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Assessment of Exposure Models for Bioengineered Microorganisms

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ABSTRACT: Environmental risk assessment performed for chemicals involves exposure assessment, effects assessment, and risk integration. The nature of living microorganisms (growth, replication, and survival), as compared to chemicals, requires that their biological attributes be integrated into risk assessment. Biological attributes have generally been dealt with in effects assessment and not exposure assessment models. Thus, exposure assessment models that characterize source, transport, transformation, and fate (effective environmental concentration, area, and duration of exposure) of genetically engineered microorganisms must incorporate biological factors along with physicochemical factors.

A study to assess the state of the art of exposure models for organisms and microorganisms in air, soil, and water was undertaken. Mathematical models developed during the past 15 years were organized into three categories: organism population dynamics, source features and transport, and management and control. One hundred forty-eight models were examined, and 56 were judged to have potential as exposure models. These 56 were screened to 31 models that were then evaluated against eight components that the ideal biotechnology model should have: (*a*) five state/process components (organism population, source application, exposure site, movement, and imposed management) and (*b*) three software components (user friendliness, availability/implementability, and flexibility). Each model was rated by individual components, combinations of two components, and total state/process components. An ideal exposure assessment model with high scores in all components was not found.

Combining two or more models so that the strong components of one compensate for the weak components of another was concluded to be the best approach for obtaining a predictive model for microorganisms. Potential couplings were ascertained from among the 31 models. Most of these couplings would combine an organism population dynamics

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model with a model from the U. S. Environmental Protection Agency's Graphical Exposure Modeling System (source and transport oriented models).

KEY WORDS: aquatic toxicology, exposure model, exposure assessment, genetically engineered microorganisms, bioengineered microorganisms, biotechnology, graphical exposure modeling system (GEMS)

In recent years, scientists have developed methods for altering the genetic composition of organisms in the laboratory. Although it is impossible to foresee all the possible uses of such bioengineered life forms, scores of organisms offering considerable commercial potential in areas such as medicine, agriculture, and chemical synthesis/degradation have already been developed. Many commercial uses as well as the field testing that precedes commercialization involve deliberate release of these new organisms into the environment. There is a need to ensure that they are environmentally compatible [*1*].

While many approaches have been developed to predict the fate, distribution, and effects of chemicals in the environment, such chemical models are not capable of predicting the behavior of organisms that, unlike chemicals, have the capacity to replicate. Furthermore, in examining the potential for toxic effects of chemicals, several lines of evidence are reasonably predictive. For example, in evaluating potential carcinogenicity of a chemical, one can utilize laboratory animal bioassay data, short-term mutagenicity studies, structural similarities to known carcinogens, as well as epidemiologic studies. Unfortunately, analogous evidence having comparable reliability and pertinence in predicting ecologic catastrophies or epidemics or both does not exist for microorganisms [*2*].

Because so many factors can contribute to the survival, growth, or failure of a genetically engineered organism in the environment, it is doubtful if even a complex model could include all of the contributing factors. Nonetheless, a reasonable comprehensive tool that can aid in risk assessments of these organisms is needed. As a first step toward meeting that need, we have identified and reviewed many available models representative of the types required and have appraised the possibility of using these models in exposure assessment of genetically engineered organisms. The results of the study are presented herein.

Procedure

General criteria for identifying potential models were established along with specific criteria for rating the potential models. A numerical scoring system, based on these specific criteria, was devised. The following discussion describes the model selection procedure, including details of both the general and specific criteria and the scoring system.

Model Selection

As a first screening step, it was established that to have potential application for exposure assessment, models need to be quantitative, capable of computer simulation, and applicable to microorganisms in ecosystems.

Quantitative—Models should describe a system in terms of mathematical formulas. Qualitative models, such as verbal descriptions of interactions between competing organisms, were excluded.

Capable of Computer Simulation—Models should use mathematical formulas where appropriate constants can be estimated from data. Highly theoretical models were consequently excluded.

