SMALL-SCALE FRESHWATER TOXICITY INVESTIGATIONS

Small-scale Freshwater Toxicity Investigations

Volume 1 – Toxicity Test Methods

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Dr. Blaise obtained university diplomas from the U. of Montréal (B.A., 1967: biology and chemistry), U. of Ottawa (B.Sc., 1970: cell biology; M.Sc., 1973: environmental microbiology) and U. of Metz (D.Sc., 1984: ecotoxicology). He is a member of the editorial board for two scientific journals (*Environmental Toxicology*; *Ecotoxicology and Environmental Safety*) and holds membership in both the biologists' (Association des Biologistes du Québec) and microbiologists' (Association des Microbiologistes du Québec) associations of the province of Québec. He regularly attends and makes presentations during major venues held in the field of ecotoxicology (SETAC: Society of Environmental Toxicology and Chemistry; SECOTOX: Society of Ecotoxicology and Environmental Safety; ATW-Canada: Aquatic Toxicity Workshop-Canada; ISTA: International Symposium on Toxicity Assessment). Dr. Blaise has (co)authored over 100 scientific articles in internationally refereed journals, as well as having written several book chapters, reviews, and various government technical reports.

He recently co-edited, with Canadian colleagues, a book dedicated to small-scale toxicity testing (Wells, P., K. Lee and C. Blaise (eds.), 1998. *Microscale testing in Aquatic Toxicology Advances, Techniques and Practice*. CRC Lewis Publishers, Boca Raton, Florida, 679 pages). He was scientific organizer of the 10th International Symposium on Toxicity Assessment (ISTA 10), hosted by the Saint-Lawrence Centre where he works, and held in Quebec City, August 26-31, 2001. He further co-edited with another Canadian colleague, a special edition of *Environ. Toxicol.* (Volume 17 [3]: 2002, special issue) highlighting selected papers presented at the ISTA 10 venue.

Jean-François Férard, D.Sc., is a professor at the University of Metz (Lorraine province of France), where he heads a research team (RT) which is part of a C.N.R.S. (Centre National de la Recherche Scientifique) research unit for Ecotoxicity and Environmental Health (E.S.E). He also manages an undergraduate school program dedicated to Environmental Engineering. His teaching duties involve fundamental and applied Ecotoxicology, Physiology and Physiotoxicology, Cell Biology and other related disciplines.

In the field of ecotoxicology, his RT was involved in the behavior of metals, PAHs and complex mixtures in air, water and soil compartments and their effects on different organisms (bacteria, algae, crustaceans, plants, arthropods, etc.). His actual research endeavors are more specifically focused on the development of metal-resistance (*e.g.* phytochelatin) and genotoxic (*e.g.* comet assay) biomarkers. He also promotes knowledge and use of toxicity tests by organizing an annual course entitled "Ecotoxicity and carcinogenicity of chemicals" which provides a theoretical and practical view of numerous toxicity tests to decision-makers, industrialists and consultants. Since 1974, he has markedly contributed to numerous research programs that have successfully lead to i) development and validation of different ecotoxicity tools (*e.g.* new toxicity test methods, trophic chain models, biomarkers), ii) hazard/risk assessment schemes and iii) links between field and laboratory studies. These undertakings were financially supported by the European Economic

Community, the French ministry of the Environment, and agencies such as the French Water Agency of the Rhin-Meuse Watershed, the French Agency for Environment and Energy Ressources.

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Preface

Developed, developing and emerging economies worldwide are collectively contributing multiple stresses on aquatic ecosystems by the release of numerous contaminants. This in turn demands that basic toxicological information on their potential to harm living species be available. Hence, environmental protection programs aimed at preserving water quality must have access to comprehensive toxicity screening tools and strategies that can be applied reliably and universally.

While a good number of toxicity testing procedures and hazard assessment approaches have been published in the scientific literature over the past decades, many are wanting in that insufficient detail is available for users to be able to fully understand the test method or scheme and to be able to reproduce it successfully. Even standardized techniques published in recognized international standard organization documents are often lacking in thoroughness and *minutiae*. Paucity of information relating to biological test methods may be consequent and trigger several phenomena including generation of invalid data and resulting toxicity measurements, erroneous interpretation and decision-taking with regards to a particular chemical or environmental issue, or simply abandonment of testing procedures. Clearly, improperly documented toxicity testing methods can be detrimental to their promotion and use, as they open the doorway to unnecessary debate and criticism as to their *raison d'être*. Furthermore, this situation can indirectly contribute to delaying, minimizing or eliminating their application, thereby curtailing the important role toxicity testing plays in the overall protection and conservation of aquatic ecosystems.

The ″cry for help″ that we have often heard from people having encountered difficulties in properly conducting biological tests was the primary trigger that set off our desire to edit a book on freshwater toxicity testing procedures in the detailed manner described herein. We feel this book is rather unique in that it includes 1) a broad review on toxicity testing applications, 2) comprehensive small-scale toxicity test methods (Volume 1) and hazard assessment schemes (Volume 2) presented in a designated template that was followed by all contributors, and 3) a complete glossary of scientific/technical terms employed by editors/contributors in their respective chapters.

