDT experience, one would expect EPA to have developed a framework for evaluating the risks associated with releasing living organisms into the environment.

- ✓ As in past development of risk assessment guidelines, EPA should assemble experts from inside and outside of the Agency to advise it on how best to assess the risks associated with this emerging technology. EPA should move cautiously before granting any approvals, waiting until it has in place a good method to determine how, when and which new creatures could safely be released into the environment.
- ✓ The EPA Administrator should direct the EPA Risk Assessment Council to immediately begin production of official agencywide guidance on ecological and health risk assessment for biotechnology. All EPA Programs, including the Office of Research and Development and EPA regional offices, should participate in development of this guidance. EPA should solicit input from scientists in other federal agencies. Via public notice, input should be sought from all interested parties outside the Agency, including environmental groups, health professionals, industry representatives and organic growers. This guidance should:
  - be completed as quickly as possible, and as units are completed, they should be released as interim guidance;

- include guidance for decisionmakers to help them understand the hazards of releasing organisms into the environment, especially the significance of genetically engineered organisms and those that cannot be contained or controlled once released;
  - should also explain for decisionmakers what type of releases carry “significant” risk; and
  - describe in detail the elements of a thorough and responsible risk assessment for introduction of genetically engineered organisms into the environment, including:
    - a. guidance on adequate data for identification of hazards and assessment of risk from loss of biodiversity, creation of weedy species, and indirect effects, such as development of antibiotic resistance or resistance to toxins incorporated into plants;
    - b. methods for quantifying risk, genetic stability, exposure, containment and eradication; and
    - c. examples of appropriate characterization of risks, including the significance of uncharacterized DNA.
- ✓ The EPA Administrator should direct EPA’s Office of Research and Development to conduct literature searches and surveys in order to summarize current public and professional opinion on the use of biotechnology so that Agency decisionmakers will understand the social and ethical context in which they make determinations of “acceptable” risk for biotechnology.
  - ✓ EPA should state as its official science policy that there is *no* genus whose organisms can always be assumed to be safe for introduction. As a corollary, EPA should establish the science policy that “If the parent organism is generally recognized as safe, it does *not* necessarily follow that an organism genetically engineered from that parent is also safe.”
  - ✓ EPA should withdraw from its proposed TSCA Test Rule the waiver of its authority to regulate the *Rhizobia* and *Bradyrhizobia* genera.
  - ✓ EPA should state that the burden of demonstrating safety falls on applicants, not on EPA. Valid, appropriate research on toxicity and pathogenicity must be used to support claims of safety. It should detail the tests that will be required in the risk evaluation process.
  - ✓ EPA should declare a moratorium on the release of new organisms until appropriate risk assessment guidance and test requirements are developed.
  - ✓ Concurrent with the risk assessment guidance, risk management guidance should be developed to address how, when and who within EPA will coordinate the

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Agency's risk assessment process with FDA, CDC and USDA.

- ✓ The Administrator should direct the TSCA and FIFRA Programs to develop risk management policies clearly defining how risks are to be incorporated into its cost-benefit analysis of applications for the release of genetically engineered organisms. This should address the issue of whether *any* risk will be acceptable if a product is not clearly efficacious and beneficial to the public and the manufacturer.
- ✓ The BSAC should be incorporated into the Science Advisory Board, and appropriate committees should be formed to advise the Agency on issues relating to introduction of new species (both genetically engineered and natural non-indigenous) and genetically engineered products.
- ✓ The EPA Administrator should prohibit Agency staff from drafting scientific reports for the SAB and BSAC.
- ✓ The Administrator should prepare and propose to Congress legislation that would give EPA clear authority to regulate living organisms as such under TSCA (as opposed to new chemicals). The legislation should give EPA the authority to make rules and regulations that address the unique properties of living organisms.
- ✓ The Administrator should direct the Agency's Office of Research and Development to do research that can be used to answer questions about the fate and transport of, containment and eradication of, and gene transfer to or from genetically engineered organisms.
- ✓ The Administrator should direct the Assistant Administrator for International Activities to coordinate with the European Union and other countries a moratorium on releases until common safeguards (risk assessment guidance, containment and control) can be established.