Applicable to Microorganisms in Ecosystems—Models should either directly model microorganisms or be adaptable to evaluating populations of microorganisms. Models that dealt with processes within an organism, such as respiration (physiological models) or models oriented toward mechanical or electrical systems were excluded.

These general criteria aided in formulating key words for use in a mechanized literature search that scanned the past 15 years. Models were also identified by scanning such key journals as *Ecological Modeling* and *Journal of Environmental Management*, and by contacting people who had made important contributions to the computer simulation of ecosystems and their components. A total of 148 models were chosen that had potential as exposure models. Further screening reduced this number to 56 models.

Models selected through the initial screening criteria were categorized based on their emphasis toward organism population dynamics (O), source features and transport (S), or control and management (M). While models could fall in more than one category, consideration of the major emphasis of the model allowed for each of the models to be easily placed into a single category. Examples of models that were classified as organism oriented are the simulations of microbial decomposition of organic material in prairies [*3,4*] and the spruce budworm and gypsy moth population dynamics models [*5,7*]. The Graphical Exposure Modeling System (GEMS) models [*8*] and an aerial insecticide spray application model [*9*] were placed in the source and movement category. The GEMS models are a group of "user friendly" models that predict the movement and behavior of chemicals in the environment and can be used to assess the risks associated with these chemicals. The last class, models oriented toward management, included simulation of the effects of releasing sterile male insects into natural populations [*10*] and models on the efficacy of insecticides applied under different temperature regimes [*11*].

Representative models were selected from these classes, roughly proportional to the number in each class, and then evaluated according to the criteria of desirable model attributes and software components as detailed later.

Specific evaluation criteria were developed based on the components of an "ideal" exposure assessment model (Fig. 1). Criteria for both state variables

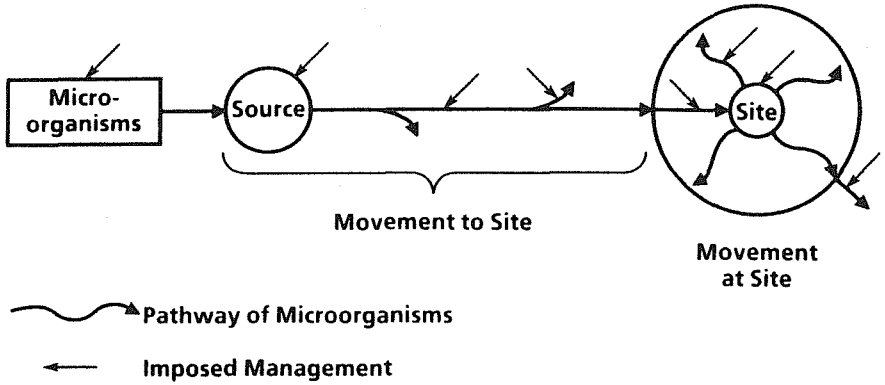

FIG. 1—*Relationships of five components in the ideal model for transfer of bioengineered microorganisms.*

and processes, and for the level of software development were established (Table 1).

A. Organism Population Characteristics—The organism population characteristics component defines the population biology of the organism being modeled and how the organism interacts with other naturally occurring biological agents in its environment. These characteristics include population changes (birth and death processes, immigration, emigration), population structure, genetics, and intra/interspecific interactions. A basic model considers only population changes over time. When included, population genetics can range from a simplistic view of a fixed "normal" genotype (that is, population genetics essentially not considered) to a population where gene frequencies change through time. Population structure can also be considered across a spectrum varying from a homogeneous viewpoint to one that considers different ages, sizes, or sexes of the organism. Intra/interspecific interactions affect birth, death, and migration rates through processes such as competition for substrates, predation, parasitism, and density-dependent actions.

B. Source/Application Characteristics—This describes both where the elements originate and the processes that move them to the exposure site. (Element represents either microorganisms or chemicals.) This component allows assessment of the initial concentrations (densities) of the element at the exposure site. The source may be a point, line, multipoint, or a broad application. Examples of sources are a cooling tower, an aerial spray application, or the discharge from a sewage treatment plant. The elements might be carried by a single medium or a combination of media. The rate of release or application may be stepped fashion, continuous, or constant, with delivery singly or several times at different rates.