Indeed, the book provides information on the purposes of applying toxicity tests and regroups 15 validated toxicity test methods (Volume 1) and 11 hazard assessment schemes (Volume 2) for the benefit and use of the scientific community at large. Academia (students, professors), government (environmental managers, scientists, regulators) and consulting professionals (biologists, chemists, engineers) should find it of interest, because it encompasses, into a single document, comprehensive information on biological testing which is normally scattered and difficult to find. It should be, for example, very useful for (under)graduate courses in aquatic toxicology involving practical laboratory training. In this respect, it can be attractive, owing to some of its contents, as a laboratory manual for learning purposes or for undertaking applied research to assess chemical hazards. As a further example, it can also prove useful for environmentalists who wish to select the most appropriate test(s) or scheme(s) for future decision-taking with regards to protection of aquatic ecosystems. In short, all groups directly or indirectly involved with the protection and conservation of freshwater environments will find this book appealing, as will those who simply wish to become familiar with the field of toxicity testing.

We are grateful for the financial support given to us in the production of this book by Environment Canada (Centre Saint-Laurent, Québec region, Environmental Conservation), the University of Metz (Metz, France) and IDRC (International Development Research Centre, Ottawa, Ontario, Canada). For their assistance in many dedicated ways which facilitated our tasks and ensured the timely completion of our book, we extend our thanks to the following persons: Mr. Andrés Sanchez and Dr. Jean Lebel (IDRC); Ms. Jacinthe Leclerc, Dr. Alex Vincent and Dr. André Talbot (Centre Saint-Laurent); Ms. Sylvie Bibeau and Dr. Laura Pirastru (University of Québec in Montréal). We are also very appreciative of the dedicated professional help provided us by Anna Besse and Judith Terpos of **Springer Publishers** in guiding us through the editorial process.

Again, how could we not extend our appreciation to all of our devoted colleagues who accepted our invitation to contribute a chapter to this book? They number 54 in total and represent 11 countries including Argentina, Australia, Canada, Chile, Columbia, Denmark, France, Germany, Poland, Switzerland and the U.S.A. Needless to say that it is owing to their outstanding career experience and interest to promote their know-how that ***Small-scale Freshwater Toxicity Investigations (Volume 1 and Volume 2)*** has now become a reality. Last but not least, the ultimate acknowledgment must go to our other estimated colleagues who acted as peer-reviewers for all manuscript contributions and who significantly contributed to their final quality.

We are convinced that this book fills an important scientific gap that will stimulate international use and application of small-scale toxicity tests, whether for research, monitoring, or educational purposes. May the "blue planet" and its aquatic species ultimately profit from such endeavours!

Christian Blaise and Jean-François Férard

January, 2005

Foreword

Much has been said and done since the International Decade for Water and Sanitation of the 1980s to improve access to sufficient and safe drinking water in developing countries. Although we are nowhere near achieving universal access to this basic human need, progress has been accomplished. Technology has played an important role, but another critical legacy of the Decade has been a much better recognition and understanding of the social factors linked to sustainable access to safe drinking water for communities in developing countries.

One of the empowering factors has been the development of simple and affordable technologies for monitoring microbial water quality. Because they are inexpensive and are not dependent of sophisticated laboratories, such technologies have made their way into areas where electrical power has yet to reach and have allowed communities to perform their own water quality monitoring. The identification of specific micro-organisms are less important to rural inhabitants than an alarm system which they can depend on to consistently alert them to fecal contamination of their water supply. With water-borne diarrhea still causing the second highest mortality and morbidity toll in Third World countries (mainly infants and young children) the precautionary principle remains the only responsible strategy for poor communities.

Although fecal contamination of drinking water is still a serious problem in developing countries, it is not the only risk that need concern their populations and ecosystems. Both natural and anthropogenic processes are known to cause another kind, but no less dangerous contamination: recent surveys have shown for example that upwards of 36 million people in the Indian sub-continent are drinking water contaminated by arsenic; such contamination is also known to occur in the Southern Cone of Latin America and in areas of China. In Bangladesh, sadly, this problem has been compounded by altruistic efforts of AID agencies, digging wells to offer an alternative to fecally contaminated surface waters. Alas, the geologic makeup of the region has caused underground water to be heavily laced with Arsenic. Serious pathological manifestations have now been reported in affected areas. Some areas of India have also reported high fluoride concentration in well water leading to severe fluorosis in children and adults alike, with severe skeletal malformations and attendant physiological problems.

Human activity has also exacerbated this problem: Mercury contamination related to gold mining in frontier areas of South America; contamination of both surface and ground water by agricultural inputs such as pesticides and fertilizers; increased chemical pollution by recently implanted industries; global pollution by persistent chemicals used in industrialized countries such as PCBs and bromine-containing fire retardants. Unquestionably, the past and continuing release of toxicants of this nature to receiving waters, one of earth’s crucial compartments, by way of numerous (non) point sources of pollution, have equally impaired the health of aquatic biota and even adversely affected the biodiversity of some of its communities (*e.g.*, invertebrates and fish). Indeed, while microbiological pollution poses predominantly a risk to human health, chemical contamination represents a much more global threat to all components of the ecosystem, with a potential for more profound and enduring consequences.