## APPENDIX ONE

# BSAC STATEMENTS CITING INADEQUATE DATA FOR RMBPC-2

p.3: "... no specific tests of the regulation of the newly introduced DNA have been presented ..."

p.4: "This predicted lack of advantage might be tested more directly by analysis for stability of the *nif* region of the insert of RMBPC-2 recovered after field growth ..."

p.5: "Conjugation does appear to be the most likely route of transfer ... However, in the absence of direct tests for transfer of the introduced DNA, this conclusion should be considered provisional."

p.6: "Currently there are not sufficient data available to generate a number representing the probability of conjugative transfer of the cassette from RMBPC-2.

"Direct experimentation with genetic exchange in soil or soil/plant microcosms is possible and would provide more concrete evidence for or against exchange from RMBPC-2.

"... one member considered this an essential step before approval of release and another thought such tests should

generally be part of testing prior to commercial release of genetically modified micro-organisms ... ."

"Negative evidence in carefully done tests would add confidence that tests had been done to safeguard against untoward effects."

p.6: "Only one field trial from rhizosphere colonization is presented for RMBPC-2." Also, in the absence of information on recovery efficiencies, the plate count data provided cannot be said to show that the introduced genes had no significant effect on persistence of RMBPC-2. However, simple replanting of alfalfa at previous test sites, or most probable number analysis ..., followed by comparison of nodule occupancy by RMBPC-2 and the commercial inoculant should remove any remaining uncertainties."

p.7: "The information provided is not adequate to establish that RMBPC-2 is similar to other *Rhizohium meliloti* strains in ability to disseminate."

p.8: "No statistical test of confidence intervals are provided for the data reviewed in the draft EA assessment ..."

p.8: "... the data analyzed in the draft risk assessment are inadequate to reach conclusions regarding the survival or dissemination of RMBPC-2 upon large scale release ..."

p.8: "While available knowledge suggests that the inserted sequences are unlikely to affect the survival, dissemination or nodulation competitiveness of strain PC ..., the field test data are not adequate to establish this."

p.13: "... because of the limited data set, it is not possible to say with certainty that the specific yields obtained were typical of the prevailing conditions,.... . The limited data set...do not permit an evaluation of agronomic efficacy as compared to the parent strain."

p.13: "However, greenhouse testing of mesquite and sweet clover ... for relative competitiveness could remove any remaining uncertainties."

p.14: "that RMBPC-2 would have adverse effects on yields of other legumes ... although there are no data presented in the risk assessment to address this question. Testing ... would remove any remaining uncertainties."

p.15: "... little or no data were presented on the behavior of RMBPC-2 itself in terms of persistence, dissemination, competitiveness, and effects on non-target plants."

p.15: "A 'reseeding' test ... would determine whether populations of RMBPC-2 would remain at low levels after return of the host plant species."

p.15: The data provided in the draft risk assessment are few; but suggest RMBPC-2 either is no more effective than other rhizobial inoculants or only marginally better under certain soil conditions."

APPENDIX TWO

EXAMPLES OF INSTANCES WHERE OPINION  
FORMED THE BASIS OF CONCLUSION IN  
THE RMBPC-2 RISK ASSESSMENT

The commercialization request for *Rhizobium meliloti* RMBPC-2, includes many instances where opinion formed the basis of the conclusions reached in the RMBPC-2 risk assessment. The following examples are drawn from the risk assessment.<sup>130</sup>

p.6: "The *dctA* gene encodes a ... protein permease that allows for the transport of C4-dicarboxylic acids ... across the bacterial membrane, and is **UNLIKELY** (emphasis added) to perform any additional functions ... The *dct* fragment also contains a small amount of uncharacterized DNA. The uncharacterized DNA is of low concern since these *dct* sequences **APPEAR** (emphasis added) to be conserved between *R. meliloti* and *R. leguminosarum*." (the donor host).

p.7: "Each of the two 500bp fragments encodes the 5S rRNA protein of *E. coli*, but it is **UNCERTAIN** (emphasis added) whether the sequence is, or can be, expressed in *R. meliloti*."

p.8: "The RMBPC-2 construct **APPEARS** (emphasis added) to be stable."

p.8: "Transformation is also **UNLIKELY** (emphasis added) due to the number of barriers to transformation **IN GENERAL** (emphasis added). In addition, natural transformation **PROBABLY** (emphasis added) has not been observed for *Rhizobium*."

p.14: "... the issue of gene transfer of the *aadA* gene to pathogens treated using streptomycin or spectinomycin is **PROBABLY** (emphasis added) not significant in light of the fact that the *aadA* gene used in constructing RMBPC-2 ... is **PRESUMED** (emphasis added) to be present in many human pathogen populations."