C. Exposure Site Characteristics—This component provides information

TABLE 1—*Evaluation factors for potential biotechnology models.*

Model Attributes/Criteria
I. Model Components
A. Organism population characteristics
1. Population genetics
2. Population structure
3. Intra/interspecific interactions
B. Source/application characteristics
1. Variable sources
2. Variable media
3. Variable rates/frequencies
C. Exposure site characteristics
1. Different locations in medium
2. Multimedia
3. Medium variables
D. Movement characteristics at the exposure site
1. Different distribution patterns
2. Multimedia
3. Variable rates
E. Imposed management characteristics
1. Variable sources
2. Variable media
3. Variable rates/frequencies
II. Software Components
A. User friendly
1. Documentation
2. Graphics
B. Available and implementable
1. Written in commonly used language
2. Compatible on both mainframe and microcomputers
3. Available
C. Flexible (modifiable)
1. Program structure (subroutines)
2. Link to other computer packages
3. Potential for application to exposure tests

about the location where the material is deposited and includes a physical description of how the site is modeled. The site may be multimedia, such as a forest ecosystem with vegetation, soil, water, and air, or a city where air and water may be the two primary media. The site may also be considered homogeneous within a medium, or it may have several levels of resolution in the vertical and horizontal planes, or both. For example, a model might cover several vertical levels in a forest-canopy, midcrown, understory, and litter layer. In a lake, an advanced level of resolution would include the ability to model the epilimnion, thermocline, hypolimnion, and the sediments. Physicochemical variables might include temperature, pH, and moisture—factors that influence the population biology or chemical processes of the element. The model may include these variables dynamically as functions that vary with time or statically by using constants.

D. Movement Characteristics—Movement describes the dispersion of the major elements in the model and is considered only at and from the site, not to the site (the latter is considered under source/application characteristics). Transport may be through a single medium, such as water, or the element may move through several different media, for example, microbes moving via wind forces and leaching processes. An element may be distributed in a fixed pattern, for example, a certain percentage moving to an adjacent area, or it may be randomly distributed to a variety of areas. The patterm might also be directional, such as leaching and percolation processes in the movement of elements through the soil.

E. Imposed Management Characteristics—This component covers human alterations to the element, to its environment and to the processes that move it. These management characteristics may have a negative effect, such as the addition of microbial predators, or a positive effect, for example, the addition of an essential nutrient or fertilizer that enhances growth and reproduction. Several types of management characteristics may be possible, involving several sources such as aerial application or hand application. Delivery may be through a single medium or several different media, especially if the model allows for the simultaneous use of several management techniques during a single execution of the program. Finally, the rates and frequency of application may vary with the type of control measure used. For example, a virus used to kill bacteria might be introduced once at a single point in the population from which an epizootic is initiated. In another case, a microbial agent with a rapid mortality may have to be applied several times over the entire population.

Turning to criteria for the software components from the standpoint of the state of development of the model for general use, the software should offer the following features.

1. "User Friendly" Input/Output—Major considerations are documentation about how to use the model and whether graphical outputs were possible. Tables or a similar form of output were assumed to be part of every model. For time-varying parameters, tables can be cumbersome, especially over long periods of time, hence the desirability of graphs to show trends and relationships among the parameters of interest.

2. Availability and Implementability—The model should be written in a language that is commonly available on micro and mainframe computers. Languages such as BASIC, FORTRAN, PL1, and PASCAL are generally available; while simulation languages like SIMSCRIPT and SIMPCOMP are not. In addition, the compatibility of the model for use on both microcomputers and mainframe is desirable, especially with the expanding availability of microcomputers. The general availability of models and their available form were also considered. Certain models may not be obtainable; others may be available only as computer listings, while others may be available on magnetic tape or disk.

3. Flexibility—This component considered the potential for linkage between two or more models, the ease of which this could be done, and the potential applicability of the model to exposure monitoring.