In most cases, laboratory analytical methods exist to detect such chemicals and to quantify them. However, they can be time consuming and very expensive. No one could even propose that screening programs could be set up for routine water testing which would be both timely and affordable. In fact, this would not be feasible for industrialized countries either. How is one to test water for safety from chemicals, then? One approach is to perform routine analysis for specific chemicals in a given area where they are presumed to exist. Therein lies a cautionary tale: in the early nineties, the British Geological Survey (BGS) carried out a survey of well waters in Bangladesh (in relation to the well digging program discussed earlier), seeking data on iron and phosphorus which were presumed to contaminate the water. No attempts were made to measure other toxic compounds such as arsenic, which we now know constituted a major contaminant. Following the appearance of severe arsenic poisoning in the affected area, Bangladesh sued the agency for failing to warn users that the toxic metal was present in well water. The BGS was cleared by a British court of any wrong doing, since the former had performed the assays for which their services had been retained – and which did not include assays for other contaminants. Could this situation have not been avoided if a test had been applied to evaluate the overall toxicity of water, irrespective of the contaminant present? What about waters which exhibit contamination by multiple chemicals: individual measurements may not give an assessment of the true toxicity if these chemicals act in synergy rather than in an additive fashion.

Thus, some environmental scientists suggest that tests be used that measure “toxicity” rather than individual contaminants. Toxic samples could then be further assayed for specific contaminants if necessary to identify point sources and/or water treatment procedures. Relatively rapid, affordable and dependable assays would be a boon for developing country communities, in the same way as earlier rapid tests were for fecal contamination. The latter have proven to be usable in a sustainable manner in developing country communities, empowering them to monitor water safety and to act appropriately when necessary.

Bioassays appeared to fit the bill to perform this service to monitor chemical contamination. They have been around for a while. Until relatively recently, however, they remained in the realm of the laboratory. Only over the last two decades have they found a niche in testing for toxic chemicals in water and sediment, but not yet specifically as a tool for routine water quality monitoring. As ***Small-scale Freshwater Toxicity Investigations (Volume 1 and Volume 2)*** amply demonstrates, the science has now come of age. Assays based on bacteria, microscopic or multi-cellular algae, protozoa, invertebrates and vertebrates (freshwater fish cell cultures) are discussed in Volume 1 of this book. Of equal importance to my mind, Volume 2 of the book describes hazard assessment schemes that are based on combinations of the various bioassays, the so-called “battery” of tests. Indeed, all organisms are not similarly sensitive to given toxics. For instance, algae are likely to be very sensitive to herbicides albeit at levels which are unlikely to represent a danger to humans, while vertebrate cells may be less so. Thus, testing the sample on a series of organisms is more likely to reflect an overall toxicity. Whether one is to assess the risk to aquatic organisms or human beings, it is important to monitor the toxicity of samples on more than one trophic level.

Another significant advance is the development of a number of schemes to combine the results of toxicity testing on multiple trophic levels into indices which could be used to standardize results from one sample to another, from one area to another. ***Small-scale toxicity testing for freshwater environments*** presents a number of such schemes, and for this the editors should be congratulated. Only through such approaches can we begin to promote the use of these techniques more generally, especially if we are to encourage their use by field workers who have at best a limited experience of analytic laboratory techniques. Along with the other excellent chapters on hazard assessment schemes described in this book, the paper by Ronco, Castillo and Diaz-Baez *et al.* is significant to my mind because these authors have been working with municipal governments of Latin America (Argentina, Chile and Mexico) to promote WaterTox©. This is a battery of tests which they developed with colleagues elsewhere in Latin America, Canada, India and the Ukraine, with support from the International Development Research Centre (IDRC), the National Water Research Institute (Burlington, Ontario, Environment Canada) and the Saint-Lawrence Centre (Montreal, Quebec, Environment Canada). Results produced by this network of superb scientists have been extremely well received and, in some countries, governments are already incorporating batteries of bioassays in the national water quality testing programs (notably the Ukraine, Mexico and Chile).

All of this bodes very well for the future of bioassays, and for their transfer to poorer communities of the Third World where perhaps they are most needed.

Gilles Forget
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OVERVIEW OF CONTEMPORARY TOXICITY TESTING

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Preamble

In co-editing this book on ***Small-scale Freshwater Toxicity Investigations (Volume 1 and Volume 2)*** we felt it would be of value to bring to light the numerous types of publications which have resulted from the development and use of laboratory bioassays over the past decades. Knowing why toxicity testing has been conducted is obviously crucial knowledge to grasp the importance and breadth of this field.

