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.

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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 (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,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).

1.2 CHEMICAL CONTAMINANTS

It has been estimated that as many as 250,000 man-made chemicals could possibly enter different compartments of the biosphere and cause adverse effects on ecosystem and human health (OSPAR, 2000). Out of concern for ensuring the protection of aquatic biota, a large number of scientists internationally have turned to bioassays as primary means of assessing the hazard (and risk) posed by these substances. Indeed, the scientific literature abounds with hundreds of publications dealing with toxicity testing of various classes of (in)organic chemicals. While it is beyond the intentions of this chapter to discuss all of these, papers have been selected that reflect the types of chemicals having undergone toxicity assessment. In general, published articles show that test organisms and biotic levels described are the same as those employed for assessing environmental samples.

Representative investigations involving toxicity assessment of metals, ions and oxidizing agents are highlighted in Table 6. Varied toxicological objectives have been pursued to evaluate metals singly or in groups of two or more with one toxicity test or with a test battery. The benefits of these initiatives to enhance our knowledge of undesirable effects that can be directed toward specific biotic levels (*e.g*., Holdway et al., 2001), to identify useful sentinel species (*e.g*., Madoni, 2000), or to promote useful (Couture et al., 1989) or potentially safer clean-up technologies (Leynen et al., 1998) should be fairly obvious.

Table 6. Studies involving toxicity assessment of metals, ions and oxidizing agents.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
One metal:		
Aluminium	TT: four species of invertebrates are exposed to Al over a pH range of 3.5 to 6.5.	I,I,I,I (Havas and Likens, 1985)
Cadmium	TT: a simple microcosm experiment associating two biotic levels conducted in a Petri dish allows measurement of reproduction effects on daphnids following Cd contamination of either their food source (algae) or of their water medium.	I (Janati-Idrissi et al., 2001)
Chromium (Cr^{+6})	TT: luminescent bacteria are exposed to assess the influence of pH speciation of chromium on toxicity response.	B (Villaescusa et al., 1997)

Table 6 (continued). Studies involving toxicity assessment of metals, ions and oxidizing agents.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
One metal:		
Copper	TT: comparison of effects occurring at molecular (DNA profiling) and population (ecological fitness parameters including acute and chronic toxicity) levels for *Daphnia magna.*	I (Atienzar et al., 2001)
Gallium	TT: assessment of inter-metallic elements used in making-high speed semiconductors such as gallium arsenic with *Cyprinus carpio.*	F (Yang and Chen, 2003)
Lead	TBA: assessment of toxicity, uptake and depuration of lead in fish and invertebrate species.	F,F,I,I (Oladimeji and Offem, 1989)
Manganese	TT: assessment at three levels of water hardness with *Ceriodaphnia dubia* and *Hyalella azteca.*	I,I (Lasier et al., 2000)
Mercury	TT: assessment of 10 mercury compounds to determine their relative toxicities to luminescent bacteria.	B (Ribo et al., 1989)
Nickel	TT: assessment with 12 species of freshwater ciliates to determine which could become, based on observed sensitivity, a good bio-indicator of waters polluted by heavy metals.	P (Madoni, 2000)
Selenium	TT: assessment of selenium compounds and relationships with uptake in an invertebrate species.	I (Maier and Knight, 1993)
Silver	TBA: assessment of toxicity to fish and invertebrates under a variety of water quality conditions.	F,I (La Point et al., 1996)
Uranium	TT: assessment of depleted uranium on the health and survival of *C. dubia* and *H. azteca.*	I,I (Kuhne et al., 2002)
Zinc	TT: assessment the influence of various ions and pH on phytotoxicity response.	A (Heijerick et al., 2002)

Table 6 (continued). Studies involving toxicity assessment of metals, ions and oxidizing agents.

Assessment category	***Type of bioanalytical application***[a]	***Biotic levels employed***[b,c] ***(and reference)***
One metal:		
Zirconium	TBA: assessment of zirconium ($ZrCl_4$), considered of use as a P-precipitating agent to reduce the eutrophication potential of pig manure wastes to receiving environments.	A,B,F (Couture et al., 1989)
Two metals:		
Cadmium, Zinc	TT: assessment of their acute and chronic toxicity to two *Hydra* species.	I,I (Holdway et al., 2001)
Three metals:		
Arsenic, Cobalt, Copper	TT: assessment of relationships between acute toxicity and various experimental variables (*e.g.*, metal concentration in water, time of exposure, bioconcentration factor) with two fish species.	F,F (Liao and Lin, 2001)
Four metals or more:	TT: assessment of the adequacy of cultured fish cells (Bluegill BF-2) for toxicity testing of aquatic pollutants.	Fc (Babich and Borenfreund, 1987)
Ions:	TT: assessment of the phytotoxicity of high density brines (calcium chloride and calcium bromide) to *L. minor*.	L (Vujevic et al., 2000)
Rare earth elements:	TT: assessment of the aquatic toxicity of rare earth elements (La, Sm, Y, Gd) to a protozoan species.	P (Wang et al., 2000)
Oxidizing agents:	TBA: assessment of the acute toxicity of ozone, an alternative to chlorination to control biofouling in cooling water systems of power plants, to fish larvae of three species and to *D. magna*.	F,F,F,I (Leynen et al., 1998)

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), F (fish), Fc (fish cells), I (invertebrates), L (*Lemnaceae*, duckweed: small vascular aquatic floating plant), and P (protozoans).
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”.

The toxicological properties of chemicals representing various classes and structures of organic substances have also been assessed by a series of bioassays at different levels of biological organization (Tab. 7). Featured in this table is but the tip of the iceberg in terms of the types of studies that have been conducted to further our knowledge about the hazards of anthropogenic molecules. While industrial progress has markedly enhanced the quality of life on this planet through production of countless xenobiotics synthesized for multiple human uses (*e.g.*, diverse household products and pharmaceuticals), it has also increased the risk linked to their discharge and fate in aquatic systems. Understanding their potential for adverse effects through the conduct of bioassays is clearly a first step in the right direction.

Table 7. Examples of studies involving toxicity assessment of organic substances.

Assessment category (and product tested)	***Type of bioanalytical application[a], biotic levels employed[b,c] (and reference)***
Acaricide (Tetradifon)	TT: I (Villarroel et al., 1999)
Adjuvants (several used as surfactants for aquatic herbicide applications)	TT: F (Haller and Stocker, 2003)
Anti-fouling paint (TBT)	TBA: A,I (Miana et al., 1993)
Aromatic hydrocarbon (*para*-methylstyrene)	TBA: A,F,I (Baer et al., 2002)
Cationic fabric softener (DTDMAC)	TBA: A,B,B,I,I,I (Roghair et al., 1992)
Chelator ([S,S]-EDDS)	TBA: A,A,F,I (Jaworska et al., 1999)
Detergents and softeners (26 detergents and 5 softeners)	TT: I (Pettersson et al., 2000)
De-icing / anti-icing fluids	TT: B (Cancilla et al., 1997)
Disinfectant (Mono-chloramine)	TBA: F,I (Farrell et al., 2001)
Dyes (Fluorescein sodium salt, Phloxine B)	TT: I (Walthall and Stark, 1999)
Fatty acids (C_{14} to C_{18})	TT: A (Kamaya et al., 2003)
Fire control substances (Fire-Trol GTS-R and LCG-R, Phos-Chek D75-F and WD-881, Silv-Ex)	TBA: A,I (McDonald et al., 1996)
Flame retardant (Brominated diphenyl ether-99)	TBA: A,I (Evandri et al., 2003)
Fungicide (Ridomil plus 72)	TBA: F,I (Monkiédjé et al., 2000)
Herbicide (Atrazine)	TT: I (Dodson et al.,1999)
Household products (Abrasives, additives, disinfectants)	TBA: A,B,B,F,F,I (Bermingham et al., 1996)
Insecticide (Glyphosate)	TT: L (Lockhart et al., 1989)

Table 7 (continued). Examples of studies involving toxicity assessment of organic substances.