Scoring Procedure

Using the factors shown in Table 1, each model was rated on the basis of the model components just described. If the component was present, a positive sign (+) was recorded; if the component was absent, a negative sign (−) was recorded. If no information was available to assess the component, NI was entered. Software components were noted but not factored into the quantitative ratings.

To arrive at comparable ratings for each model, scores were totaled in three ways. First, we scored model components (both alphabetic and numeric categories in Table 1) with each plus (+) receiving equal weight. The total number of pluses was divided by 20 (highest possible score) and then multipled by 10 to put the score on a scale of 1 to 10. This score was recorded as the *total score*. For example, if a model received five pluses, distributed as follows:

one in the alphabetic category for organism
one in the numeric category for organism—population structure
one in the alphabetic category for site
one in the numeric category for site—medium variables
one in the alphabetic category for management

the total score would be $(5/20) \times 10 = 2.5$.

Second, component scores were also calculated for each category (organism, source, site, movement, and management) and recorded (the component scores equal the total score).

Component scores for the preceding example would be as follows:

organism	$2/20 \times 10 = 1.0$
source	$0/20 \times 10 = 0$
site	$2/20 \times 10 = 1.0$
movement	$0/20 \times 10 = 0$
management	$1/20 \times 10 = 0.5$

Third, category scores were calculated by taking the total pluses in the alphabetic component categories (equal weighting), dividing by 5 (highest possible score), and multiplying by 10 to put the score on a 1 to 10 scale. For the preceding example, the category score would be $(3/5) \times 10 = 6.0$, since three component categories—organism, site, and management—were modeled.

The scores were then used to determine the models with the best total score, category score, and component score.

Results

Of 56 models that passed initial screening criteria, 31 (Table 2) were evaluated using the criteria just detailed. The majority of these models were written during the past 15 years (1972 to present). Organism-oriented models dominate the list (60%), followed by source and movement models (30%), and finally management-oriented models (10%).

Average values calculated for the total scores and within category or component scores were similar for the three classes of models, as shown in Table

TABLE 2—*Model author, description, and number code of 31 models, evaluated as potential candidates for use in exposure assessment.*

Authors Names	Model Description	Model Code	Reference
	ORGANISM ORIENTED (O)		
Bazin et al, 1976	microbes and soil	2	(*12*)
Benefield and Molz, 1984	activated sludge microbes	3	(*13*)
Botkin et al, 1970	forest growth	5	(*14*)
Curds, 1971	microbes predator-prey	7	(*15*)
Curry et al, 1980	weevils	8	(*16*)
de Figueirdo et al, 1975	mosquito control	9	(*17*)
Hunt, 1977	decomposition	14	(*3*)
Jones, 1977	eastern spruce budworm	16	(*5*)
Leonard, 1977	selection and plant pathogens	17	(*18*)
McNamee et al, 1983	gypsy moth	18	(*5*)
Ogawa and Mitsch, 1979	fish populations	20	(*19*)
Patten, 1972	short grass prairie	21	(*4*)
Potter, 1979	forest planning simulator	22	(*20*)
Sheehan, 1984	western spruce budworm	25	(*6*)
Shugart and West, 1977	forest succession	26	(*21*)
Williams, 1971	microbial populations	29	(*22*)
Zadocks and Kampmeijer, 1977	crop populations	31	(*23*)
	SOURCE AND MOVEMENT ORIENTED (S)		
Bonazounatas and Wagner, 1981	SESOIL-GEMS	4	(*24*)
Burns et al, 1982	EXAMS-GEMS	6	(*25*)
Dumbauld et al, 1980	aircraft spray dispersion	10	(*9*)
Goodman and Tucker, 1971	water quality	11	(*26*)
Hetrick and McDowell-Boyer, 1984	TOX-SCREEN-GEMS	13	(*27*)
Jang et al, 1983	bacteria transport	15	(*28*)
Miller et al, 1979	soil loss	19	(*29*)
Raridon et al	ATM-TOX-GEMS	23	(*30*)
Sorber et al, 1979	bacterial aerosols	27	(*31*)
Vithayathil et al, 1979	nutrient and pesticide drainage	28	(*32*)
Yeh, 1981	AT123D-GEMS	30	(*33*)
	MANAGEMENT ORIENTED (M)		
Barclay and Mackauer, 1980	sterile insect release	1	(*10*)
Harrison et al, 1982	sterile insect release	12	(*34*)
Reichenbach, 1985	budworm control	24	(*11*)

