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## The Scientist's Role in the Controversy Over Genetic Engineering, Regulation and Utilization of Microorganisms: A Symposium Presented at the 100th Annual Meeting of the Iowa Academy of Science, Iowa State University, Ames, Iowa April 21-23, 1988: Introduction

Arthur K. Weissinger  
*Iowa State University*

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## The Scientist's Role in the Controversy Over Genetic Engineering, Regulation and Utilization of Microorganisms

A Symposium Presented at the  
100th Annual Meeting of the Iowa Academy of Science,  
Iowa State University, Ames, Iowa April 21-23, 1988<sup>1,2</sup>

### Introduction

ARTHUR K. WEISSINGER<sup>3</sup>  
Chair, Biotechnology Section

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As we begin, I would first like to thank the Iowa State University Agricultural Bioethics Committee, and especially Drs. David Kline and Mike Warren, for their support of the symposium. We are very lucky, I think, to have this kind of bioethics group associated with a university doing this kind of work. I think that's a relatively rare phenomenon and is a very important one.

The introduction of technologies for the direct genetic manipulation or alteration of organisms offers tremendous promise of improved agronomic prosperity through the enhancement of existing agricultural systems and through development of alternative products and methods. These technologies also pose philosophical, socioeconomic, and environmental questions. A number of these questions were considered at the bioethics symposium that was held here at Iowa State last November, the purpose of which was to examine the social, ethical, regulatory, and legal issues associated with the application of cell culture, molecular biology, and other so-called biotechnologies to agriculture. I believe that it was especially useful because it dealt with questions which scientists don't normally find within the scope of their work. I was gratified personally by the level at which these non-scientists were aware of these technologies — what is happening and how they affect the world. To balance and complement that set of discussions, some of us in the Biotechnology Section in the Academy decided that it would be helpful to examine the use of these new technologies from the standpoint of scientists who are actually involved in the work. Our time is rather limited, so we have chosen to study one example of the release of an organism, specifically the release of genetically modified microorganisms.

While these organisms clearly have tremendous positive potential, their containment is not as straightforward as, for example, the control of genetically engineered crop plants. I would tell you, however, that I do work on genetic engineering of crop plants myself, and some of the questions regarding their release are not as straightforward as we would like to think. I have been asked, for example, if we introduce a gene for herbicide resistance into a crop plant, what is the

probability that that gene will escape from that crop plant by outcrossing and form a herbicide-resistant weed species in the process. I think for crops like corn where there are no naturally hybridizing native plants in the U.S., it's a fairly straightforward question. For sunflowers, the question may be harder to answer. I have developed a stock answer for farmers who ask me that question. I think that this is a very, very good question to ask if somebody is going to sell you such materials. Ask it often, loudly, and demand answers. And when people give you answers, make sure that they do not include the words "quarantine", "National Guard" or "flame thrower". It's pretty important, I think.

In the case of genetically engineered microorganisms, some of the questions around the release of these organisms have been summed up in a series of five questions posed by Martin Alexander, an ecologist at Cornell. These are: Will a released organism survive in the environment? Will it multiply? Will it spread beyond its original area of application? Can it transfer its genetic material to other organisms? Will the original organism or any of those that might pick up its genes prove harmful?

The implicit assumption is that once microorganisms are released, they are thereafter part of the ecosystem, and they are under the control, if you will, of natural forces and no longer under the control of humans. How then do scientists involved in this work view the modification and release of organisms? Part of the purpose of this discussion today is to point out that many of these questions are amenable to experimentation, but it is difficult to gain permission for scientists who are qualified to do those experiments to actually carry them out.

We are very fortunate today in having three noted workers in this area, Dr. Donald Dean, Dr. Steven Lindow, and Dr. Anne Vidaver. In addition, we are fortunate to have with us two noted Iowa citizens, Dr. Donald Huffman and the Honorable Mr. Paul Johnson, who will question the panel after they have described their research and their understanding of the situation.

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<sup>1</sup>Because of the timeliness of this subject, this symposium is being published as an edited version of the transcript of talks given by the invited speakers. The editors thank Dr. Ruth Swenson, 1989-90, President of the Iowa Academy of Science, for her work in serving as special editor for these manuscripts.

<sup>2</sup>This symposium was sponsored by the Agricultural Bioethics Program at Iowa State University with support from the State of Iowa, the Joyce Foundation, and the Northwest Area Foundation.