Assessment category (and product tested)	***Type of bioanalytical application[a], biotic levels employed[b,c] (and reference)***
Lubricant additives (Ashless dispersant A and B, Zinc dialkyldithiophosphate)	TT: A (Ward et al., 2002b)
(Tri *n*-butyl phosphate)	TBA: A,B (Michel et al., 2004)
Nitromusks (Ambrette, Setone, Moskene,Tibetene, Xylene)	TBA: A,B,I (Schramm et al., 1996)
Narcotics (*n*-alkanols)	TT: B (Gustavson et al., 1998)
Organochlorides (PCBs)	TT: B (Chu et al., 1997)
Organosulfur compounds (several benzothiophenes)	TBA: B,I (Seymour et al., 1997)
Pesticide (Cyromazine)	TT: I,I (Robinson and Scott, 1995)
Pharmaceutical compound (β-Blockers)	TBA: F,I,I,I (Huggett et al., 2002)
Phenolic compounds (Pentachlorophenol)	TBA: A,B,I,S (Repetto et al., 2001)
Phtalate esters (several)	TT: I,I,I (Call et al., 2001)
Solvents (Mono-, Di- and Tri PGEs)	TBA: A,B,F,F,F,I,I,L (Staples and Davis, 2002)
Surfactant (Genapol OX-80)	TT: A (Anastácio et al., 2000)
Volatilecompounds (*N*-nitrosodiethylamine, *N*-nitrosodimethylamine)	TBA: A,A,F,I,I (Draper III and Brewer, 1979)
Wood preservative (Bardac 2280)	TBA: F,F,F,F,I,I,I,I (Farrell et al., 1998)

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), F (fish), I (invertebrates), L (Lemnaceae, duckweed: small vascular aquatic floating 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".

Several papers have also reported toxicity data for a variety of metals and organic substances simultaneously. Reasons for conducting such investigations include 1) establishing the concentrations at which chemicals exert their adverse effects (*e.g.*, at the ng/L, μg/L or mg/L levels), 2) estimating environmental risk based on measured toxicity endpoints and predicted environmental concentrations for specific chemicals and 3) defining toxicant concentrations harmful for specific biotic levels and/or assemblages of species within each level.

Studies have assessed the toxicological properties of one or more heavy metal(s) with one or more organic substance(s). Examples include copper and diazinon (van der Geest et al., 2000), cadmium and pentachlorophenol (McDaniel and Snell, 1999), several heavy metals (Cd, Cu, Ni, Pb, Zn) and organic (Chlorpyrifos, DDT, DDD, DDE, Dieldrin) toxicants (Phipps et al., 1995), and two metals (Cu, Zn) and eight surfactants (Dias and Lima, 2002). Again, test organisms employed for toxicity assessment are similar to those discussed previously and investigators make use of one or more biotic levels to undertake their evaluations.

Chemical toxicity assessment should also take into consideration the combined effects that groups of chemicals can have on living organisms. Indeed, contaminants are not discharged singly in aquatic systems but are joined by many others whose composition will depend on the origin of (non)point sources of pollution affecting particular reaches of receiving waters (*e.g.*, industrial, municipal and agricultural sources). The recognition that groups of chemicals can interact together to produce a resulting effect that can reduce (antagonistic effect) or exacerbate (synergistic effect) that of substances tested singularly has prompted scientists to appraise the toxicity characteristics of mixtures.

Published articles indicate that work has focussed on (binary, ternary, etc.) mixtures including metals, organics as well as metal/organic cocktails. For metals, examples include toxicity testing of various mixtures with algae (Chen et al., 1997), bacteria (Mowat and Bundy, 2002) and micro-invertebrates (Burba, 1999). For organics, mixtures have been assessed belonging to groups such as antifouling agents (Fernandés-Alba et al., 2002), herbicides (Hartgers et al., 1998), pesticides (Pape-Lindstrom and Lydy, 1997), and manufactured munitions (Hankenson and Schaeffer, 1991). For (in)organic mixtures, metal/pesticide (Stratton, 1987), metal/composted manure (Ghosal and Kaviraj, 2002), as well as metal/miscellaneous organic (Parrott and Sprague, 1993) combinations offer additional examples of interaction assessments. Because appraising mixtures of compounds (singularly and in binary, ternary or other combinations) is more laborious in time and effort than for single compounds, toxicity testing has, in most cases, been conducted with a single test organism, as opposed to the use of a test battery. Algal, bacterial and micro-invertebrate tests have thus far been favoured in this respect.

Another active field of research intended to estimate the toxic properties of organic compounds lies in the determination of their quantitative structure-activity relationships (QSAR). The rationale for this work is based on the fact that molecules will enter living organisms to exert adverse effects depending on their elemental composition and structure. In brief, QSARs are regression equations relating toxicological endpoints (*e.g.*, LC50s, EC50s, IC50s, NOECs) to physicochemical properties within a class of compounds. A good number of QSARs, for example, are determined with the octanol-water coefficient (K_{ow}), a well-known predictor of the tendency of a compound to be bio-accumulated. QSARs have several potential uses, some of which include 1) predicting the effects of newly-synthesized chemicals, 2) priority ranking of chemicals destined for more elaborate toxicity testing, 3) assistance in deriving water quality guidelines and 4) rapidly estimating toxicity for specific compounds when toxicity test data are unavailable (Environment Canada, 1999).

A quantitative structure-activity relationship (QSAR), for example, has been shown for aliphatic alcohols, where 96h-LC50s for fathead minnows are related to their K_{ow} status (Veith et al., 1983). Other QSARs based on K_{ow} have been reported for several classes of organics with test species including algae, invertebrates and fish (Suter, 1993). Hydrophobicity-based QSARs were also generated for fish and invertebrates with a set of 11 polar narcotics (Ramos et al., 1998) and for bacteria, fish and protozoan test organisms with a large set of (non)polar narcotic classes of chemicals (Schultz et al., 1998). QSARs were also employed to predict the biodegradation, bioconcentration and toxicity potential of more than 5000 xenobiotics (industrial chemicals, pesticides, food additives and pharmaceuticals) having a potential for release into the Great lakes basin (Walker et al., 2004). This study, in particular, illustrates the usefulness of QSARs as a cost-effective pre-screening adjunct to (significantly more expensive) monitoring studies that can then be prioritized towards those chemicals having the potential to persist and bio-accumulate in aquatic species. In these and other recent QSAR-based investigations of chemicals (Junghans et al., 2003; Choi et al., 2004; Schultz et al., 2004), it is noteworthy to mention that small-scale toxicity tests conducted with algae, bacteria, invertebrates and protozoans are used frequently.