Our tracking of publications involving toxicity testing was carried out with several databases (Poltox, Current Contents, Medline, Biosis and CISTI: Canada Institute for Scientific and Technical Information) and key words tailored to our objectives. In undertaking our search of the literature, we exclusively circumscribed it to articles or reports dealing with toxicity testing performed in the context of freshwater environments – obviously the focus of this book. Excluded from this review are publications describing sub-cellular bioassays (*e.g.*, assays conducted with sub-mitochondrial particles or where specific enzymes are directly exposed to contaminants) and those carried out with recombinant DNA (micro)organisms (*e.g.*, promoter/reporter bacterial constructs) and biosensors. These essentially newer techniques are unquestionably of interest and will be called upon to play increasingly useful roles in the area of small-scale environmental toxicology in the future, but they are clearly beyond the primary aims of this book.

C. Blaise and J.-F. Férard (eds.), Small-scale Freshwater Toxicity Investigations, Vol. 1, 1-68.

While this review cannot be judged exhaustive, it is nevertheless representative of toxicity tests developed and applied at different levels of biological organization to comprehend toxic effects associated with the discharge of xenobiotics to aquatic environments. In reading this chapter, it is our hope that readers will get a broad sense of the versatile ways in which bioassays have been used by the scientific community at large and of the genuine role they play - along with other tools and approaches in ecotoxicology - in ensuring the protection and conservation of the freshwater aquatic environment.

Introduction

Laboratory toxicity tests have been developed and conducted over the past decades to demonstrate adverse effects that chemicals can have on biological systems. Along with other complementary tools of ecotoxicology available to measure (potential or real) effects on aquatic biota (*e.g.*, microcosm, mesocosm and field study approaches with assessment of a variety of structural and/or functional parameters), they have been, and continue to be, useful to indicate exposure-effect relationships of toxicants under defined, controlled and reproducible conditions (Adams, 2003).

Among their multiple uses, acute and chronic bioassays have served, for example, to rank and screen chemicals in terms of their hazardous potential, to undertake biomonitoring studies, to derive water quality criteria for safe release of single chemicals into aquatic bodies and to assess industrial effluent quality in support of compliance and regulatory statutes.

Because of the pressing contemporary need to assess an ever-growing number of chemicals and complex environmental samples, the development and use of small-scale toxicity tests (also called "micro-scale toxicity tests" or "microbiotests") have increased because of their attractive features. Simply defined as "a test involving the exposure of a unicellular or small multicellular organism to a liquid or solid sample in order to measure a specific effect", small-scale tests are generally simple to execute and characterized by traits which can include small sample volume requirements, rapid turnaround time to results, enhanced sample throughput and hence cost-effectiveness (Blaise et al., 1998a).

Small-scale toxicity tests are numerous and their relative merits (and limitations) for undertaking environmental assessment have been amply documented (Wells et al., 1998; Persoone et al., 2000). The small-scale toxicity tests methods described in this book and the hazard assessment schemes into which they can be incorporated are certainly representative of the field of small-scale aquatic toxicology and of tests and approaches being applied actively in today's world.

Our scrutiny of publications identified in the literature search has enabled us to uncover the various ways in which laboratory toxicity tests have been applied, many of which are small-scale in nature. We have assembled papers based on their application affinities and classified them into specific sections, as shown in Figure 1. This classification scheme essentially comprises the structure of this chapter and each section is subsequently commented hereafter.

Main categories of aquatic bioassay applications based on representative publications involving toxicity testing

1. Liquid media toxicity assessment

- **1.1 Environmental samples**
- **1.2 Chemical contaminants**
- **1.3 Biological contaminants**

2. Sediment toxicity assessment

- **2.1 Assessment of areas of concern**
- **2.2 Critical body residues and links to (sub)lethal toxicity responses**

3. Miscellaneous studies/initiatives linked to aquatic toxicity testing applications (liquid media and sediments)

- **3.1 Endeavors promoting development, validation and refinement of toxicity testing procedures**
    - **3.1.1 Test method development**
    - **3.1.2 Inter-calibration exercises**
    - **3.1.3 Comparative studies**
    - **3.1.4 Factors capable of affecting bioassay responses**
- **3.2 Initiatives promoting the use of toxicity testing procedures**
    - **3.2.1 Review articles, biomonitoring and HAS articles**
    - **3.2.2 Standardized test methods and guidance documents**

Figure 1. Presentation pathway for the overview on toxicity testing exposed in this chapter.

In discussing the developments and applications of bioassays to liquid media and to sediments, we have placed some emphasis on the types of chemicals and environmental samples that have been appraised, on the types and frequency of biotic level(s) employed, as well as on the relative use of single species tests as opposed to test battery approaches.

