

## Assessing the Potential Impact of Planned Releases of Bioengineered Organisms Using Modeling and Nonmodeling Approaches

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Accepted for publication 31 October 1990 (submitted for electronic processing).

Planned releases of organisms modified by genetic engineering into the environment are now a reality in the United States, and it is likely that the number of such releases will increase in the near future as many research projects enter a "product" phase (26). Scientists called on to make recommendations on potential effects of planned releases often have insufficient data, and approaches need to be found that accommodate this lack of empirical data but still allow predictions of potential outcomes. In this regard, both subjective and objective (modeling) approaches have been used in similar situations requiring prediction of impact or risk (4,6,11) and offer much potential in assisting with the evaluation of bioengineered organisms. However, a realistic assessment of the current state of knowledge is that "standardized protocols do not exist for predicting either the kinds of risks or the magnitudes that could be associated with planned introductions of genetically engineered organisms into the environment" (26).

A planned release is the intentional introduction of a bioengineered organism into the natural environment. The potential impact of a planned release has to be considered at different levels of biological organization, i.e., populations, communities, and ecosystems. Impact is commonly construed as effects, although, more accurately, these are estimated as both impact (changes in system structure, system processes, externalities) and risk (the probabilistic assessment of the magnitude of the impact). In this paper, actual and potential techniques for impact assessment, using quantitative approaches, nonquantitative approaches, and a combination of both, are discussed.

### QUANTITATIVE APPROACHES

The potential impact of any organism or product introduced into the environment is a classic statement of the rationale for systems modeling: changing one aspect of a system may well produce changes, or the need for change, in other parts of the system, such that the whole is more than the sum of its parts (24). Many of the impending releases of bioengineered organisms will introduce them into complex and dynamic ecosystems (26). In such systems, changes could occur at any and many levels or sites, such that a systems model offers the only technique to capture all this complexity. Furthermore, modeling allows ex ante evaluation of changes in systems that, for all practical purposes, do not yet exist. As Andow et al (2) succinctly put it, "accurate estimation of risk requires testing in the field, but testing in the field requires prior estimation of risk." The ecological literature shows that many models have been developed for simulating the spatial and temporal dynamics of single- and multiple-species systems. In agriculture, models exist for major crops, many key pests, and even their interactions (22). These models of pest or crop dynamics and of losses have many applications in practical decision making, for example, in pest management. Such models may in turn be used for estimating the benefits of changing the system through the introduction of a bioengineered organism.

The use of different types of models for estimating the potential impact of bioengineered organisms introduced into the environ-

ment is exemplified by the work with non-ice-nucleating bacteria by Andow et al (1,2). These genetically modified bacteria were the subject of early controversies about their release for use in reducing frost damage on potatoes in California (16). In preparation for the series *New Developments in Biotechnology* (26), the U.S. Office of Technology Assessment (USOTA) funded a study on the potential environmental impact of widespread releases of non-ice-nucleating bacteria in agriculture (1). The study had several components, among which were estimates of 1) impacts in a worst-case scenario of worldwide release and of capabilities for survival, competitive ability, adaptive value, dispersal, and spread of non-ice-nucleating bacteria, 2) the effects of non-ice-nucleating bacteria on local and global precipitation processes, and 3) potential potato yield benefits from the use of non-ice-nucleating bacteria. Parts 1 and 3 are used here as illustrations of the use of different model types in impact assessment.

**A worst-case scenario.** A worst-case scenario was evaluated for the release of non-ice-nucleating bacteria for 1 yr with assumptions of maximum bacterial growth, complete displacement of ice-nucleating-active (INA) bacteria on cropland, and 100% movement of non-ice-nucleating bacteria into the atmosphere (1). The steps taken by the authors to arrive at their assessments were as follows: 1) identification of qualitative and quantitative information on INA bacteria; 2) estimation of the area of frost-vulnerable cropland in the United States and worldwide to determine the total cropland that could benefit from the use of non-ice-nucleating bacteria; 3) quantification of natality, mortality, and immigration and emigration rates of non-ice-nucleating bacteria, especially *Pseudomonas syringae*; 4) quantification of the processes and rates of dispersal and spread of non-ice-nucleating bacteria at different levels of scale; and 5) quantification of the adaptive value of non-ice-nucleating bacteria, especially their competitive abilities.