1.3 BIOLOGICAL CONTAMINANTS

Besides the many hazards looming on aquatic life owing to the uncontrolled discharge of a myriad of chemicals, exposure to plants or microbes may also place it at risk. Indeed, toxicity tests conducted within the last decade on plant substances/extracts, and on microbes or their products (*e.g.*, metabolites), to investigate their biopesticide or toxicity potential, have indicated that species of different levels of biological organization can be adversely affected by such biological contaminants (Tab. 8). Since undesirable ecological effects to aquatic communities could result from exposure to naturally-produced chemicals or micro-organisms, documenting their toxicity potential via bioassays is fully justified.

As future applications with natural and/or genetically-modified plants and micro-organisms are expected to increase in the future (*e.g.*, for bioremediation treatments of contaminated soils, wastewaters, sediments), so will toxicity assessment programs to insure the protection of aquatic biota. In Canada, for example, information is now required to appraise new microbes (and their products) in terms of their toxicity potential toward aquatic organisms, and standardized toxicity test methods are being developed and recommended for this purpose (Environment Canada, 2004a). Risk assessment of biological contaminants is clearly an area that will receive sustained attention in the coming years.

Table 8. Examples of studies involving toxicity assessment of biological contaminants.

Assessment category and product tested	***Type of bioanalytical application[a], biotic levels employed[b,c] (and reference)***
Biopesticides	
Aquatic plant: essential oils from *Callicarpa americana*	TBA: A,A,A,B,B,B,B,B,S,S (Tellez et al., 2000)
Aquatic plant: phenanthrenoids from *Juncus acutus*	TT: A (DellaGreca et al., 2002)
Aquatic plant: essential oils from *Lepidium meyenii*	TBA: A,A,I,S,S (Tellez et al., 2002)
Aquatic plant: antialgal furano-diterpenes from *Potamogetonaceae*	TT: A (DellaGreca et al., 2001)
Aquatic plant: ent-labdane diterpenes from *Potamogetonaceae*	TBA: A,I,I,I,I (Cangiano et al., 2002)
Bacterium: *Bacillus thuringiensis*	TT: I (Manasherob et al., 1994); TT: I (Kondo et al., 1995)
Fungus: *Metarhizium anisopliae*	TT: B (Milner et al., 2002)
Biotoxins	
Cyanobacteria	
Microcystis aeruginosa	TBA: B,I (Campbell et al., 1994)
Anabaena sp., *M. aeruginosa*, *Microcystis* sp., *P. aghardii*, *P. rubenscens*	TT: I (Törökné, 2000; Törökné et al., 2000)
M. aeruginosa, *M. wesenbergii*	TBA: B,B,B,I,I,I,I,P (Maršálek and Bláha, 2000)
Cyanobacterial blooms	TBA: I,I,P,P (Tarczynska et al., 2000)
Pathogenic bacteria: *Aeromonas hydrophila*, *Flavobacter* spp., *Flexibacter columnaris*	TT: F (Geis et al., 2003)
Odor and taste compounds of microbial origin	
Geosmin, 2-methyliso-borneol	TT: Fc (Gagné et al. 1999)

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), F (fish), Fc (fish cells), I (invertebrates), 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".

2. Sediment toxicity assessment

2.1 ASSESSMENT OF AREAS OF CONCERN

In today's world, sediment contamination continues to be a growing environmental issue. Indeed, the deposition of numerous (in)organic chemicals in aquatic systems stemming from various types of anthropogenic activities (urban, industrial, agricultural) has the potential to adversely affect aquatic biota. Once deposited, resuspension of contaminated sediment *via* both natural (*e.g.*, flood scouring) and man-made (*e.g.*, dredging, navigation, open water deposition) activities can further harm living organisms by increasing their contact with (and uptake of) deleterious chemicals. Integrated strategies to assess the toxic potential of contaminated sediments, such as the sediment quality triad approach (see Volume 2, Chapter 10) continue to favour the presence of a strong bioanalytical component within investigation schemes.

Our literature review has shown that sediment toxicity assessment has received marked attention over the past decades and that bioassays have been largely used for this purpose. Contaminated environments, for instance, have triggered many studies conducted to detect and quantify sediment toxicity, to determine the extent of its impact, and to enhance understanding of its short and long-term effects on aquatic communities.

To give readers a first insight into the ways in which toxicity tests have been applied for sediment assessment, we have regrouped publications dealing with sediments collected from areas of concern (Tab. 9) and those collected from other lotic and lentic environments, also impacted by pollutant discharges, where combined chemical-biological analyses were performed (Tab. 10). Sediments were collected from lakes and rivers to undertake initial assessment of sites, to study effects of diverse (in)organic contamination, as well as to investigate various toxicity aspects linked to oil spills and flooding events (Tab. 9). A number of studies also explored relationships between specific contaminants and observed toxicity effects (Tab. 10).

Table 9. Studies with field-collected sediments: assessment of areas of concern.

Assessment objective, type of bioanalytical application[a] and tested sediment phase(s)		***Biotic levels employed[b,c] (and reference)***
Areas impacted by wastewaters: with sediments potentially contaminated by (in)organic pollution		
Ammonia effects	TT: overlying water, pore water	I (Bartsch et al., 2003)
Initial/preliminary assessment of sites	TT: whole sediment	B (Onorati et al., 1998)
	TT: overlying water	I,I (Rediske et al., 2002)
	TT: whole sediment	I (Bettinetti et al., 2003)
	TT: whole sediment	I,I (Collier and Cieniawski, 2003)
	TBA: elutriate	A,B,I,I,I (Sloterdijk et al., 1989)
	TBA: pore water, whole sediment	B,I (Munawar et al., 2000)
Metal contamination	TT: overlying water	I,I,I (West et al., 1993)
	TT: spiked sediment, whole sediment	I,I (Dave and Dennegard, 1994)
	TT: pore water	I (Besser et al., 1995)
	TT: pore water	I (Deniseger and Kwong, 1996)
	TT: pore water	I (Call et al., 1999)
	TT: pore water	I (Hill and Jooste, 1999)
	TT: overlying water, pore water	I (Bervoets et al., 2004)
	TBA: pore water, whole sediment	B,F,F,I,I,I,I (Kemble et al., 1994)
	TBA: overlying water, pore water, whole sediment	B,I,I,I,I,S (Burton et al., 2001)
Metal and organic contamination	TT: whole sediment	I,I (Nebeker et al., 1988)
	TT: elutriate	A (Lacaze et al., 1989)
	TT: whole sediment	B,B (Kwan and Dutka, 1992)
	TT: whole sediment	I,I (Jackson et al., 1995)
	TT: elutriate	I (Bridges et al., 1996)
	TT: elutriate, pore water, whole sediment	I,I (Ristola et al., 1996)
	TT: whole sediment	B (Svenson et al., 1996)
	TT: pore, elutriate, whole sediment	I,I,I,I,I (Sibley et al., 1997b)
	TT: whole sediment	A (Blaise and Ménard, 1998)
	TT: OE[d], whole sediment	B (Salizzato et al., 1998)

Table 9 (continued). Studies with field-collected sediments: assessment of areas of concern.