p.16: "Although nodulation outside the specific cross-nodulation groups has been reported, it **APPEARS** (emphasis added) that this non-specific nodulation is limited and that *R. meliloti* does not **CONSISTENTLY** (emphasis added) nodulate these plants."

p. 18: "The indigenous *Rhizobia* specific for those legumes **MAY** (emphasis added) be

<sup>130</sup>Risk Assessment: Commercialization Request for P-92-403 *Rhizobium meliloti* RMBPC-2, December 1994.

able to outcompete an alfalfa-specific rhizobial strain, such as RMBPC-2, for nodulation.”

p.18: “It is also **UNLIKELY** (emphasis added) that RMBPC-2 would come into contact with these other crops in adjacent fields because of the geographic distribution of **SOME** (emphasis added) (e.g., fenugreek), and because the movement of *R. meliloti* in soil **APPEARS** (emphasis added) to be quite limited.”

p.18: “Even though RMBPC-2 **MAY** (emphasis added) be capable of nodulating **SOME** (emphasis added) wild legumes, and **MAY** (emphasis added) be ineffective in nitrogen fixation in those plants, there is also little concern for decreased growth of non-crop leguminous plants resulting in decreased biological diversity. First, since movement of *Rhizobia* is **USUALLY** (emphasis added) quite limited, there **MAY** (emphasis added) be little contact between RMBPC-2 and native leguminous species.

p.20: “... *Brucella*. These organisms do not **APPEAR** (emphasis added) to possess characteristics permitting them to engage in exchanges of genetic material.”

p.20: “While the use of these two antibiotics (streptomycin and spectinomycin) **APPEAR** (emphasis added) to be very limited for the treatment of animal diseases, **THERE ARE NO RELIABLE SOURCES OF STATISTICS ON CURRENT USE OF VETERINARY ANTIBIOTICS IN THE U.S.** (emphasis added).”

p.25: “... recombinant *Rhizobia* were studied for aerial, horizontal and vertical movement. There was little aerial dispersal ... on **THE DAY** (emphasis added) of planting ... nor on **THE DAY** (emphasis added) of termination ... Therefore, this lack of movement lessens the probability for coming in contact with this organism.”

p.25: “Since the parent strain seems to be a fairly competitive one, RMBPC-2 **SHOULD ALSO BE** (emphasis added).”

p.28: “Since rhizobial numbers **UNDOUBTEDLY** (emphasis added) decline during the processing operations and the field application step, these numbers represent a worst-case scenario for release.”

p.30: “All field evidence points to a strain that functions within the normal bounds of rhizobial inocula ... It **MAY** (emphasis added) be poorer than some strains under a **FEW** (emphasis added) circumstances, and significantly better under different circumstances ... This product **MAY** (emphasis added) be successful under those circumstances where it was reported to do best ...”

p.30 “... the nitrogen fixed during, and left in the field after rotation with RMBPC-2 **MAY** (emphasis added) reduce the need for chemical fertilizer in subsequent rotational field crops ...”

p.30: “... the genetic manipulations which lead to strain RMBPC-2 **SHOULD** not (emphasis added) alter the legume host range of strain RMBPC-2 ...”

## APPENDIX THREE

# ACTIONS BY CALGENE AND FDA TO SUPPORT SAFETY CLAIMS FOR THE FLAVRSAVR™ TOMATO

### Calgene was required to:

1. Provide data that APH(3')II, the protein formed in the genetically engineered FlavrSavr™ tomato, is:

- ☛ inactivated by stomach acid
- ☛ degraded by digestive enzymes and
- ☛ not modified by glycosylation

2. Conduct protein and DNA sequence comparisons using sequences in 4 data bases to establish that APH(3')II does not have significant homology to any proteins listed as food allergens or toxins in those data bases.

3. Conduct an acute mouse feeding toxicity study.

4. Conduct experiments to determine if APH(3')II in fresh tomatoes would render orally administered kanamycin ineffective:

- ☛ in vitro studies with tomato extract containing APH(3')II and kanamycin
- ☛ under normal gastric conditions

☛ under simulated abnormal gastric conditions

5. Analyze residual neomycin levels over 56 days in medicated cottonseed and rape seed feed meals both with and without APH(3')II (the protein would also be formed by genetically engineered cotton and rape seed plants).