The study concluded that all particles can nucleate ice if it is cold enough; however, at temperatures above  $-5^{\circ}\text{C}$  there are few such sources, the main ones being living and dead bacteria. The most important INA bacteria, as far as plant agriculture is concerned, are *P. syringae*, *P. fluorescens*, *P. viridiflava*, and *Erwinia herbicola*. Andow et al (1) estimated that, of 648 *P. syringae* strains reportedly tested by researchers, 353 (54.5%) could be considered INA strains. The probability of discovering an INA strain in a new species was calculated as  $P = 0.014$ , compared to the probability of discovering an INA strain in an already identified species such as *P. syringae*, assuming the same number of strains were tested. These figures have to be regarded as starting points in an analysis, as they indicate the microbiological environment in which non-ice-nucleating bacteria would have to operate. The same authors estimated the area of cropland to which non-ice-nucleating bacteria might be applied to be about 110 million hectares in the United States and 710 million hectares worldwide. The total leaf area of frost-vulnerable cropland was calculated to be 330 million hectares in the United States and 3,300 million hectares worldwide. Non-ice-nucleating bacteria were estimated to occupy 25% of the potential environmental sites, and of these at least 0.24% of the sites have fewer than 100 INA bacteria. The study also showed that the intrinsic population growth rate of *P. syringae* is variable, with

daily rates from -1.61 to 5.61 per day. Dispersal was considered to occur during mechanical operations and on dry days with numbers like  $2.2 \times 10^8$  cfu/ha from pulverized potato haulm. The authors divided dispersal into five spatial scales: micro, local, meso, continental, and global. They concluded that different models are needed for these scales: for micro dispersal, passive diffusion; for local dispersal, a turbulent/Gaussian plume model; for meso dispersal, patches of habitat with uniform dispersal among patches; for continental dispersal, patches of habitat randomly varying in quality, joined by a stochastic dispersal process; and for global dispersal, no formal model. Bacterial deposition by rain on leaves was considerably higher (about 740-4,200 cfu/50 cm<sup>2</sup> of leaf area per hectare) than under dry conditions. The probability of survival is likely to be higher under wet than dry conditions. Ice nucleation was also postulated to provide a competitive advantage to INA bacteria. Andow et al (1) then integrated their information into a bacteria spread model resembling the diffusion-reaction model of Levin (15) and structured like the EPIMUL model of Kampmeijer and Zadoks (12). Simulations with the model (1) led the authors to conclude that in a worst-case scenario, with a homogeneous environment, in which all cropland received non-ice-nucleating bacteria for 50 yr, the probability that these bacteria could totally displace INA bacteria would be about 0.39. Under more constrained conditions, the probability would drop to between 0.005 and 0.05. In heterogeneous environments, non-ice-nucleating bacteria could only totally displace INA bacteria if large areas (e.g., 100,000 km<sup>2</sup>) were inundated with non-ice-nucleating bacteria early in a growing season.

**Potential benefits to potato from the use of non-ice-nucleating bacteria.** Another aspect of the above-mentioned study (1) dealt with estimating the economic benefits to be derived from the potential use of non-ice-nucleating bacteria in agriculture. Potatoes are a major frost-sensitive food crop, with about 513,000 ha grown in the United States, and were among the first to be involved in the controversy over planned release (5,16). Spring and fall frosts are known to cause damage to potatoes in parts of the United States such as northern California, Idaho, Minnesota, and North Dakota. The authors estimated potential losses using a simple potato crop model called SPUDGRO, which was developed in Minnesota and validated in several states (9,10). From the loss estimates, the potential economic benefits to be derived by reducing these losses through non-ice-nucleating bacteria were determined (1). Four production sites were selected for study: Fargo, North Dakota; Pocatello, Idaho; Mount Shasta, California; and Bangor, Maine (2). Potato growth and development were simulated for each site.