Assessment objective, type of bioanalytical application[a] and tested sediment phase(s)		***Biotic levels employed[b,c] (and reference)***
Areas impacted by wastewaters: with sediments potentially contaminated by (in)organic pollution		
Metal and organic contamination	TT: overlying water	I (Call et al., 1999)
	TT: overlying water	I (Martinez-Madrid, 1999)
	TT: overlying water, whole sediment	I,I,I,I (Munawar et al., 1999)
	TT: overlying water	I,I,I,I (Cheam et al., 2000)
	TT: pore water	I (Kemble et al., 2002)
	TBA: pore water	B,I,I (Giesy et al., 1988)
	TBA: overlying water, whole sediment	A,B,B,B,B,I (Dutka et al., 1989)
	TBA: elutriate, whole sediment	A,I (Gregor and Munawar, 1989)
	TBA: pore water, whole sediment	B,I,I,I (Giesy et al., 1990)
	TBA: elutriate, pore water, whole sediment	A,B,B,F,I(8x) L, Pl (Ross et al., 1992)
	TBA: pore water, whole sediment	B,I,I,I (Hoke et al., 1993)
	TBA: elutriate, OE[d]	B,I,S (Lauten, 1993)
	TBA: elutriate, whole sediment	B,I,I (Moran and Chiles, 1993)
	TBA: elutriate, whole sediment	A,A,B,F,I,I (Naudin et al., 1995)
	TBA: pore water	B,B,I,I (Heida and van der Oost, 1996)
	TBA: overlying water, pore water	F,I,I (Watzin et al., 1997)
	TBA: pore water, whole sediment	A,B,I,I (Carter et al., 1998)
	TBA: pore water, whole sediment	A,B,B,B,I,I,I (Côté et al., 1998a)
	TBA: overlying water, whole sediment	B,I,I,I,S,S,S (Rossi and Beltrami, 1998)
	TBA: elutriate, OE[d]	B,I (Hong et al., 2000)
	TBA: pore water	A,B,I,I,I,I,P (Persoone and Vangheluwe, 2000)
	TBA: elutriate, OE[d]	A,B,B,I (Ziehl and Schmitt, 2000)

Table 9 (continued). Studies with field-collected sediments: assessment of areas of concern.

Assessment objective, type of bioanalytical application[a] and tested sediment phase(s)		***Biotic levels employed[b,c] (and reference)***
Areas impacted by wastewaters: with sediments potentially contaminated by (in)organic pollution		
Metal and organic contamination	TBA: whole sediment	B,I,I (Ingersoll et al., 2002)
	TBA: pore water	B,I,I,I,I (Lahr et al., 2003)
	TBA: pore water, whole sediment	B,I,I (Munawar et al., 2003)
Organic contamination	TBA: OE[d]	A,B,I (Santiago et al, 1993)
	TBA: pore water	B,I (Pastorok et al., 1994)
	TBA: elutriate, pore water	B,I (Hyötyläinen and Oikari, 1999)
Areas impacted by oil spill events		
Diesel fuel spill	TT: whole sediment	I,I (Keller et al., 1998)
Oil sands	TT: overlying water	F (Tetreault et al., 2003)
Oil pollution	TT: seepage water, whole sediment	I,I (Wernersson, 2004)
Simulated oil spill experiment	TT: whole sediment	B (Ramirez et al., 1996)
	TT: OE[d]	B (Johnson et al., 2004)
	TBA: whole sediment	B,B,B,I (Mueller et al., 2003)
	TBA: whole sediment	A,B,B,I,I (Blaise et al., 2004)
Areas impacted by flooding events		
Metal and organic contamination	TT: whole sediment	I (Kemble et al., 1998)
	TBA: overlying water, whole sediment	F,I,I (Hatch and Burton, 1999)

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), F (fish), 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) Organic (solvent) extract.

Table 10. Studies with field-collected sediments: assessment of areas of concern where combined toxicity and contaminant analysis studies were undertaken.

Assessment objective, type of bioanalytical application[a], tested sediment phase(s) and type of chemical analysis		***Biotic levels employed[b,c] (and reference)***
Lake sediments	TT: pore water Organic analysis	B (Guzzella et al., 1996)
	TT: elutriate, OE[d] Organic analysis	Fc (Gagné et al., 1999b)
	TT: whole sediment Organic analysis	I,I (Marvin et al., 2002)
River sediments	TT: whole sediment Heavy metal and organic analysis	I, Bc (Canfield et al., 1998)
	TT: overlying water, whole sediment Heavy metal and organic analysis	I,I,I,I (Bonnet, 2000)
	TT: pore water Heavy metal and organic analysis	I (Cataldo et al., 2001)
	TT: overlying water Heavy metal analysis	F (Bervoets and Blust, 2003)
	TT: whole sediment Organic analysis	I,I (Cieniawski and Collier, 2003)
	TBA: elutriate Organic analysis	A,B,F,I (Bradfield et al., 1993)
	TBA: elutriate Organic analysis	B,I (McCarthy et al., 1997)
	TBA: OE[d], pore water, whole sediment NH_3, heavy metal and organic analysis	A,B,B,B,B,Fc,I,I,I,I,I,I (Côté et al., 1998a,b)
	TBA: whole sediment Heavy metals	B,I,I,I (Richardson et al., 1998)

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), Bc (various benthic communities), F (fish), Fc (fish cells), and I (invertebrates).
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) Organic (solvent) extract.

Of the 75 studies reported in Tables 9 and 10, less than half (n = 34) 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 = 41). This contrasts somewhat with bioassay applications for liquid media assessment, where TBAs comprised nearly 54% (101/188) of reported studies (Tables 1-3). Again, test and biotic level selection may be based on a variety of reasons and study objectives (*e.g.*, practicality, cost, personnel availability) and have influenced a preference for conducting TT assessments. Another factor may lie in that there were (and still are) less toxicity tests whose use is validated for undertaking sediment appraisals. With the exception of those conducted with several benthic invertebrates, most other tests conducted with other groups (*e.g.*, algae, bacteria, fish) were first developed and intended for liquid media assessment (*e.g.*, chemicals and polluted waters). Unlike invertebrate tests, their use to evaluate different liquid compartments associated with whole sediment (*i.e.*, interstitial waters, elutriates, organic extracts of whole sediment) was generally less frequent until the early 1990's when more small-scale assays were developed and validated for sediment toxicity assessment (Wells et al., 1998). Yet another factor is linked to the fact that sediments, unlike liquid samples, comprise several phases that can be assayed (pore waters, elutriates, whole sediment and organic extracts thereof). Ideally, all of these phases should be assessed with a relevant battery of tests for a comprehensive understanding of the sediment's full toxicity potential. In reality, however, scientists will make choices based on laboratory capability for testing and study objectives. When TBAs are used, they are mostly conducted with two or three trophic levels (Tab. 11), similarly to those TBAs performed to study liquid media (Tab. 4).