3. The average scores recorded for the organism population components were similarly high for both Class O and M models (organism-oriented and management-oriented, respectively). Class S models (source and movement oriented) had, in comparison, low scores in the organism category. Conversely, in the source category scores, the trend was exactly opposite. Site characteristic scores were similarly high for both Class O and S models, while they were low for Class M models. Management scores were obviously highest among the management-oriented models.

The organism-oriented models were generally weak in source and movement characteristics. Source and movement models were weak in organism characteristics since these models are primarily oriented toward chemicals in the environment. Management-oriented models are primarily directed toward control of organisms, hence their high scores in the organism category. These models also scored low in the source and movement categories. Table 4 shows the specific models that scored highest at each level of assessment. Total scores were relatively low for all models. On the 1 to 10 scale, the highest

TABLE 3—*Mean scores for the three classes of models evaluated for the different levels of assessment.*

Model Class	*N*	Mean Scores: Total Score	Category Score	Organism Score	Source Score	Site Score	Movement Score	Management Score
O[a]	17	3.2	7.2	1.4	0.1	1.1	0.4	0.2
S[b]	11	4.0	7.8	0.1	1.2	1.4	1.0	0.1
M[c]	3	2.6	6.6	1.4	0.1	0.4	0.0	0.9

[a]O = organism oriented.
[b]S = source and movement oriented.
[c]M = management oriented.

TABLE 4—*Models that scored high in the different levels of assessment. Models are listed as codes (see Table 1 for codes).*

Range of Scores	Total Score	Category Score	Organism Score	Source Score	Site Score	Movement Score	Management Score
Highest Score	4, 13	11, 12	31	4,10, 13, 23	4, 8, 10, 13, 18	4, 6, 13	8, 18
Next highest score	8, 18, 25	8, 15, 18, 24, 25, 27 31	1, 5, 8, 9, 12, 16, 17, 18 20, 25 26, 29	6, 11, 30	6, 14, 16, 19 22, 23, 25, 28, 30	15, 16, 25	1, 12, 25

total score recorded was 6.0 for the TOX-SCREEN-GEMS [*13*] and SESOIL models [*4*] (Table 4). The low scores reflect the models' strength in one or two areas and weaknesses in the others.

An ideal exposure assessment model that scored high in all five categories was not found. As a matter of fact, all five major components were included in only two models: water quality [*11*] and forest planning [*22*]. However, their respective total scores were only 3.6 and 3.2, hence the level of resolution in each of the respective component categories was not high.

Table 4 lists those models with the highest scores. For example, Model 31 (crop population) scored highest in the organism component. It was one of only three models that incorporated population genetics.

By contrast, models that had the highest scores in the source component were SESOIL [*4*], aircraft spray dispersion [*10*], TOX-SCREEN [*13*], and ATM-TOX [*23*]. These models are oriented toward chemical dispersion and hence scored low (0.0) in the organism category. More details are available through a study of Table 4.

Discussion

The ideal exposure assessment model will possess adequate software and will have attributes that meet the model-component criteria: (1) population structure, changes, genetics, and interactions of the organism; (2) element origin and movement to the exposure site; (3) physical description of the exposure site; (4) dispersion at the exposure site; and (5) control or management processes that may enhance or deplete the microorganism population. Further, it will offer fixed and, more importantly, variable functions.

No model that satisfies all these conditions has been identified. A method for resolving this deficiency is to adapt or couple two models to meet needs in specific situations. The first approach is to adapt chemical transfer models to model microorganism dispersion. The best candidate models are SESOIL-GEMS [*4*], EXAM-GEMS [*6*], and TOX-SCREEN-GEMS [*13*]. These chemical exposure models have all the basic components necessary for an exposure model (source, transport, fate), but they cannot model biological changes in microorganism population numbers such as reproduction and mortality (Fig. 2). This deficiency is the fundamental difference between microbiological and chemical exposure models.

