Microbiotests in Aquatic Ecotoxicology: Characteristics, Utility, and Prospects

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ABSTRACT

Small-scale biological tests (microbiotests) have steadily increased in development and application over the last 30 years in the field of aquatic ecotoxicology. Multitrophic level assessment requirements, attractive features of microbiotests, and the constant search for simplicity and cost efficiency of testing are reasons explaining the expanding use of microbiotests. In this article, the major characteristics that advantageously confer popularity on microbiotests are presented and 25 currently applied aquatic toxicity microbiotests are listed. Conducted with bacteria, protozoans, microalgae, small invertebrates, and fish cell lines, these microbiotests represent a realistic cross section of those that are now becoming an essential part of ecotoxicological assessment. Microbiotests can be profitably employed for ranking and screening chemicals, for novel applications enabling rapid detection of ecotoxic effects in complex liquid samples, and for increasing the cost efficiency and diagnostic potential of hazard assessment schemes. Microbiotesting research, development, and applications will continue to surge in the 1990s, driven, among other factors, by the imperative need for cost effectiveness in environmental programs. Research in the fields of ecotoxicology, biotechnology, and immunochemistry should provide interesting breakthroughs to further enhance the specificity and diagnostic value of microbiotests.

INTRODUCTION

Significant leaps have marked the development and application of biological tests in the field of aquatic ecotoxicology over the past three decades. From their modest (but essential) beginnings in the 1960s, primarily triggered by the urgent need to assess the suspected impact of industrial pollution, biological tests have steadily grown in quality and quantity in the 1970s and 1980s. At the dawn of the 1990s, they now collectively represent an important ecotoxicological tool with which anthropogenic stresses on the environment can be diagnosed (Leclerc

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and Dive, 1982; Environment Canada, 1984; Persoone *et al.*, 1984; Blaise *et al.*, 1988; Munawar *et al.*, 1989). In particular, microbiotests (small-scale tests) are being increasingly applied (Maciorowski *et al.*, 1980), because of their advantages over the more traditional macrobiotests (e.g., fish tests). After proposing a broad definition for the term "microbiotest," this paper discusses (1) the attractive features that are contributing to their augmenting popularity, (2) the types of aquatic microbiotests presently employed, (3) novel application possibilities with such tests, and (4) prospects for future research and development in the area of microbiotests.

CHARACTERISTICS OF MICROBIOTESTS

In the field of aquatic ecotoxicology, a microbiotest can be broadly defined as involving the exposure of a unicellular or small multicellular organism to a liquid sample in order to measure a specific effect. Other terms such as "small-scale test," "microtest," and "second-generation biotest" also allude to this definition. Based on this rather large view of what constitutes a microbiotest, a wide array of (micro)organisms can be employed to undertake aquatic toxicity studies. These may comprise representatives of different biological levels such as bacteria, protozoa, microalgae, fungi, yeasts, and even small invertebrates (e.g., rotifers, water fleas, roundworms).

The need for multitrophic level ecotoxicity assessment is one basic factor that has driven the evolution of biotesting toward increased use of microbiotests. The continuing search for simplicity and cost efficiency of biotesting, allowing large-scale screening of xenobiotics (singularly or in mixtures) in the process, is another.

There are undoubtedly many attractive features of microbiotests which are contributing to their present stardom. Table I lists and explains several major advantages popularizing their use. It should be obvious that all existing microbiotests do not possess all of these features, and that possessing more or less of these is test dependent. Not so obvious, perhaps, is that particular microbiotests can indeed incorporate most, if not all, of these advantages, as will be discusssed later in this section.

Table II displays a comprehensive cross section of microbiotests presently applied in aquatic studies to rank and screen chemicals, wastewaters, and various environmental matrices. While they represent microassays specifically familiar to the author, such multitrophic tests are but the tip of the iceberg of a much more formidable army of available microbiotests. Recent publications in the area of biological testing are certainly convincing in confirming the diversity of existing