Table 11. Frequency of the number of biotic levels employed in test battery approaches (TBA) for sediment assessment based on the 34 TBA papers classified in Tables 9 and 10.

TBA studies undertaken with:	***Number and frequency (%)***
Two biotic levels	18/34 (52.9)
Three biotic levels	11/34 (32.4)
Four biotic levels	4/34 (11.8)
Five biotic levels	0/34 (0)
Six biotic levels	1/34 (2.9)

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 for sediment assessment (Tab. 12). With an overwhelming majority, invertebrates have unquestionably been the most commonly employed, even more so than for liquid media assessment (Tab. 5). The conduct of solid phase tests on whole sediment with invertebrate species explains their preferential selection as test organisms. Bacterial tests rank second in utilization, likely owing to the frequent use of sediment direct contact bioluminescence inhibition assays whose development began in the early 1990s (Brouwer et al., 1990). Algae and fish have also been used by some workers, in part to study the potential impact of contaminants on water column organisms owing to sediment resuspension.

Several phases associated with sediments are evaluated for their toxic potential as Tables 10 and 11 indicate. Whole sediment and pore water stand out as phases that are most frequently investigated (Tab. 13). Because sediments act as contaminant sinks where both readily-soluble and adsorbed toxicants can be present, it is not surprising that whole sediments should be the compartment to receive marked attention, as the (endo)benthic community lives in intimate contact with this matrix and therefore vulnerable to adverse effects. Man-made activities that cause sediments to move (*e.g.*, dredging) can spread contaminants back into the water column and pose a threat to pelagic organisms. Hence, testing sediment phases including elutriates, interstitial waters and overlying waters are fully justified and these have been amply tested as well. Organic extracts of whole sediment, purported by some to lack environmental relevance because they can extract persistent (lipophilic) compounds that would normally stay sequestered *ad infinitum* in sediments, can nevertheless indicate possible long-term effects for benthic organisms.

Table 12. Frequency of use of specific biotic levels employed in toxicity testing (TT) and test battery approaches (TBA) for sediment assessment based on the 75 papers classified in Tables 9 and 10.

TT and TBA studies undertaken with:	***Number and frequency (%)***
Algae	16/222* (7.2)
Bacteria	53/222 (23.9)
Fish	9/222 (4.1)
Invertebrates	136/222 (61.3)
Lemnaceae (duckweed)	1/222 (< 1)
Plant (*H. verticulata*)	1/222 (< 1)
Protozoans	1/222 (< 1)
Seeds	5/222 (2.3)

*Total number of single species tests reported in the 75 papers classified in Tables 9 and 10 (= sum of number of A,B,F,I,L,P,Pl,S tests indicated in the "Biotic levels employed" column).

Table 13. Testing frequency of specific sediment phases for sediment toxicity assessment based on the 75 papers classified in Tables 9 and 10.

Sediment phase	***Number and frequency (%)***
Elutriate	16/109* (14.7)
Overlying water/seepage water	17/109 (15.6)
Pore water	28/109 (25.7)
Organic extract	7/109 (6.4)
Whole sediment	41/109 (37.6)

*Total number of times different sediment phases have been assayed in the 75 papers classified in Tables 9 and 10 (= sum of number of sediment phases indicated in the "Assessment objective…" column).

2.2 CRITICAL BODY RESIDUE STUDIES AND LINKS TO (SUB)LETHAL TOXICITY RESPONSES

During exposure to contaminated sediments, test organisms can concentrate chemicals in their tissue and exhibit measurable (sub)lethal effects linked to accumulated substances. In the field of sediment toxicity assessment, it is noteworthy to mention that some studies have been conducted to characterize both exposure and biological effects in parallel. Exposure to contaminants can be gauged by measuring their concentrations in water/sediment and tissue, and effects can be estimated with endpoints such as survival and growth. These studies are important, for example, to detect threshold concentrations at which chemicals begin to exert adverse effects. As such, they can be useful to recommend effective chemical quality standards that will be protective of aquatic life.

CBR (critical body residue) studies include research on metals, organics and contaminants in mixtures. For instance, cadmium toxicity was appraised with the midge, *Chironomus tentans*, exposed to spiked-sediments that were stored for different periods of time (Sae-ma et al., 1998). Decreases in toxicity effects (lethality) and Cd accumulation in midge tissue with storage time suggested that decreased bioavailability of this metal had occurred. This work clearly illustrated the influence of sediment storage time on organism toxicity response and the impact it could have on test results. Effects of fluoranthene, a PAH (polycyclic aromatic hydrocarbon) congener, were appraised in benthic copepods exposed to dosed sediments for ten days (Lotufo, 1998). Relationships were found between organism health (survival, reproductive and grazing capacity) and fluoranthene concentration in both sediment and tissue. This study was therefore able to more closely pinpoint the NOEL (no observed effect level) concentration of this chemical for this group of biota. Another initiative in CBR studies sought to find out whether the AVS (acid-volatile sulphide) content of sediments collected in areas impacted by mining activities might influence the bioaccumulation of metals (Zn, Cu) and toxicity to the midge *C. tentans* (Besser et al., 1996). Results indicated differences in metal uptake in organisms based on AVS content and showed that growth inhibition was more markedly linked to Zn than Cu. Recommendations called for considering AVS concentrations in metal-contaminated sediments, because of the importance it can have on uptake by biota and subsequent toxicity responses. These investigations indeed confirm the usefulness of CBR-like approaches for evaluating hazard and risk to sediment-dwelling organisms from metals and organic pollutants.

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

3.1 ENDEAVOURS PROMOTING THE DEVELOPMENT, VALIDATION AND REFINEMENT OF TOXICITY TESTING PROCEDURES

There are literally hundreds of publications that, directly or indirectly, have contributed to the development, validation and refinement of bioassay techniques both for liquid and solid media assessment. These papers incorporate initiatives that have dealt with 1) test method development, 2) inter-calibration exercises, 3) comparative studies and 4) factors capable of affecting bioassay responses. Anyone familiar with the world of toxicity testing would likely not disagree with the statement that "the perfect bioassay is not of this world" and that developers of these instruments of ecotoxicology simply do their utmost to make each test "as least imperfect as possible". To reach this latter stage, assurance of reproducibility, demonstration of scope of use and understanding confounding factors capable of influencing toxicity responses are some of the issues that must be addressed. Hereunder, examples of such studies are given to reveal some of the ways in which they have contributed to the science of small-scale toxicity testing by enhancing its diagnostic tools.