1. Liquid media toxicity assessment

1.1 ENVIRONMENTAL SAMPLES

Articles related to toxicity testing of waters, wastewaters and other complex media are separated into three groups: studies involving toxicity testing of wastewaters and solid waste leachates (Tab. 1); studies involving toxicity testing of specific receiving media and sometimes including wastewaters (Tab. 2); studies combining toxicity/chemical testing and sometimes integrating other disciplines to assess waters, wastewaters and solid waste leachates (Tab. 3). While some investigations have strictly sought to measure bioassay responses after exposure to (waste)waters (Tables 2 and 3), an equally important number have combined toxicity and chemical testing in an attempt to establish a link between observed effects and putative chemical stressors present in appraised samples (Tab. 3). In both cases, a wide variety of point source effluent wastewaters of diverse industrial and municipal origins, as well as solid matrix leachates and various receiving media have been assessed. On the industrial scene, pulp and paper wastewaters appear to have received more overall attention than other industrial sectors, very likely owing to the fact that the forestry industry is a major enterprise internationally. Historically, also, pulp and paper mills were notorious for their hazardous discharges to aquatic environments (Ali and Sreekrishnan, 2001), although secondary treatment application has greatly reduced their toxicity (Scroggins et al., 2002b).

Table 1. Studies involving toxicity testing of wastewaters and solid waste leachates.

Assessment category	***Type of bioanalytical application^{a}***	***Biotic levels employed^{b,c} (and reference)***
Industrial effluents		
Dyeing factory	TT	B (Chan et al., 2003)
Electrical utilities	TBA	B,F,I (Rodgers et al., 1996)
Metal plating	TT	P (Roberts and Berk, 1993)
	TBA	B,F,I (Choi and Meier, 2001)
Mining	TT	B,B,B (Gray and O'Neill, 1997); F (Gale et al., 2003)
	TBA	B,B,F,I,I,I,I (CANMET, 1996); A,A,B,F,F,I,L (CANMET, 1997b); I,F (CANMET, 1998); Bi,F,I,I (Milam and Farris, 1998); A,F,I,L (Scroggins et al., 2002a);
Oil refinery	TT	B (Riisberg et al., 1996)
	TBA	A,A,F (Roseth et al., 1996); A,B,F,F,I,I,I,L,S (Sherry et al., 1997)
Pulp and paper	TT	F (Gagné and Blaise, 1993); B (Oanh, 1996); F (Bennett and Farrell, 1998); F (Parrott et al., 2003); F (Sepúlveda et al., 2003); F (van den Heuvel and Ellis, 2002)
	TBA	A,B,F (Blaise et al., 1987); B,B,B,I (Rao et al., 1994); A,B,L (Oanh and Bengtsson, 1995); A,B,B,F,I (Ahtiainen et al., 1996); A,B,F,F (Priha, 1996); B,F,F,I,I,I,I (Côté et al., 1999); A,F,F,I (Scroggins et al., 2002b); B,I (Pintar et al., 2004)
Tannery	TT	B,B (Diaz-Baez and Roldan, 1996)
	TBA	A,B,I,I,I,I,I (Isidori, 2000)
Textile	TT	I (Villegas-Navarro et al., 1999)

Table 1 (continued). Studies involving toxicity testing of wastewaters and solid waste leachates.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
Industrial effluents		
Various effluents	TT	F (Blaise and Costan, 1987); B (Tarkpea and Hansson, 1989); B (Svenson et al., 1992); I (Seco et al., 2003)
	TBA	B,F,F,F,F,F,I (Williams et al., 1993); B,F,I (Gagné and Blaise, 1997); B,I,I (Jung and Bitton, 1997); B,I (Liu et al., 2002)
Wood industry	TT	F (Rissanen et al., 2003)
Municipal effluents	TT	B,B,B,B,B (Codina et al., 1994); I (Monda et al., 1995); Fc (Gagné and Blaise, 1998a); Fc (Gagné and Blaise, 1999); B (Sánchez-Mata et al., 2001)
	TBA	B,B,I (Arbuckle and Alleman, 1992); A,B,F,P (George et al., 1995); B,B,F,Fc (Dizer et al., 2002); F,I (Gerhardt et al., 2002a)
Municipal and industrial effluents	TT	B (Asami et al., 1996); Fc (Gagné and Blaise, 1998b) ; Fc,Fc,F (Gagné and Blaise, 1998c)
	TBA	F,F,I,I,I (Fisher et al., 1989); F,F,I,I,I (Fisher et al., 1998); B,I (Doherty et al., 1999); B,F,I,I,S (Castillo et al., 2000); A,A,B,I,I,P (Manusadžianas et al., 2003)
WWTP (waste water treatment plants)	TT	B (Hoffmann and Christofi, 2001); B (Paixão and Anselmo, 2002)
	TBA	B,F,I (Sweet et al., 1997)
Solid waste leachates	TT	A (McKnight et al., 1981); B (Bastian and Alleman, 1998); B (Coz et al., 2004)
	TBA	B,B,B,F,F,I,I (Day et al., 1993); A,B,I,I,I,L,P (Clément et al., 1996); A,B,I,I,Pl,Pl,Pl (Ferrari et al., 1999); A,I,I,P (Törökné et al., 2000); A,A,B,B,I,I,P,S (Sekkat et al., 2001)

a) TT (toxicity testing): a study undertaken with test(s) at only one biotic level. TBA (test battery approach): a study involving tests representing two or more biotic levels.

b) Levels of biological organization used in conducting (or describing) TT: A (algae), B (bacteria), Bi (bivalve), F (fish), Fc (fish cells), I (invertebrates), L (*Lemnaceae*, duckweed: small vascular aquatic floating plant), P (protozoans), Pl (plant), and S (seed germination test with various types of seeds, *e.g.*, *Lactuca sativa*).

c) A study reporting the use of more than one toxicity test at the same biotic level is indicated by additional lettering (*e.g.*, use of three different bacterial tests is coded as “B, B, B”.