<sup>3</sup>Current address: Department of Crop Science, North Carolina State University, Raleigh, NC 27695.

Let's consider the following. We have in this package a bacterium which is creating a survival mechanism for itself, a spore, and it is creating an insecticidal protein with which it hopes (in teleological terms) to create a niche for itself. Otherwise, it would be just like *Bacillus cereus*. *Bacillus thuringiensis* is about 90% similar to *Bacillus cereus*, which is a common soil microorganism.

If, somehow, we engineered this organism not to make the spore, but to make only the diamond-shaped crystal with its lattice of protein sub-units, this crystal by itself is no longer a microbial pest control agent; it is now a biochemical agent, as I described earlier. It is not living, and it has a finite lifetime in the environment. There are plenty of things for the biochemist and the microbial geneticist like myself to get excited about — the understanding of how this protein functions as a pesticide and the engineering of it to make better products from it.

Now we are going to discuss the current regulatory mechanisms that EPA uses for the registration of microbial pesticides. First of all, with all kinds of products, there is first identification of the product itself (e.g., as a protein), then how it is manufactured, including discussion of the formulation and any other ingredients that are added by the company that is making it. In some cases, diatomaceous earth is added as a carrier. In other cases, they add molasses as a sticking agent or more high tech versions to get the toxin to stay on the plant longer and to extend its insecticidal properties. The information required includes various analyses of the sample, certification of the limits (exactly what the toxic range is), analytical methods that are used to study the products, and physical and chemical properties of the toxin. Samples must be deposited. So this is the start — a very general review of what is expected of any kind of registration.

Now we come to a multi-tier system in which we have various tiers of tests. In general, if the first tier is passed without undue effect on the animal system that is being tested, the EPA, to save expenses, does not require further tier testing. However, if, for example, in the acute oral dermal inhalation examinations on animal models, the agent has harmful side effects, the other tiers of testing are necessary before the agent can be registered.

In the case of the microbial products, all of these passed easily on the first tier. Massive testing has been done. The first registry of microbial pesticides was in 1961. Since then, as I've indicated earlier, others have been tested, and so far, *Bacillus* has been a very safe model system in passing tests.

Finally, we now have ecological effects that are being tested in order to have the registration of microbial pesticides. Again we have the tier system, including avian oral tests, wild mammal tests, fresh water fish testing, fresh water aquatic invertebrate testing, estuary marine animal testing, plant studies, and non-target insect tests. If these are insecticides, what is their range, what is their effect on non-target insects?

Some manufacturers are a little concerned that these microbial pesticide tests are so specific. My particular line of research is the definition of the specificity of particular proteins, and some of them are really quite specific. One particular gene that looks almost like another gene produces a protein which has 100-fold more activity against one insect than another. This has very interesting consequences from a biochemical molecular genetic standpoint, but farmers may not have the same patience with these microbial agents. They want what they have had before — a quick fix, something that kills all of the insects and knocks them down tomorrow. When they wake up tomorrow morning, they want to see all those insects dead and off their crops. Some of these microbial agents don't work like that. First of all, they must be eaten by the insect, then they cause paralysis of the insect, but the current products don't cause killing in every case. For example, the cotton bollworm, the corn ear worm (*Heliothis zea*) is a great target for control. Practically everyone who is working on these microbial agents wants to make a protein toxin that is more specific to

*Heliothis zea*. At the present time, *Heliothis zea* can be intoxicated. We can tell that because after eating a sufficient amount of the protein, it doesn't continue developing, it doesn't gain weight, it doesn't eat plants any more, but it's still there, and if you touch it, it wiggles. This is not exactly what farmers have come to expect of pesticides, but I think that as they become aware of both the importance of getting away from chemical pesticides and as their extension agents explain to them how this pesticide works, they will begin to understand how to use it.

These are some of the current regulatory mechanisms, and I think that as they exist, they are very sufficient to test new products in terms of their impact on the environment and their toxicity to non-target organisms — man or other insects.

Let's talk a little about their current use. This is one of the most interesting aspects of what I want to discuss — the current use of *Bacillus thuringiensis* as a pest control agent. Data available from Canada show for various years the total forest acreage that has been treated to control spruce budworm. Pine tussock moth is also used. In 1979, only about 10% of all pesticide treatment used *Bacillus thuringiensis* and 90% was chemical. At the present time, about 75% of all pesticides used to control these major forest pests in Canada is *Bacillus thuringiensis*. I don't think Americans realize that it is such a popular agent in Canada, which I consider a little more modern country, in some cases, on the use of ecologically sound pest control measures.