3.1.1 Test method development

To guarantee that reliable procedures are consistently employed to generate toxicity data, it is first essential that sufficient effort be directed toward the development of reproducible toxicity test methods whose results will remain unchallenged. Those that are featured in this book are representative of dependable micro-assays presently in use internationally. Many other small-scale toxicity test methods have been developed at various levels of biological organization. These include bioassays conducted with **algae** (Daniels et al, 1989*; Radetski et al., 1995; St-Laurent and Blaise, 1995; Chen et al., 1997; Blaise and Ménard, 1998*; Persoone, 1998; Tessier et al., 1999; Geis et al., 2000), **bacteria** (Bitton et al., 1994; Blaise et al., 1994; Bulich and Bailey, 1995; Kwan, 1995*; Bulich et al., 1996; Botsford, 1998; Lappalainen et al., 1999*; Ulitzur et al., 2002; Gabrielson et al., 2003), **fish cells** (Ahne, 1985; Pesonen and Andersson, 1997; Sandbacka et al., 1999), **invertebrates** (Snell and Persoone, 1989; Oris et al., 1991; Kubitz et al., 1996*; Benoit et al., 1997*; Johnson and Delaney, 1998; Chial and Persoone, 2002*; Gerhardt et al., 2002b*; Tran et al., 2003), ***Lemnaceae*** (Bengtsson et al., 1999; Cleuvers and Ratte, 2002a), **protozoans** (Dive et al., 1991; Larsen et al., 1997; Berk and Roberts, 1998; Twagilimana et al., 1998; Gilron et al., 1999) and **yeast** (Ribeiro et al., 2000).

*(tests applying to sediment toxicity testing)

For freshwater solid media investigations, efforts have also been directed towards the development of formulated sediments (also called "artificial" or "synthetic" sediments) to assess their adequacy for conducting contaminant-spiked sediment toxicity studies (Suedel and Rodgers, 1994; Kemble et al., 1999). Among other uses, formulated sediments can be useful to recommend realistic sediment quality criteria for (in)organic substances. Different types of formulated sediments have been employed to evaluate both metal- spiked (Gonzalez, 1996; Harrahy and Clements, 1997; Chapman et al., 1999; Péry et al., 2003) and organic-spiked (Fleming et al., 1998; Besser et al., 2003; Lamy-Enrici et al., 2003) contaminants.

3.1.2 Inter-calibration exercises

Beyond test development and validation, inter-calibration exercises (also known as "round robin" or "inter-laboratory exercises") are mandatory steps that must be undertaken if a toxicity test method is intended for standardization. These exercises further contribute to test validation by insuring reproducibility of results among different laboratories. In most cases, they also contribute to test method improvement and refinement (*e.g*., Thellen et al., 1989; Dive et al., 1991; Persoone et al., 1993).

For example, inter-calibration exercises have been undertaken with **algae** (Thellen et al., 1989), **bacteria** (Ribo, 1997; Ross et al., 1999*), **fish cells** (Gagné et al., 1999a), **invertebrates** (Cowgill, 1986; Persoone et al., 1993; Burton et al., 1996*; Hayes et al., 1996), **protozoans** (Dive et al., 1990), and **test organisms of several biotic levels** (Rue et al., 1988; Ronco et al., 2002).

*(tests applying to sediment toxicity testing)

If toxicity tests fulfill the scientific criteria set out by inter-calibration exercises, they can then be considered for the standardization process. If this process is followed, an official toxicity test method document is eventually produced that ensures proper conduct of biological tests (see Section 3.2.1).

3.1.3 Comparative studies

Comparative studies involving toxicity tests abound in the scientific literature. There are many reasons compelling ecotoxicologists to conduct work of this nature, some of which are directed 1) to assess the performance, sensitivity and relevance of individual bioassays undertaken on various chemicals and (liquid and solid) media to specify their scope of use, 2) to optimize the diagnostic potential of bioassay batteries to broaden hazard detection (insure that tests in a battery are complementary and not redundant) and 3) to promote the application of novel assays capable of high throughput for cost-effective screening of (complex) environmental samples.

As an overview, **studies carried out with liquid media** have been launched to compare **bioassay responses** (Finger et al., 1985; Blaise et al., 1987; Kaiser and McKinnon, 1993; Ross, 1993; Isomaa et al., 1995; Dodard et al., 1999; Lucivjanskà et al., 2000; Brix et al., 2001a; Nalecz-Jawecki and Sawicki, 2002; Mummert et al., 2003; Sherrard et al., 2003; Tsui and Chu, 2003), **different endpoints** (Dunbar et al., 1983; Fernández-Casalderrey et al., 1993; Pauli and Berger, 1997; Froehner et al., 2000; Snell, 2000; Weyers and Vollmer, 2000; Jos et al., 2003), **responses of laboratory test organism species and endemic species and/or laboratory bioassay responses and field results** (Koivisto and Ketola, 1995; Traunspurger et al., 1996; van Wijngaarden et al., 1996; Jak et al., 1998; Crane et al., 1999; Tchounwou and Reed, 1999; Dyatlov, 2000; Milam et al., 2000; Pascoe et al., 2000; Bérard et al., 2003), **and bioassay and biomarker endpoints** (Gagné and Blaise, 1993; Nyström and Blanck, 1998; Connon et al., 2000; Perkins and Schlenk, 2000; De Coen and Janssen, 1997; Bierkens et al., 1998; Sturm and Hansen, 1999; den Besten and Tuk, 2000; Guilhermino et al., 2000; Maycock et al., 2003; Taylor et al., 2003).

In **studies conducted with sediments**, comparisons have been reported for **artificial (formulated) and natural sediments** (Barrett, 1995; Fleming et al., 1998), **bioassay and biomarker endpoints** (Gillis et al., 2002), **bioassay responses** (Ahlf et al., 1989; Becker et al., 1995; Day et al., 1995a; Kwan and Dutka, 1995; Suedel et al., 1996; Barber et al., 1997; Day et al., 1998; Fuchsman et al., 1998; Guzzella, 1998; Huuskonen et al., 1998; Côté et al., 1998a,b; Vanderbroele et al., 2000; Watts and Pascoe, 2000; Chial et al., 2003; Milani et al., 2003; Mueller et al., 2003; Petänen et al., 2003), **different endpoints** (Suedel et al., 1996; Watts and Pascoe, 1996; Sibley et al., 1997a; Pasteris et al., 2003; Landrum et al., 2004; Vecchi et al., 1999), **different sediment phases** (Harkey et al., 1994), **responses of laboratory test organism species and endemic species** (Conrad et al., 1999) **and/or laboratory bioassay responses and field results** (Reinhold-Dudok et al., 1999; Bombardier and Blaise, 2000; Peeters et al., 2001; den Besten et al., 2003) and **sediment collection techniques** (West et al., 1994).

3.1.4 Factors capable of affecting bioassay responses

Toxicity testing developers and users have also devoted significant energy to the understanding of specific factors capable of confounding (micro-) organism responses and/or interfering with data interpretation (*e.g.*, pH, temperature, light, growth medium, natural contaminants such as NH_3, H_2S, or grain size in case of solid phase tests).