Table 2. Studies involving toxicity testing of specific receiving media and sometimes including wastewaters.

Assessment category	***Type of bioanalytical application[a]***	***Biotic levels employed[b,c] (and reference)***
Groundwater	TBA	A,B,B,I (Dewhurst et al., 2001)
Lake	TT	I (Kungolos et al., 1998)
	TBA	A,B,B,I,S (Okamura et al., 1996); A,I (Angelaki et al., 2000)
River/Stream	TT	I (Viganò et al., 1996); Bi,I (Stuijfzand et al., 1998); I (Jooste and Thirion, 1999); I (Lopes et al. 1999); I,I (Pereira et al., 1999); I (Sakai, 2001); I (Schulz et al., 2001); A (Okamura et al., 2002); I (Sakai, 2002a); I (Williams et al., 2003)
	TBA	A,B,F,I (Wilkes and Beatty-Spence, 1995); B,B,B,I,I (Dutka et al., 1996); A,F,F,I,L (CANMET, 1997c); A,I (Baun et al., 1998); B,B,I (Sabaliunas et al., 2000); A,B,I,I,I (Van der Wielen and Halleux, 2000)
Wetland	TT	B (Dieter et al., 1994)
Specific types of environmental samples		
Packaged water	TT	P (Sauvant et al., 1994)
Pond	TT	I,I,I (Lahr, 1998)
Rainwater	TT	I (Sakai, 2002b)
Rice field	TBA	A,I (Cerejeira et al., 1998)
Runoff water	TT	A (Wong et al., 2001); I (Boulanger and Nikolaidis, 2003)
	TBA	B,B,I (Marsalek et al., 1999); A,B (Heijerick et al., 2002)
Diverse types of environmental samples [d]	TT	B (Coleman and Qureshi, 1985); I (Samaras et al., 1998); I (Lechelt, 2000); A (Graff et al., 2003); Fc (Schweigert et al., 2002)

Table 2 (continued). Studies involving toxicity testing of specific receiving media and sometimes including wastewaters.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
Diverse types of environmental samples [d]	TBA	B,B,I (Cortes et al., 1996); B,I (Pardos et al., 1999a); A,I,I,L,P (Blinova, 2000); A,I,I,P (Czerniawska-Kusza and Ebis, 2000); A,I,I,P (Dmitruk and Dojlido, 2000); A,I,I,I (Isidori et al., 2000); B,I,I,P (Stepanova et al., 2000) A,I,I,S,S (Arkhipchuk and Malinovskaya,2002); A,I,I,S (Diaz-Baez et al., 2002); A,I,I (Mandal et al., 2002); A,I,I,S (Ronco et al., 2002)

a) TT (toxicity testing): a study undertaken with test(s) at only one biotic level. TBA test battery approach): a study involving tests representing two or more biotic levels.

b) Levels of biological organization used in conducting (or describing) TT: A (algae), B (bacteria), Bi (bivalve), F (fish), Fc (fish cells), I (invertebrates), L (*Lemnaceae*, duckweed: small vascular aquatic floating plant), P (protozoans), and S (seed germination test with various types of seeds, *e.g.*, *Lactuca sativa*).

c) A study reporting the use of more than one toxicity test at the same biotic level is indicated by additional lettering (*e.g.*, use of three different bacterial tests is coded as "B, B, B".

d) Includes samples such as potable/surface waters, as well as industrial effluents, soil/sediment/sludge extracts, landfill leachates and snow, where individual studies report testing one or more sample type(s).

Table 3. Studies combining toxicity/chemical testing and sometimes integrating other disciplines to assess waters, wastewaters and solid waste leachates.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
Industrial effluents		
Chemical plant	TT	B (Chen et al., 1997)
	TBA	B,I,I,I (Guerra, 2001)
Coal industry	TBA	A,I,I,I (Dauble et al., 1982); F,I,I (Becker et al., 1983)
Coke	TBA	A,B (Peter et al., 1995)
Complex munitions	TBA	A,A,A,A,F,F,F,F,I,I,I,I (Liu et al., 1983)
Mining	TT	I,I (Fialkowski et al., 2003)
	TBA	F,I (Erten-Unal et al., 1998); A,B (LeBlond and Duffy, 2001)
Pharmaceutical	TBA	A,B,B,B,F,I (Brorson et al., 1994); B,I (Tišler and Zagorc-Koncan, 1999)