Microorganisms, as they move from the source to the exposure site, undergo processes that affect not only the population concentrations but also changes in the characteristics of the population. Alterations of the population characteristics involve altered virulence that is analogous to chemical transformation (Fig. 2). Changes in population concentrations include dilution, which acts on both chemicals or microorganisms, and the unique processes of mortality and reproduction. These processes, which produce changes in the microoganism populations and in the characteristics of the population, are

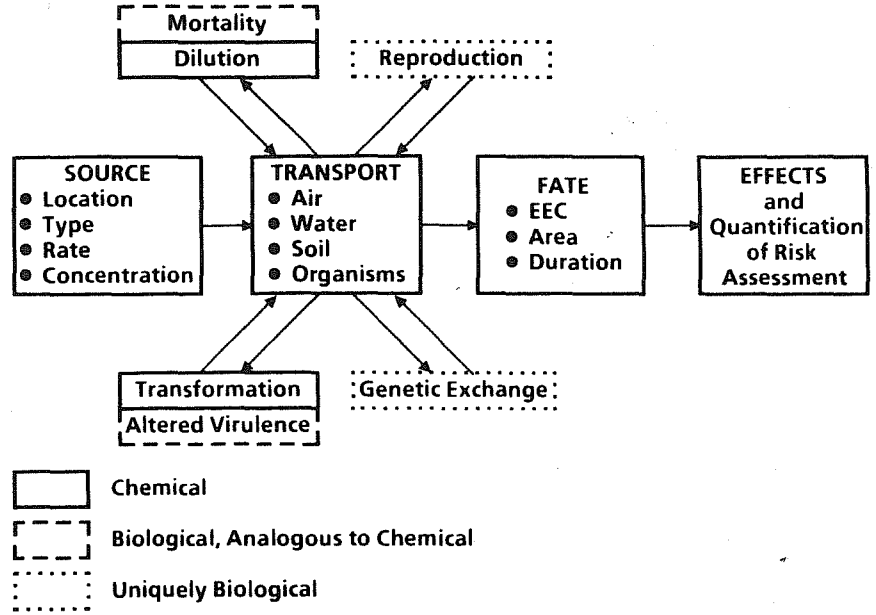

FIG. 2—*Exposure assessment of bioengineered microorganisms.*

critical in determining the estimated environmental concentration (EEC). They also affect the distribution of the EEC over space and time (area and duration).

Another unique attribute of microorganisms is their ability to transfer genetic material from one strain or species to another. The microorganism that was deliberately released for purposes of increasing food production may not persist; however, its traits may be passed to another organism in the same environment. These traits may lead to either negative or positive impacts.

By taking advantage of the similarities between chemical and microbiological exposure assessment, the models used in chemical exposure assessment could be adapted to handle the unique processes of microorganisms' reproduction and mortality. A subroutine could be added that handles increase in mass and, in some cases, virulence as the microorganism population grows, or conversely, attenuation of the microorganism mass in a hostile environment.

To meet part of this need for treatment of organisms, animal-population models, in particular, can be adapted to microorganism models. One of the best to start with is mosquito control [*9*]. There are 12 additional organism-oriented models from which to choose.

Living populations, whether microorganisms, plants, or animals, exhibit fundamental similarities, such as reproduction and mortality. Therefore, when these fundamental similarities are addressed in a model, often only the rates at which the processes occur need to be adjusted. One important adjustment in an adaptation from animals to microorganisms is the rate at which new organisms are assembled.

The second approach is to use existing strong models in new combinations, that is, couple two or more models. Most of the models evaluated focused on one or more of the attributes. The best model(s) for each of the attributes were:

Organism	crop populations
Source	GEMS series/aircraft spray
Site	GEMS series/weevils/aircraft spray/gypsy moth
Movement	GEMS series
Control	weevils/gypsy moth.

This list suggests that the crop populations model and the recurrent GEMS series model could be combined to make a powerful new model.