In fact, any aspect of testing likely to impact toxicity results (*e.g.*, stimulatory effects in the case of algal toxicity assays, or sample colour interferences in the case of a toxicity endpoint measured by photometry) have been a focus of concern, as have been ways of minimizing, eliminating or circumventing particular problems or limitations that may be test-specific. In brief, seeking thorough understanding of a test's capabilities and limitations has been considered paramount for proper toxicity assessment (and final data interpretation) and marked efforts have been directed toward this goal.

With this purpose in mind, investigations have explored the influence of such factors as **acid volatile sulfides** (Sibley et al., 1996*; Long et al., 1998*), **alkalinity** (Lasier et al., 1997*), **ammonia** (Besser et al., 1998*; Newton et al., 2003*), **colored samples** (Cleuvers and Weyers, 2003), **equilibration time** (Lee et al., 2004*), **experimental design** (Naylor and Howcroft, 1997*; Bartlett et al., 2004*), **fluid dynamics** (Preston et al., 2001), **food** (Sarma et al., 2001; Gorbi et al., 2002; de Haas et al., 2002*; Antunes et al., 2004; de Haas et al., 2004*); **grain size** (Guerrero et al., 2003*), **genetic variability** (Baird et al., 1991; Barber et al., 1990; Barata et al., 1998), **gut contents** (Sibley et al., 1997c*), **heavy metal speciation** (Gunn et al., 1989*; Ankley et al., 1996*), **humic/fulvic acids** (Ortego and Benson, 1992; Alberts et al., 2001; Guéguen et al., 2003; Koukal et al., 2003; Ma et al., 2003), **intermittent or short exposures to contaminants** (Hickey et al., 1991; Brent and Herricks, 1998; Naddy and Klaine, 2001, Broomhall, 2002), **life-cycle stage/age** (Williams et al., 1986; Stephenson et al., 1991; Watts and Pascoe, 1998*; Hamm et al., 2001), **light regime** (Cleuvers and Ratte, 2002b), **organic matter content** (Ankley et al., 1994*; Lacey et al., 1999*; Besser et al., 2003*; Guerrero et al., 2003*; Lamy-Enrici et al., 2003*; Mäenpää et al., 2003*; VanGenderen et al., 2003), **pH** (Fisher and Wadleigh, 1986; Fu et al., 1991; Svenson and Zhang, 1995; Rousch et al., 1997; Franklin et al., 2000; Peck et al., 2002*; Long et al., 2004), **phosphorus** (Van Donk et al., 1992; Mkandawire et al., 2004), **potassium** (Bervoets et al., 2003*), **pre-exposure to contaminants** (Bearden et al., 1997; Muyssen and Janssen, 2001, 2002; Ristola et al., 2001*; Vidal and Horne, 2003*), **sand** (Thomulka et al., 1997), **sediment**

indigenous animals (Reynoldson et al., 1994*), **sediment processing** (Day et al., 1995b*), **sex** (Sildanchandra and Crane, 2000), **solvents** (Calleja and Persoone, 1993; Fliedner, 1997), **choice of statistical tests** (Isnard et al., 2001), **sulfates** (Brix et al., 2001c), **sulfur** (Jacobs et al., 1992*; Pardos et al., 1999b*), **suspended solids** (Herbrandson et al., 2003a,b), **temperature** (Fisher, 1986; Broomhall, 2002; Buchwalter et al., 2003; Heugens et al., 2003), **test exposure time** (Suedel et al., 1997; Naimo et al., 2000*; Froehner et al., 2002; Feng et al., 2003), **test medium** (Vasseur and Pandard, 1988; Guilhermino et al., 1997; Samel et al., 1999), **test organism inoculum density** (Moreno-Garrido et al., 2000; Franklin et al., 2002), **UV irradiation** (Bonnemoy et al., 2004), **water chemistry/quality** (Persoone et al., 1989; Jop et al., 1991; van Dam et al., 1998; Karen et al., 1999; Clément, 2000; Bury et al., 2002; Graff et al., 2003), **water hardness** (Fu et al., 1991; Baer et al., 1999; Verge et al., 2001; Charles et al., 2002; Gensemer et al., 2002; Naddy et al., 2003; Long et al., 2004), **water-sediment partitioning** (Stewart and Thompson, 1995*).

*(tests applying to sediment toxicity testing)

3.2 INITIATIVES PROMOTING THE USE OF TOXICITY TESTING PROCEDURES

For over three decades, the use of bioassays for toxicity testing has steadily increased and become an indispensable component of aquatic environmental assessment. In this section, specific types of publications are presented as important contributions that have 1) promoted the use of ecotoxicology testing in the biomonitoring, regulatory and compliance arena, 2) disseminated information and understanding relating to toxicity testing issues, 3) favoured technology transfer of test methods internationally and 4) provided overall sound scientific support to facilitate decision-making aimed at environmental protection and conservation.

3.2.1 Review, bio-monitoring and HAS articles

Review articles are particularly useful to synthesize research work that has been undertaken in different spheres relating to toxicity testing. By exposing the state of the art for a selective field, these articles will often circumscribe the limitations, advantages and scope of use of bioassays which then leads to their proper and effective application. Some examples of review articles include papers on **concept/management/policy** (MacGregor and Wells, 1984; U.S. EPA and Environment Canada, 1984; Sergy, 1987; Cairns and Pratt, 1989; Maltby and Callow, 1989; Blaise, 2003), as well as several others on specific trophic groups including **algae** (Blaise, 1993; Lewis, 1995; Sosak-Swiderska and Tyrawska, 1996; Blaise et al., 1998b; Blaise, 2002), **bacteria** (Bennett and Cubbage, 1992b*; Bitton and Koopman, 1992; Kross and Cherryholmes, 1993; Painter, 1993; Bitton and Morel, 1998; Ross, 1998; Doherty, 2001*), **fish cells** (Babich and Borefreund, 1991;Fentem and Balls, 1993; Denizeau, 1998; Fent, 2001; Castaño et al., 2003), **invertebrates** (Burton et al., 1992; Ingersoll et al., 1995*; Snell and Janssen, 1995, 1998; Chapman, 1998*; CANMET, 1999) and **protozoa** (Gilron and Lynn, 1998; Sauvant et al., 1999; Nicolau et al., 2001; Nalecz-Jawecki, 2004).

Other reviews have also encompassed **different levels of toxicity tests** (Giesy and Hoke, 1989*; Bennett and Cubbage, 1992a; CANMET, 1997a; Blaise et al., 1998a; de Vlaming et al., 1999; Blaise et al., 2000; Girling et al., 2000; Janssen et al., 2000; Repetto et al., 2000).

*applying to sediment toxicity assessment

Various papers expounding the value of **biomonitoring, routine and/or regulatory testing** have also advanced the practice of bioassays. Some of these include articles on **drinking water assessment** (Forget et al., 2000), **single chemical or mixture assessment** (Altenburger et al., 1996; Aoyama et al., 2000), **surface water assessment** (Canna-Michaelidou et al., 2000; Marsalek and Rojickova-Padrtova, 2000; Ruck et al., 2000), **wastewater assessment** (OECD, 1987; Blaise et al., 1988; Mackay et al., 1989; Hansen, 1993; Johnson et al., 1993; Stulhfauth, 1995; Kovacs et al., 2002), **sewage treatment plant performance assessment** (Fearnside and Hiley, 1993), and **sediment quality assessment** (Nipper, 1998).