Table 3 (continued). Studies combining toxicity/chemical testing and sometimes integrating other disciplines to assess waters, wastewaters and solid waste leachates.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
Industrial effluents		
Pulp and paper	TBA	B,I,F (Dombroski et al., 1993); B,F,I (Leal et al., 1997); B,F,I (Middaugh et al., 1997); A,B,B,F,I (Ahtiainen et al., 2000); B,I,I,P,P (Michniewicz et al., 2000)
Resin production	TBA	A,B,F,I (Tišler and Zagorc-Koncan, 1997)
Tannery	TT	I,I (Cooman et al., 2003)
	TBA	B,I (Fernández-Sempere et al., 1997); B,I (Font et al., 1998)
Tobacco plant	TBA	A,B,B,B,B,P,P (Sponza, 2001)
Water based drilling muds	TBA	A,I (Terzaghi et al., 1998)
Oily waste		
Olive oil	TBA	B,I,I (Paixão et al., 1999)
Oil refinery	TT	B (Aruldoss and Viraraghavan, 1998)
	TBA	A,B,B,F,F,I,I,I,L,S (Sherry et al., 1994); B,F,I (Bleckmann et al., 1995)
Oil-shale	TT	B,B,B (Kahru et al., 1996)
	TBA	B,B,I,I,P (Kahru et al., 1999); A,B,B,B,I,I,I,P (Kahru et al., 2000)
Composting oily waste	TBA	B,B,B,B,B,I,I,I,L,S (Juvonen et al., 2000)
Municipal effluents	TT	B (Pérez et al., 2001)
	TBA	B,B,Pl,Pl,S (Monarca et al., 2000)
WWTP (waste water treatment plant)	TT	B (Chen et al., 1999); I (Kosmala et al., 1999); B,B,B (Gilli and Meineri, 2000); B (Svenson et al., 2000); B (Wang et al., 2003)
	TBA	F,I (Fu et al., 1994); A,Fc,I (Pablos et al., 1996); B,B,B,B,P (Ren and Frymier, 2003)
Leachates		
From agricultural production solid waste	TT	B (Redondo et al., 1996)
From industrial solid waste	TT	L (Jenner and Janssen-Mommen, 1989); B (Coya et al., 1996); I,I (Rippon and Riley, 1996); I,I,I,I,I,I (Canivet and Gibert, 2002)

Table 3 (continued). Studies combining toxicity/chemical testing and sometimes integrating other disciplines to assess waters, wastewaters and solid waste leachates.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
Leachates		
From industrial solid waste	TBA	A,B,I (Lambolez et al., 1994); B,B,B,B,L,S,S,S (Joutti et al., 2000); A,B,I (Malá et al., 2000); A,B,B,I (Vaajasaari et al., 2000)
From municipal solid waste	TBA	A,A,B,I,I,S (Latif and Zach, 2000); A,B,B,F,I,I (Rutherford et al., 2000); A,B,I (Ward et al., 2002a)
Miscellaneous types of environmental samples [d]	TT	I (Gasith et al., 1988); I (Doi and Grothe, 1989) B (Bitton et al., 1992); I (Jop et al., 1992); A (Wong et al., 1995); B (Hao et al., 1996); I (Blaise and Kusui, 1997); B,B (Hauser et al., 1997); I (Eleftheriadis et al., 2000); F (Liao et al., 2003); I (Kszos et al., 2004); A,I,I,P,S (Latif and Licek, 2004)
	TBA	F,I,I (Tietge et al., 1997); A,B,I,I,I (Kusui and Blaise, 1999); A,A,I,I,P (Manusadžianas et al., 2000)
Natural waters		
Floodplain	TBA	B,I,I,I (de Jonge et al., 1999)
Groundwater	TBA	A,B,I,P,P,P (Helma et al., 1998); B,F,I (Gustavson et al., 2000)
Rivers and streams	TT	A (Guzzella and Mingazzini, 1994); Bi,I,I (Crane et al., 1995); I (Bervoets et al., 1996); A,A (O'Farrell et al., 2002)
Wetland	TT	B (Boluda et al., 2002)

a) TT (toxicity testing): a study undertaken with test(s) at only one biotic level. TBA (test battery approach): a study involving tests representing two or more biotic levels.

b) Levels of biological organization used in conducting (or describing) TT: A (algae), B (bacteria), Bi (bivalve), F (fish), Fc (fish cells), I (invertebrates), L (*Lemnaceae*, duckweed: small vascular aquatic floating plant), P (protozoans), Pl (plant), and S (seed germination test with various types of seeds, *e.g., Lactuca sativa*).

c) A study reporting the use of more than one toxicity test at the same biotic level is indicated by additional lettering (*e.g.*, use of three different bacterial tests is coded as "B, B, B".

d) Includes samples such as storm waters, river waters, as well as industrial/municipal effluents, sludge extracts, where individual studies report testing one or more sample type(s).