TABLE I
Attractive features of microbiotests

Feature	Explanatory remark
Inexpensive *or* cost efficient	Cost is test dependent and can vary from a few dollars to several hundred dollars in Canadian currency
Generally not labor intensive	As opposed to steps involved in undertaking fish bioassays, for example
High sample throughput potential	When automation technology can be applied
Cultures easily maintained or maintenance free	Freeze-drying technology or cryptic life form preservation, for example
Modest laboratory and incubation space requirement	As opposed to a specialized laboratory essential for fish bioassays, for example
Insignificant postexperimental chores	Owing to disposable plastic ware, which is recycled instead of having to be washed for reuse, as in the case of large experimental vessels
Low sample volume requirements	Often, a few milliliters suffice to initiate tests instead of liters
Sensitive/rapid responses to toxicants	Short life cycles of (micro)organisms enable end-point measurements after just minutes or several hours of exposure to toxic samples
Precise/reproducible responses	High number of assayed organisms, increased number of replicates, and error-free robotic technology are contributors to this feature
Surrogate testing potential	Microbiotests are adequate substitutes for macrobiotests in some cases
Portability	Cases where microbiotests are conveniently amenable to being applied in the field

microtesting procedures and applications (Dive and Leclerc, 1982; Persoone *et al.*, 1984; Zimmermann and Taylor-Mayer, 1985; Bitton and Dutka 1986; Babich and Borenfreund, 1987; Munawar *et al.*, 1989).

Of the 25 microbiotests reported in Table II, several possess attractive characteristics that markedly enhance their simplicity to perform

TABLE II
Examples of applicable bacterial (B), protozoan (P), microalgal (M), invertebrate (I), and fish cell line (F) toxicity microbiotests

Test organism (test name)	Reference
B_1: *Salmonella typhimurium* (Ames test)	Ames *et al.*, 1975
B_2: *Pseudomonas alcaligenes* (Dehydrogenase activity test)	Bitton *et al.*, 1986
B_3: *Spirillum volutans* (Motility inhibition test)	Dutka, 1986
B_4: *Photobacterium phosphoreum* (Microtox test)	Bulich *et al.*, 1981
B_5: *Photobacterium leiognathi* (Mutagenicity test)	Ulitzer, 1986
B_6: *Escherichia coli* (TOXI-Chromotest)	Reinhartz *et al.*, 1987
B_7: *Escherichia coli* (SOS-Chromotest)	Fish *et al.*, 1987
B_8: *Bacillus cereus* (ECHA biocide monitor)	Dutka and Gorrie, 1989
P_1: *Colpidium campylum* (Growth inhibition test)	Dive and Leclerc, 1975
P_2: *Tetrahymena pyriformis* (Respiratory inhibition test)	Slabbert and Morgan, 1982
P_3: *Tetrahymena pyriformis* (Chemoattraction inhibition test)	Roberts and Berk, 1990
M_1: Multispecies (Metal uptake test)	Hassett *et al.*, 1981
M_2: *Chlorella kessleri* (Algal growth potential and growth inhibition test)	Lukavsky, 1983; 1985
M_3: *Selenastrum capricornutum* (Flask growth inhibition test)	Joubert, 1983 U.S. Environmental Protection Agency, 1989
M_4: *Selenastrum capricornutum* (Microplate growth inhibition test)	Blaise *et al.*, 1986
M_5: Multispecies (Toxicity fluorescence microtest)	Blanck, 1987
I_1: *Brachionus plicatilis* (Marine rotifer lethality test)	Snell and Persoone, 1989a
I_2: *Brachionus rubens* (*calyciflorus*) (Freshwater rotifer lethality test)	Snell and Persoone, 1989b
I_3: *Daphnia magna* (Cladoceran lethality test)	Poirier *et al.*, 1988
I_4: *Ceriodaphnia reticulata* (Cladoceran 7-day life cycle test)	Mount and Norberg, 1984
I_5: *Artemia salina* (Mysid shrimp lethality test)	Vanhaecke and Persoone, 1981

TABLE II (Continued)

Test organism (test name)	Reference
I_6: *Panagrellus redivivus* (Nematode lethality/mutagenicity test)	Samoiloff *et al.*, 1983
I_7: *Hydra attenuata* (Teratogenicity test)	Wilby *et al.*, 1986
F_1: Rainbow trout RTG2 gonadal cells (Cytotoxicity test)	Denizeau and Marion, 1984
F_2: Rainbow trout hepatocytes (Cytotoxicity test)	Ahne, 1985

and cost efficiency. Microbiotests harboring these practical features are shown in Table III. Bacterial tests, in particular, stand out in terms of the features considered over tests conducted with other types of bioindicators. Indeed, tests B_6 and B_7 encompass all five desirable attributes. It is equally of interest to note that invertebrate microtests I_1, I_2, and I_5 offer all five features as well.