Articles proposing new **hazard assessment schemes** (HAS) for liquid or sediment assessment have equally paved the way for the employment of test batteries in ecotoxicity appraisals. Some describe systems for evaluating **water/wastewater** (Blaise et al., 1985; Heinis et al., 2000; Ronco et al., 2000 ; Persoone et al., 2003), **chemicals** (Fochtman et al., 2000; Garay et al., 2000; Girling et al., 2000; Pica-Granados et al., 2000; Brix et al., 2001a,b,c) and **sediments** (Ingersoll et al., 1997; Côté et al., 1998b). These effects-based indices, varied in their concepts and objectives, demonstrate novel ways of utilizing groups of bioassays to deal with "real-life" environmental situations. As such, they highlight schemes that are complementary to the robust and validated HAS approaches described in Volume 2 of this book.

3.2.2 Standardized test methods and guidance documents

Finally, marked efforts have been undertaken nationally and internationally to publish **standardized toxicity test methods** and several standards organizations (*e.g.*, ASTM, ISO, OECD) have been very active in the production of documents too numerous to reproduce in this chapter. Publishing official test methods is not a simple task and can require a substantial amount of time and energy from dedicated scientists. Again, standardized toxicological method documents are crucial to environmental assessment as they ensure proper use of testing, (inter)national consistency and acceptance, as well as reliability of test results owing to the quality control and assurance components that are integrated in such protocols.

Test method standardization (TMS) calls for several actions that involve 1) preparation of a formal draft test method document for each bioassay intended for standardization, 2) a critical review by an expert subcommittee, 3) the preparation of a final draft test method, 4) an international peer review of each test method, 5) an inter-calibration exercise of the final draft test method, 6) finalization of each test method and 7) the formal publication of the toxicity test method document. Environment Canada (EC) has been particularly active in biological test method standardization and has thus far contributed 18 standardized aquatic and sediment toxicity methods, eight and three of which apply to acute/chronic freshwater liquid (tests with algae, bacteria, fish, invertebrates, and *Lemnaceae*) and solid (tests with bacteria and invertebrates) media assessment, respectively (IGETG, 2004). As a complement to TMS, EC has also produced several **guidance documents** that provide assistance on matters related to choice of reference toxicants (Environment Canada, 1990), sampling and spiking techniques for sediments (Environment Canada, 1994, 1995), interpretation of results (Environment Canada, 1999) and statistical considerations for toxicity tests (Environment Canada, 2004b).

Other **standardized/validated test methods** reported in the literature include acute/chronic tests performed with **algae** (*e.g*, OECD, 2002a; ISO, 2003), **fish cells** (Gagné and Blaise, 2001), **invertebrates** (Borgmann and Munawar, 1989*; Trottier et al., 1997; Pereira et al., 2000*; OECD, 2001*a,b), ***Lemnaceae*** (OECD, 2002b), and with **toxicity tests conducted at different trophic levels** (Nebeker et al., 1984*; U.S. EPA, 2002a,b).

*applying to sediment toxicity assessment

Additionally, **miscellaneous guidance/technical documents** have reported on various aspects linked to ecotoxicity that give advice on:

- choice of bioassays for general contaminant assessment (Calow, 1989);
- criteria to select tests for effluent testing (Grothe et al., 1996; Johnson, 2000);
- choice of species and endpoints for appraising pharmaceuticals (Länge and Deitrich, 2002);
- proper application of algal, bacterial and invertebrate tests (Santiago et al., 2002);
- approaches, design and interpretation of sediment tests (Ross and Leitman, 1995; Ingersoll et al., 2000; Wenning and Ingersoll, 2002; MacDonald and Ingersoll, 2002a,b).

4. Conclusion(s)

Small-scale freshwater toxicity testing is but a modest fraction of a diverse array of scientific activities connected to the field of ecotoxicology. Yet, within this still emerging discipline, few will argue the fact that tools and approaches developed to measure the undesirable effects that countless chemicals (alone or in mixtures) and complex (liquid and solid) media can exert on biota have markedly contributed to aquatic ecosystem preservation. Indeed, the breadth and scope of application of bioassays thus far directed toward obtaining relevant information aimed at problem-solving and prevention of contaminant-based issues has progressed well.

While many developed countries have been effective over past decades in eliminating acute toxicity from point source discharges owing to technological improvement of industrial processes and legislation, chronic effects on aquatic biota are still very much an issue. Furthermore, as the 21rst century unfolds, many emerging and developing countries active in joining the world economy are presently creating new contaminant burdens on aquatic systems that will contribute additional acute and chronic toxicity pressures until, once again, technology and legislation repress pollution. Hence, the techniques and hazard assessment schemes featured in this book can prove to be very relevant for use in all parts of the world. As editors of this book, it is our hope that readers will grasp that an effects-based approach is primordial to deal with hazard and risk assessment of pollutants and that use of toxicity tests is an essential cog in this respect. It is also our hope that many, directly or indirectly involved in ensuring the well-being of aquatic systems, will actually use (or suggest the use of) some of the toxicity testing methods and hazard assessment schemes described in subsequent sections.

Lastly, while acute and chronic (sub)lethal toxicity effects are basic concerns that must be first dealt with and eradicated, new demands will be made on ecotoxicology to address emerging issues. Indeed, several more subtle (and potentially deleterious) effects owing to long-term exposures to low concentrations of contaminants will merit investigation (Eggen et al., 2004). Genotoxicity, teratogenicity, immunotoxicity and endocrine disruption are some of the undesirable consequences of classical (*e.g.*, metals, pesticides, organochlorides) and more recent (*e.g.*, household products and pharmaceuticals) chemical discharges into receiving waters that require urgent comprehensive assessment. Here as well, reliable and relevant standardized tools and approaches will have to be developed and applied.

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Abbreviations

ASTM	American Society for Testing and Materials
AVS	Acid-Volatile Sulphide
CANMET	Canada Center for Mineral and Energy Technology

CISTI	Canada Institute for Scientific and Technical Information
CBR	Critical Body Residue
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
EC	Environment Canada
HAS	Hazard Assessment Schemes
IGETG	Inter-Governmental Ecotoxicological Testing Group
ISO	International Standard Organisation
Kow	octanol-water coefficient
NOEC	no observed effect concentration
NOEL	no observed effect level
PCBs	polychlorinated biphenyls
PGE	Propylene glycol ether
QSAR	Quantitative Structure-Activity Relationships
OECD	Organization for Economic Cooperation and Development
PAH	Polycyclic Aromatic Hydrocarbon
[S,S]-EDDS	trisodium[S,S]-ethylene diamine disuccinate
TBA	Test Battery Approach
TMS	Test Method Standardization
TT	Toxicity Testing
U.S. EPA	U.S. Environmental Protection Agency.