Data required at each site were average daily temperature (from 30-yr records), occurrence of the first frost, and incident solar radiation. Frequency distributions of first frosts in spring and fall were determined, as well as frequency distributions of temperatures. Potato growth was simulated for three emergence dates for the cultivar Russet Burbank, and the effect of frost was assumed to cause 90% destruction of the haulm. Expected annual yield loss was calculated from the cumulative probability distribution of frost events capable of causing the most loss. For example, the study found that at Pocatello, Idaho, the probability of an 18% loss was 0.25 for a 50% emergence date of 15 June and was 0.005 for a 50% emergence date 1 June (2). The expected losses for the two emergence dates were therefore 4.5 and 0.09%, respectively. From the simulations, the authors were able to determine cumulative probability distributions for different amounts of yield loss (in kilograms per hectare) for each of the four sites and each of three emergence dates. Data could then be summarized in annual expected yield loss curves for each emergence date. For example, at Pocatello, Idaho, if the potato price is \$0.09/kg and yield is 25,200 kg/ha, then a 10% loss equals \$227/ha. The maximum possible loss in Idaho was estimated to be \$111-568/ha (2). On the assumption that the total direct costs of using non-ice-nucleating bacteria are \$37-62/ha, i.e., comparable the cost of applying pesticides, then the potential benefit-cost ratios could be between 3.0 and 9.2. Although the

above analyses were done with many assumptions, they offer a realistic methodology for the interdisciplinary cooperation in impact assessment advocated by the USOTA (26).

The studies of Andow et al (1,2) have shown that it is possible, given knowledge of bacterial ecology and crop yield physiology, to develop systems models so that some educated guesses can be made on potential ecosystem effects resulting from the release of modified organisms into the environment. As data become available from dispersal/spread studies using improved monitoring tools such as monoclonal antibodies and markers (13), these data, when incorporated into models, will further increase the validity of simulation-generated educated guesses.

## NONQUANTITATIVE APPROACHES

The development of objective procedures for impact assessment of planned releases has admittedly lagged behind regulatory needs, resulting in the development, in many countries, of so-called guidelines for evaluation (19,21,27). These guidelines are at best attempts to collate current knowledge of biological aspects of the organism (genetic, phenotypic, and ecological), the nature of a planned release (e.g., site and containment), and eradication should deleterious effects result from a release. From these are developed "points" or criteria to assist a panel of experts in deciding on the merit of the planned release.

Genetic considerations that have been identified in several countries (20,21,27) include the potential pathogenicity of the modified organism to other organisms, potential vectors, properties of the donor DNA, the type of genetic modification and its effect on the phenotype, the origin and degree of characterization of the inserted DNA, the frequency of actual and potential reversion, and the potential for horizontal transfer of genetic material. Many of these considerations are difficult to quantify, with their importance subject to interpretation, and therefore any evaluation made with them is open to criticism. Phenotypic and ecological considerations include reproduction rates of the modified organism, the quantity or number of organisms to be released, the survival potential and dispersal and spread abilities, the impact on nontarget organisms, the impact on macrobes (such as crop species), the favorability of the environment at the test site for the organism, and benefit-cost data on the purpose of the genetic modification (21). To this list may be added data on the distribution and importance of the agricultural system impacted by the modified organism (2). All the above points may be either quantified empirically or estimated with defined assumptions; they resemble the data needed to develop ecosystem models.

Biosafety aspects are strongly featured in many guidelines, with information required on containment facilities, transport and release procedures, human protection, the existence of contingency plans to cope with unexpected natural phenomena, and the level of expertise of personnel conducting the release (21). Many of these are nonquantifiable and, like some of the genetic considerations, subject to interpretation.

Concerns with worst-case scenarios, in spite of the best-intentioned efforts, have prompted requirements for the documentation of monitoring and eradication procedures, should the need for them arise (21). Some countries have also required documentation of why the particular scientific approach, i.e., genetic engineering, is needed and whether there is not an alternative with conventional techniques. Japan also distinguishes between recombinant microbes and higher plants (20). When subjective criteria are used for impact assessment, they may become an issue should any planned release go before a court of law. In the future, it may be necessary to distinguish between guidelines per se and regulatory criteria. The Australian assessment procedures (21) attempt to rate the importance of a particular point for consideration; e.g., with respect to the origin of an organism, the degree of scrutiny ranges from "less" to "more" for situations ranging from "native to Australia and release area" to "introduced," respectively. Similarly, the evaluation of contingency plans is based on whether the organism can be

eliminated entirely, whether it can be limited but not eliminated, or whether there are no effective measures proposed or available to attempt elimination (21).