The potential adaptations and couplings were recognized in the following way. First, the models with the highest and next highest scores were considered the best ones to modify. Second, a review of the model was made to understand the scope of the model. High-score and reasonable-scope models of one type were matched with high-score and reasonable-scope models of another type. Within a multimedia framework of air, water, and soil, microorganism-related models were matched with movement-related models on a media-by-media basis. The results of this methodology are displayed in Table 5. Another aspect of coupling models is to utilize submodels within a larger model. For example, the gypsy moth model has submodels that handle the propagation of microbial and viral populations.

Possible modifications of existing models are listed in Table 5. The table shows models that offer the best technical possibilities to solve noted deficiencies. Specific modifications include adaptation of the GEMS models to include a microbial component. Insect models could be adapted to microbial use, especially the mosquito model, which has the ability to model population genetics. In addition, the four microorganism models could be adjusted to better model more microorganism attributes. Several model couplings appear to be technically reasonable (from a subject view). The evaluation was organized into three parts corresponding to different couplings: (1) microorganism models with movement models, giving attention to air, water, and soil transfers; (2) microorganism models with management models; and (3) microorganism models with movement models and then with management models, that is, towards the ideal model. Note that there are 16 technically reasonable couplings. Most of them couple one of four microorganism models to a GEMS model.

TABLE 5—*List of possible technical actions for modification of organism-oriented models.*

Organism-Oriented Modifications	Best Technical Candidates (model codes in parenthesis)
	ADAPTATIONS OF MODELS
Chemical to microorganism	GEMS series (4, 6, 14, 23, 30)
Animal to microorganism	weevils (8) and mosquito control (9)
Microorganism (generic) to microorganism (specific)	microbes and soil (2), bacterial transport (15), bacterial aerosols (27), and microbial populations (29)
	COUPLING OF MODELS
Microorganism with movement:	
Air	bacterial aerosols (27) with TOX-SCREEN-GEMS (13) and bacterial aerosols (27) with ATM-TOX-GEMS (23)
Water	activated sludge microbes (3) with EXAMS-GEMS (6), microbes predator-prey (7) with EXAMS-GEMS (6), and microbial populations (29) with EXAMS-GEMS (6)
Soil (porous media)	microbes and soil (2) with SESOIL-GEMS (4), decomposition (14) with SESOIL-GEMS (4), and bacterial transport (15) with SESOIL-GEMS (4)
Multimedia	microbes and soil (2) with TOX-SCREEN-GEMS (13), decomposition (14) with TOX-SCREEN-GEMS (13), and bacterial transport (15) with TOX-SCREEN-GEMS (13)
Microorganism with management:	crop populations (31) with weevils (8), microbial populations (29) with weevils (8), and crop populations (31) with gypsy moth (18)[a]
Microorganism with movement and management:	crop populations (31) with GEMS (4, 6, 13, 23, 30) with weevil (8), and crop populations (31) with GEMS (4, 6, 13, 23, 30) with gypsy moth (18)[a]

[a]Has a microbial population subroutine.

Conclusions

A search of the literature covering the past 15 years revealed a number of models with some attributes needed to represent what might happen to engineered microorganisms when they are released to the environment. However, no model evaluated was strong in all five of the major characteristic components that would be needed. Only two models were found that incorporated all five components, but they were strong in only one area.

Of the 31 models evaluated, 28 did not include population genetics. However, there were several models that included the other attributes judged necessary for modeling organism population dynamics.

There is another group of models that have strong potential for depicting what happens at the source, at the receiving area (site), and during movement

from source to site. But because many of these models have been developed for use with chemicals, they have little capability for describing organism population dynamics. Organism population dynamics includes reproduction and mortality, which are processes that distinguish chemical exposure models from microbiological exposure models.

Most of the models (about 60%) have no capabilities for representing management and control of the microorganisms.

Combining two or more models so that the strong areas of one compensate for the weak areas of another shows promise as a method for obtaining a predictive device for microorganisms. Several combinations proposed for predicting the destiny of microorganisms were judged applicable.

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