While it is beyond our intent to discuss the main purpose(s) that prompted research groups to conduct individual investigations with particular toxicity tests, readers can access this information by consulting references of interest. Others are mentioned hereafter, however, to indicate bioanalytical endeavors that have taken place in past years. For example, Bitton et al. (1992), after developing a metal-specific bacterial toxicity assay, demonstrated its capacity to correctly pinpoint heavy-metal containing industrial wastewaters. In another venture, Roberts and Berk (1993) were motivated to undertake toxicity testing of a metal plating effluent and of a series of (in)organic chemicals in order to further validate a newly-developed protozoan chemo-attraction assay. Again, a test battery approach with chemical support to assess a coke plant effluent identified treatment methods that were superior for decontaminating the wastewater (Peter et al., 1995). In toxicity testing of tannery industry effluent samples, bacterial tests were shown to be sufficiently sensitive to act as screening tools for such wastewaters (Diaz-Baez and Roldan, 1996). In a study conducted on industrial, municipal and sewage treatment plants, toxicity testing identified chlorination as the most important contributor of toxic loading to the receiving environment (Asami et al., 1996). After a comprehensive assessment of pulp and paper mills, toxicity testing proved useful to ameliorate mill process control (Oanh, 1996). Another study conducted with three bacterial toxicity tests showed that oil-shale liquid wastes could be bio-degraded when activated sludge was pre-acclimated to phenolic wastewaters (Kahru et al., 1996). Petrochemical plant assessment using toxicity testing, chemical analysis and a TIE/TRE strategy combined to identify aldehydes as the main agent of effluent toxicity (Chen et al., 1997). Test battery assessment of a mine water discharge, which involved both toxicity testing and in-stream exposure of bivalves, helped to set a no-effect level criterion for a bioavailable form of iron (Milam and Farris, 1998). A comparison of laboratory toxicity testing and *in situ* testing of river sites downstream from an acid mine drainage demonstrated good agreement between the two approaches for the most contaminated stations (Pereira et al., 1999). A similar strategy to assess gold and zinc mining effluents confirmed the reliability of some chronic assays for routine toxicity monitoring (LeBlond and Duffy, 2001). Clearly, there are numerous reasons for conducting toxicity testing and/or chemical analysis of (waste)waters to derive relevant information that have eventually triggered enlightened decisions contributing to their improvement.

Of the 188 studies reported in Tables 1, 2 and 3, more than half (n = 101) were conducted with two or more tests representing at least two biotic levels (*i.e.*, test battery approach or TBA), as opposed to those performed with a single biotic level (n = 87). While test and biotic level selection may be based on a variety of reasons and study objectives (*e.g.*, practicality, cost, personnel availability), preference for TBAs can also be influenced by the need to assess hazard at different levels so as not to underestimate toxicity. Indeed, contaminants can demonstrate "trophic-level specificity" (*e.g.*, phytototoxic effects of herbicides) or they can exert adverse effects at multiple levels (*e.g.*, particular sensitivity of cladocerans toward heavy metals in contrast to bacteria). When TBAs are used, they are mostly conducted with two, three or four trophic levels (Tab. 4).

Whether TT (toxicity testing with single species tests at the same biotic level) or TBAs are performed, some test organisms have been more frequently used than others (Tab. 5). Invertebrates have been the most commonly employed, as had been pointed out in an earlier literature survey conducted between 1979 and 1987 (Maltby and Calow, 1989). Bacteria as well as fish and algal assays come next in frequency of use. Early standardization of invertebrate (*e.g.*, *Daphnia magna*) and bacterial test (*e.g.*, *Vibrio fischeri* luminescence assay) procedures, as well as increased miniaturization and cost-effectiveness, are likely factors explaining their popularity over the past decades. While some groups of small-scale toxicity tests (*i.e.*, fish cell, duckweed and protozoan tests) have thus far received less attention to appraise various environmental samples, recent efforts in test procedure validation and standardisation should effectively promote their use in the future (see Volume 1, Chapters 7, 8, 14 and 15).

Table 4. Frequency of the number of biotic levels employed in test battery approaches (TBAs) for complex liquid media assessment based on the 101 TBA papers classified in Tables 1-3.

TBA studies undertaken with:	***Number and frequency (%)***
Two biotic levels	39/101 (38.6)
Three biotic levels	38/101 (37.6)
Four biotic levels	19/101 (18.8)
Five biotic levels	3/101 (3)
Six biotic levels	2/101 (2)

Table 5. Frequency of use of specific biotic levels employed in toxicity testing (TT) and test battery approaches (TBA) for complex liquid media assessment based on the 188 papers classified in Tables 1-3.

TT and TBA studies undertaken with:	**Number and frequency (%)**
Algae	70/553* (12.7)
Bacteria	152/553 (27.5)
Bivalves	3/553 (< 1)
Fish	68/553 (12.3)
Fish cells	8/553 (1.5)
Invertebrates	199/553 (36.0)
Lemnaceae (duckweed)	10/553 (1.8)
Plants	3/553 (< 1)
Protozoans	23/553 (4.2)
Seeds	15/553 (2.7)

*Total number of single species tests reported in the 188 papers classified in Tables 1-3 (= sum of number of A, B, Bi, F, Fc, I, L, P, Pl, S tests indicated in the "Biotic levels employed" column).