6. Present data to demonstrate the potential for compromise of antibiotic therapy by transfer of kanXr gene, the gene that codes for the APH(3')II protein, to gut micro-organisms or intestinal epithelial cells. In these experiments Calgene measured DNA after in vitro exposure to stomach-simulating fluids and calculated worst-case transformation frequencies.

7. Present calculations for transformation from plants to micro-organisms in soil.

### Food and Drug Administration:

1. Published notice of receipts of request from Calgene for approval of use.

2. Published a policy for conditions for safe use of APH(3')II) as an aid in development of new crop varieties (57 Federal Register 22984, May 29, 1992). It contains a guideline to industry that outlines the approach for the safety evaluation of foods derived from transgenic plants.
3. Convened a public meeting of the FDA's Food Advisory Committee to undertake scientific discussion of FDA's approach to evaluating safety of whole foods produced by new biotechnologies. The Committee was asked if the approach used by FDA to evaluate the safety of the tomato was appropriate and if all relevant questions had been adequately addressed.
4. Considered whether the gene might be transferred to other organisms.
5. Acknowledged legal responsibility to review safety of food additives. Section 409© (3) (A) of the FFDCFA states that a food additive cannot be approved unless a "fair evaluations of the data available to FDA establishes that the additive is safe for that use." This requires "reasonable certainty."
6. Reviewed data and studies submitted by Calgene, and other data in its files; considered comments received in response to Federal Register notice announcing receipt of request from Calgene.
7. Researched which antibiotics affected by the Calgene gene are used therapeutically in the U.S.
8. Performed a safety evaluation. This addressed issues of:
  - ☛ direct effects of ingestion of the protein, including allergenicity
  - ☛ effect of the enzyme on the therapeutic efficacy of orally administered antibiotics.
9. Researched the number of proteins in whole foods; the toxicity of proteins; the significance of phosphorylating enzymes in the food supply; and the estimated dietary exposure to APH(3')II from tomatoes, canola oil and cottonseed oil.
10. Reviewed studies relevant to the application.
11. Reviewed the degradation of APH(3')II under simulated gastric conditions.
12. Considered the patient population likely to be exposed to aminoglycoside antibiotics.
13. Considered the potential for horizontal transfer of the kanXr gene to intestinal microorganisms and subsequent expansion of the population of antibiotic-resistant pathogens.

## APPENDIX FOUR

# DEFICIENCIES IN THE MARKET POTENTIAL EVALUATION

- ☛ EPA made no attempt to understand how this market operates. The Market Potential Evaluation (MPE) was based on information provided by Research Seeds and a few other sources. It does not appear that there was any attempt to verify the information with other competitors in the alfalfa seed and inoculant businesses.
- ☛ The MPE does not confirm Research Seeds' estimate of its market share. It concludes that since Research Seeds stated that it "controls 60% of the market," that it sells up to 168 million pounds of alfalfa seed. Actually, Research Seeds may have a 60% share of the *Rhizobia inoculant* market for alfalfa but does not have 60% of the alfalfa seed market. Alfalfa is sold by many seed companies across the U.S. Much of the seed that will be coated with RMBPC-2 will actually be sold under the Land O'Lakes label. The Nitragin Division of Liplha Chemicals, located in Milwaukee, Wisconsin, has a 40-50% share of the current *Rhizobia meliloti* inoculant market for use on alfalfa seed. Nitragin's peat carrier has been considered superior to Research Seeds' clay carrier, since clay is dusty and a significant amount of it does not stay with the seed, but falls to the bottom of the seed bag.
- ☛ The MPE does not take into account the fact that alfalfa fields are only reseeded approximately every four years.
- ☛ The MPE makes no attempt to evaluate the elasticity of the market. It assumes that the marketplace (farmers) are prepared to pay \$0.04 for the inoculant per pound of seed rather than the customary \$0.02, a doubling of price.
- ☛ The MPE claims that this product has the potential for greatly expanding the market for itself (and similar competitors) by eliminating chemical-based fertilizers, and thus leading to benefits "far in excess" of benefits to be realized from the product today. The MPE contains no data to support this.
- ☛ The MPE assumes a benefit of increased nitrogen presence in the alfalfa field such that another crop in rotation (e.g., corn) would have as much as a 14% reduction in the need for chemical fertilizers. There are no data indicating such a benefit.