We have here an example of a tremendous influx into the environment of this particular microbial agent. Just exactly how many bacteria are being put out there? It has recently been estimated that approximately 2.3 million kgs of *Bacillus thuringiensis* are released annually. I calculate that this is about  $4.5 \times 10^{20}$  bacteria or approximately the number of all the stars in the universe. We are putting out a lot of bacteria.

So we have done a very big experiment on the release of the microorganism into the environment, and we can ask from this experiment the five questions that were introduced by Arthur Weisinger, as posed by one of the most eminent microbial ecologists, Martin Alexander. What is the response? What happens to *Bacillus thuringiensis* when it is put out in nature like that? Can it mutate to become a permanent resident? Is it already a permanent resident? If you are not a microbial ecologist, I'll tell you that *Bacillus* is one of the major and most ubiquitous soil microorganisms. It is probably second only to *Xanthomonas*, so we would expect that this experiment is probably a worst case scenario. We are putting a bacterium out there that, from our naive assumptions, is the organism that could survive best in nature. As a matter of fact, that's not what happens. *Bacillus thuringiensis* is very rapidly decimated from the environment. One year after the treatment of a field, the presence of the exact strain that was placed out there is no more prevalent than could be seen from a bloom of these bacteria in a virgin forest where it was never disseminated. I'm not using this microorganism as a paradigm for release of all microorganisms, but I am saying this is what happens with *Bacillus*. You put out an extraordinarily large number of bacteria, and you find that these bacteria simply do not survive in nature in their own ecological niche.

What are the reasons for this? Part of them might have to do with the organism itself. It has been shown in numerous publications that these bacteria are particularly sensitive to ultraviolet light, they are very sensitive to plant extracts, and they are very sensitive to cold weather. Also, in more recent studies responding to the questions that we pose today (i.e., what's the effect of deliberate release of the microorganisms?), it has been shown that *Bacillus* has a very low tolerance to starvation.

Well, what is it going to starve from? Another major reason for its lack of survival is a microbial ecological one. Basically the microbial environment of the soil is not a rich one. It is a climax situation, but

unlike climax situations for plants, the climax in microbial soil means that these decomposers have decomposed almost everything there is to decompose, and the last thing they are doing is decomposing one another. That is what humus is. So when we dump a lot of these *Bacillus thuringiensis* on the soil, the ecological response is that it is not a rich environment for them. Secondly, there is a tremendous biotic resistance from the resident community of bacteria. They look upon these introduced microorganisms as food and break them down, so that the newcomers simply do not compete against the very entrenched residential population.

At the present time, I think that is what we know about why *Bacillus thuringiensis* doesn't survive, and this particular microorganism is a pretty good example of a major experiment. I don't think that we could try a grander scale experiment. Certainly if mutation were to be a problem, we could calculate that any possible gene in the organism could mutate, and we would still have between  $10^{10}$  and  $10^{14}$  microorganism mutant forms existent. For comparison, that's about the number of stars in our galaxy.

Finally, let me very quickly go through some of the benefits that we would hope to obtain. We hope to change the specificity to make the microbial agent more specific against our particular problems. *Heliothis zea*, for example, is an organism over which we would hope to

have better pest control. We hope to increase the expression of the microbial agent so that we can make more of these proteins per liter of fermentation broth, so it is cheaper to use and there is an economic incentive to get away from chemical pesticides. We hope to introduce these toxins into plants and plant epiphytes so that they become even more specific. There is little chance that we would control the monarch butterfly if these toxins were placed into a cotton plant, simply because the only insects that we wish to control are those that eat the cotton, as an example, and monarchs do not.

This is just an example of the kind of experiment that I do in my lab where I am substituting regions of one gene into another. In this way, I hope to localize where insect specificity occurs on this protein through the gene and also to improve that specificity. When we introduce genetic material into plants, the plant is making a systemic protein which, I say, is a beneficial, proteinaceous nutrient supply for humans but a very specific systemic toxin against a particular insect.

Finally, I would like to quote from Rachael Carson's book. "Specialists representing various areas of the vast field of biology are contributing — entomologists, pathologists, geneticists, biochemists, physiologists, ecologists — all pouring their knowledge and their creative inspiration into the formation of a new science of biologic control."