Partly owing to their practicality, it is not surprising to observe that microbiotests have now become an essential part of ecotoxicological assessment, and that they inspire new and varied activities in research and development. This is clearly evidenced by the recent creation of what has become a popular and dynamic biennial international symposium exclusively dedicated to ecotoxicity testing using microbial systems (Burlington, Canada, 1983; Banff, Canada, 1985; Valencia, Spain, 1987; Las Vegas, United States, 1989; Kurashiki, Japan, 1991; West

TABLE III
Practical features outstanding in the 25 microbiotests listed in Table II

Feature	Corresponding microbiotests
Available in kit format[a]	$B_{6,7,8}$, $I_{1,2,5}$
Portability	$B_{4,6,7}$, M_4, $I_{1,2,5}$
Maintenance-free bioindicator	$B_{4,5,6,7,8}$, $I_{1,2,5}$
Performed in microplates	$B_{6,7}$ P_1^b, $M_{1,2,4,5}$, $I_{1,2,5}$, F_2
Minimal training and equipment requirement	$B_{2,3,4,5,6,7,8}$, P_3, $M_{2,4}$, $I_{1,2,5,7}$, F_2

[a] These microbiotests can be purchased commercially in convenient compact kits (called TOXKITS in the case of the invertebrate tests) complete with preserved bioindicator, experimental vessels, reagents, positive controls, and instructions for use.

[b] Although not routinely undertaken in microplates, this test can be performed with these.

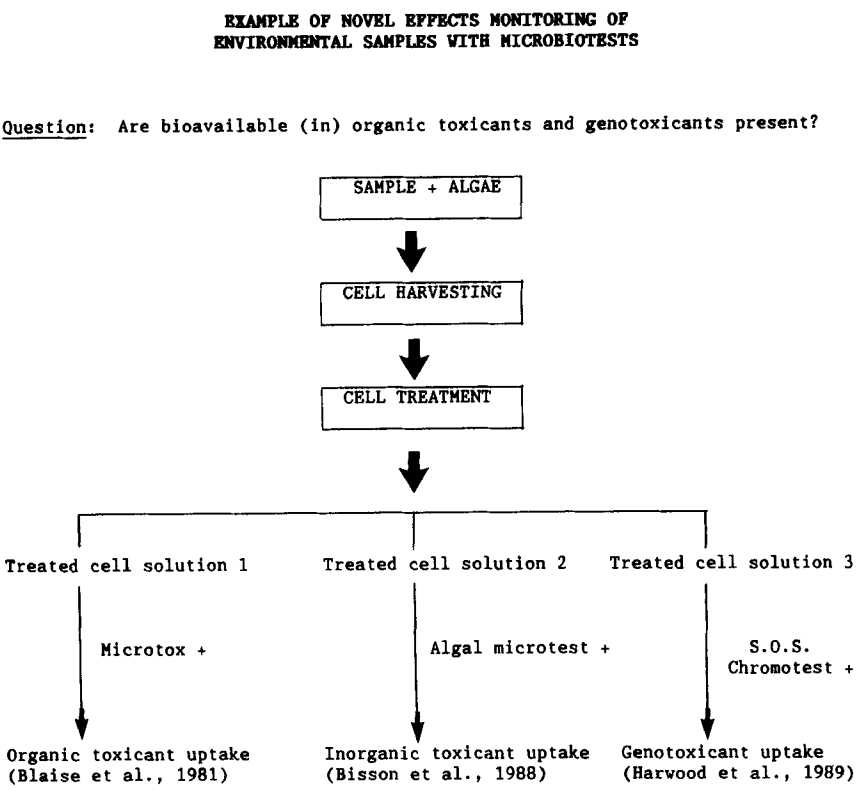

Fig. 1. Bio-availability studies of liquid samples with microalgae.

Germany, 1993; Australia, 1995). The rising effervescence in this field is manifest.