Subjective procedures have been in use for the evaluation of alternative hypotheses in scientific research per se and in its application (23). If questions are properly formulated, they have potential value in assisting with impact assessment. The questions must recognize in detail the many aspects of the biology, physical and experimental environment, and socioeconomics of a situation. This is particularly true with bioengineered organisms, as very little empirical data can be acquired until actual release has occurred.

### RISK ANALYSIS AND RISK ASSESSMENT

The term *risk* implies a probability of some hazardous effect arising from an action. In impact assessment, it may be premature to assume there is risk, and therefore to attempt to conduct risk assessment, until risk is proven! Risk identification is one step of several advocated by the USOTA for any risk assessment. The other steps are risk-source characterization, exposure assessment, dose response assessment, and risk estimation (26). Risk identification and risk-source characterization constitute risk analysis; the remaining three steps constitute risk assessment. Risk assessment has been defined as "the use of scientific data to estimate the effects of exposure to hazardous materials or conditions" (26). In practice, the examples used in the preceding sections, especially the studies by Andow et al (1,2), illustrate the estimation of system change for which a risk has to be assigned. In this regard, potential risks may be assessed in relation to possible negative effects, organism survival, reproduction, horizontal gene transfer, and dissemination. Published literature on risk assessment associated with biotechnology is lacking, and there is continuing debate on whether methods designed for assessing chemical risks and other biological risks can be applied to planned releases of bioengineered organisms (6,8,14).

Quantitative risk assessment techniques in agriculture are most commonly used by economists (4) and include the following: cost-benefit analysis, data variable estimation, sensitivity analysis, probability methods (such as PERT-Beta and Monte Carlo simulation), and analytical methods such as mean/variance ratios and stochastic dominance (25). Many biologists have difficulty translating these techniques into practical applications. In their potato study, Andow et al (1,2) used Monte Carlo simulations to generate probabilities of negative system effects (yield loss). They could have calculated (but did not calculate) mean/variance ratios associated with the use of non-ice-nucleating bacteria to reduce losses. This would have allowed a least-risk scenario to be estimated. An underlying difficulty in assessing risk resulting from ecosystem changes is the large number of candidate variables from which risk criteria may be selected. A crop ecosystem with one pest subsystem may have several hundred state variables (22,24), each of which could potentially be affected. Risk may be evaluated from data generated by probabilistic simulation of crop growth over a defined period, with criteria such as plant dry matter, yield, or economic returns (26). In most crop simulation models, the response of the crop to environmental factors and to pests is quantified as genetic coefficients that are unique for each crop cultivar (11). Bioengineered plants may therefore be compared with a reference genotype, such as a commonly grown cultivar. Currently, pest models are being coupled to software such as the Decision Support System for Agrotechnology Transfer (DSSAT) at the University of Hawaii (11) to create multiple-organism systems. The structure of these coupled models will facilitate the evaluation of system change resulting from biotechnology, e.g., increasing plant resistance to an insect. DSSAT (11) is a prototype of software that may be required if risk assessment is to be undertaken with integrated, quantitative or nonquantitative approaches. In the near future, expert systems may help interpret the results from software like DSSAT and capture some of the uncertainties associated with impact assessment of bioengineered organisms.

### DISCUSSION

Impact assessment requires both data and algorithms. The proposed National Biological Impact Assessment Program may be a mechanism to provide these data bases through a participatory effort of concerned scientists in the public and private sectors (3). Computer software for using the data bases, e.g., geographic information systems, also needs to be developed. Knowledge of microbial and higher-level systems ecology is essential for impact assessment. Yet fundamental questions, germane to any assessment concerning planned release, persist in these fields (18). A research initiative is required that will lead to the filling of knowledge gaps, and it is hoped that such a program will be preceded by a systems analysis of research needs.

The acceptance of any new technology is not based on its scientific merits alone (17). Because biotechnology augurs profound changes to society, it has expectedly come under intense public scrutiny. Not all of the public concern, however, is related to potential risk; rather, some derives from actual ignorance or misinformation on the impacts of planned releases. Fisher's comment (7, p. 221) of 1930 is particularly germane to the issues discussed in this paper: "probability is simply an expression of ignorance; risk varies inversely with knowledge." Therefore, with sufficient knowledge, there would be no need for probability distributions, since only certainty would exist.

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