UTILITY OF MICROBIOTESTS

While aquatic microbiotests are invaluable for screening and ranking ecotoxic effects, their features can contribute novel applications enabling rapid detection of potential hazards linked to liquid samples. Figure 1 illustrates how new diagnostic possibilities can be developed with microbiotests. With simple experimental protocols and modest sample volume requirements (5 L), the presence of bioavailable organic (Blaise *et al.*, 1981) and inorganic (Bisson *et al.*, 1988) toxicants, and genotoxicants (Harwood *et al.*, 1989) in liquid samples can be appraised. This procedure calls for the short-term exposure ($\leq$24 h) of an accumu-

lator species indicator (*Selenastrum capricornutum*) to the liquid sample under study. After the exposure period, harvested algal cells are treated in three different ways such that appropriate post-treatment toxicity indicator microbiotests (organic toxicity probe = Microtox test = test B_4, Table II; inorganic toxicity probe = *S. capricornutum* microtest = test M_4, Table II; genotoxicity probe= SOS Chromotest = test B_7, Table II) can be applied to each treated cell solution. Positive toxicity responses of the microbiotests to treated algal cell solutions 1, 2, and 3, respectively, indicate organic, inorganic, and genotoxic uptake by the algae, and thus infer the presence of such bioavailable toxicants in the sample (Fig. 1). Procedural details can be obtained by consulting cited references.

The above application demonstrates clearly that microbiotests can offer new opportunities for effects monitoring of chemicals and varied environmental matrices (effluents, leachates, interstitial waters, etc.). Further evidence that microbiotesting protocols and results can prove useful in this way has been documented elsewhere (Levin *et al.*, 1984; Couture *et al.*, 1985; Thomas *et al.*, 1986). Microbiotests can also contribute to increasing the cost efficiency and diagnostic potential of hazard assessment schemes (HASs). Although it is beyond the scope of this article to discuss HASs, which can vary greatly in structure, organization, and objectives, it is important to emphasize that employing a suitable battery of microbiotests to conduct HASs is essential to optimize ecotoxicity characterization and prediction (Thomas *et al.*, 1986; Blaise *et al.*, 1988; Dutka, 1988; van Coillie *et al.*, 1988).

PROSPECTS FOR MICROBIOTESTS

A safe prediction indeed is to state that biological testing, and microbiotesting in particular, will increase significantly in environmental protection activities in the future. International recognition of biotesting, and various environmental policies, regulations, and guidelines, all favor the expanded application of ecotoxicological investigations making use of (micro)biotests (Blaise *et al.*, 1988). It is also anticipated that research and development in microbiotesting will expand because of the imperative need for cost efficiency in environmental assessment (Cram, 1989). Obviously, microbiotests can play a prominent role in this matter.

We can expect future research endeavors to provide microbiotests with increasing specificity and diagnostic value. From the field of ecotoxicology, for example, comparative bioindicator studies will further validate the surrogate potential of certain microbiotests when these are applied on specific target samples. There is presently some evidence

for this potential in the literature (Blaise *et al.*, 1987); Munkittrick *et al.*, 1990). Again, novel applications with flow cytometry, a relatively recent technology, will contribute incisive diagnostic value to microbiotests by allowing differentiation of viable and nonviable cells after toxicant exposures, for example (Dorsey *et al.*, 1989). Another pioneering avenue of research will involve the "creation" of bioengineered microorganisms sensitive to specific (classes of) chemicals (Guzzo and Dubow, 1991). These "causally related microbiotests," also known as bioprobes or biosensors, will be particularly useful in the diagnostic screening of samples of unknown composition. They are likely the "third generation biotests," the development and use of which will mark the last decade of this century. Polymerase chain reaction (PCR) DNA amplification and immunoassay technologies, although not strictly falling into the realm of microbiotests, are nevertheless distinctly linked to aquatic ecotoxicological assessment. PCR-DNA amplification, for one, will prove useful in detecting the presence and fate of genetically engineered microorganisms discharged to receiving environments (Steffan and Atlas, 1988). For another, immunoassays, through the use of monoclonal antibodies, should provide cost-efficient quantitative detection of high-risk chemicals (Vanderlaan *et al.*, 1988). Hence, these latter technologies can be important adjuncts to microbiotesting activities and are therefore certainly worthy of mention. Evidently, ecotoxicology, biotechnology, and immunochemistry must continue to be essential partners in promoting environmental protection through microbiotesting.

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