Regulatory ecotoxicology testing in Canada – activities and influence of the Inter-Governmental Ecotoxicological Testing Group

Lisa N. Taylor, Kenneth G. Doe, Richard P. Scroggins and Peter G. Wells

ABSTRACT

The Inter-Governmental Ecotoxicological Testing Group (IGETG) is an ad hoc group of government scientists, technologists, data users, and scientific advisors that has been active in the development and application of ecotoxicological testing in Canada. Membership includes representatives from government laboratories that conduct toxicity testing for research and development purposes, monitor effluent discharge for compliance with regulations, and/or perform exploratory monitoring of non-regulated sectors. The original focus of the group was to support the development and application of standardized toxicity test methods under the Fisheries Act but as the group matured it broadened its focus to five goals: (1) to promote the use of ecotoxicity testing; (2) to disseminate and harmonize new knowledge and understanding of issues related to ecotoxicity testing; (3) to provide scientific support to environmental programs; (4) to develop, validate and publish toxicological test methods; and (5) to establish and implement quality assurance practices in toxicology laboratories. Since 1990, IGETG has assisted Environment Canada in standardizing 22 toxicity test methods and in developing eight guidance documents. In this context, we briefly outline the history and future of applied ecotoxicological testing in Canada illustrated by specific examples wherein standard toxicity tests are useful. This paper commemorates IGETG’s 35th anniversary.

Key words | aquatic toxicology, ecotoxicology, environmental conservation and protection, history of science, regulatory testing, toxicity test methods

INTRODUCTION

Ecotoxicity is concerned with the adverse effects of chemical substances and physical agents, alone and in combination, on aquatic, sediment dwelling and terrestrial organisms and their ecosystems. For almost 40 years, starting under the Canadian Fisheries Act in 1972, ecotoxicity testing has been an integral component of environmental conservation and protection activities in Canada, with a wide range of applications in enforcement and compliance, environmental assessments and standards setting. To date, 22 standard biological test methods and reference methods, and eight supporting guidance documents have been researched, written, and published by Environment Canada (EC) (Tables 1 and 2). They reflect the contributions and needs of researchers, data generating laboratories, regulators and end users of the data, across Canada. The EC methods have been accepted by the provincial and federal governments, and have gained international recognition (e.g., Thompson et al. 2005); and their data have been utilized by the United Nations, International Maritime Organization EHS Working Group (Wells et al. 1999).

An important feature in the acceptance of the EC methods is that there is a high degree of harmonization with biological tests prepared by the American Society for Testing and Materials, the American Public Health Association, the International Organization for Standardization, and the US Environmental Protection Agency.
<table>
<thead>
<tr>
<th>Test media</th>
<th>Organism type</th>
<th>Title of biological test method or guidance document</th>
<th>Endpoint measured</th>
<th>Test duration*</th>
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</tr>
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<tbody>
<tr>
<td>Aqueous</td>
<td>Fish</td>
<td>Acute lethality test using Rainbow Trout</td>
<td>LC50</td>
<td>96 hours</td>
<td>EPS 1/RM/9, July 1990, amended May 1996 and May 2000</td>
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<td></td>
<td></td>
<td>Acute Lethality test using Threespine Stickleback (Gasterosteus aculeatus)</td>
<td>LC50</td>
<td>96 hours</td>
<td>EPS 1/RM/10, July 1990</td>
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<td></td>
<td></td>
<td>Test of reproduction and survival using the Cladoceran Ceriodaphnia dubia</td>
<td>LC50. ICp</td>
<td>Approximately 7 days (3 broods in control animals)</td>
<td>EPS 1/RM/21, 2nd Edition, February 2007</td>
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<td></td>
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<td>Fertilization assay using Echinoids (Sea Urchins and Sand Dollars)</td>
<td>ICp and % fertilization in undiluted sample</td>
<td>10 minutes (sperm) + 10 minutes (egg plus sperm)</td>
<td>EPS 1/RM/27, 2nd Edition, February 2011</td>
</tr>
<tr>
<td>Invertebrate</td>
<td>Acute lethality test using Daphnia spp.</td>
<td>LC50</td>
<td>48 hours</td>
<td>EPS 1/RM/11, July 1990</td>
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<td></td>
<td></td>
<td>Test for measuring the inhibition of growth using the freshwater Macrophyte, Lemna minor</td>
<td>ICp</td>
<td>7 days</td>
<td>EPS 1/RM/37, 2nd Edition, January 2007</td>
</tr>
<tr>
<td>Sediment</td>
<td>Invertebrate</td>
<td>Acute test for Sediment Toxicity using marine or Estuarine Amphipods</td>
<td>% mortality or LC50</td>
<td>10 days</td>
<td>EPS 1/RM/26, December 1992, amended October, 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test for survival and growth in sediment using the larvae of freshwater midges (Chironomus tentans or Chironomus riparius)</td>
<td>% mortality, reduced growth or LC50, ICp</td>
<td>10 days</td>
<td>EPS 1/RM/32, December 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test for survival and growth in sediment using the freshwater Amphipod Hyalella azteca</td>
<td>% mortality, reduced growth or LC50, ICp</td>
<td>14 days</td>
<td>EPS 1/RM/33, December 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Test for survival and growth in sediment using Spionid Polychaete worms (Polydora cornuta)</td>
<td>% mortality, reduced growth or IC</td>
<td>14 days</td>
<td>EPS 1/RM/41, December 2001</td>
</tr>
<tr>
<td>Soil</td>
<td>Invertebrate</td>
<td>Tests for toxicity of contaminated soil to earthworms (Eisenia andrei, Eisenia fetida, or Lumbricus terrestris)</td>
<td>% mortality, reduced reproduction, avoidance, or LC50, EC50, ICp</td>
<td>48 or 72 hours; 7 days (optional); 14 days; 28 days; 56 days; 63 days (optional)</td>
<td>EPS 1/RM/43, June 2004, amended June 2007</td>
</tr>
</tbody>
</table>

(continued)
Efforts to make test methods and quality standards consistent across the country have been led and/or facilitated by the actions of the ad hoc group of federal and provincial government ecotoxicologists, now known as the Inter-Governmental Ecotoxicological Testing Group (IGETG). The IGETG serves as a mechanism by which experts at both levels of government work together to promote harmonized approaches to testing industrial and municipal effluents, chemicals, chemical mixtures and environmental samples consistent with internationally recognized quality standards.

The IGETG has five main goals:

1. To promote the use of aquatic and terrestrial ecotoxicology testing in environmental regulations and policies;
2. To disseminate and harmonize new knowledge and understanding of issues related to ecotoxicology testing, including technology transfer;
3. To provide scientific support to environmental conservation and protection programs;
4. To develop, validate, and publish toxicological test methods as part of the standardization process;

### Table 1

<table>
<thead>
<tr>
<th>Test media</th>
<th>Organism type</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td></td>
<td>Test for measuring survival and reproduction of springtails exposed to contaminants in soil</td>
<td>% mortality, reduced reproduction or LC50, ICP</td>
<td>21 or 28 days (varies with test species)</td>
<td>EPS 1/RM/47, September 2007</td>
</tr>
<tr>
<td>Aquatic, sediment and soil</td>
<td>Bacteria</td>
<td>Toxicity test using luminescent bacteria (<em>Photobacterium phosphoreum</em>)</td>
<td>IC50</td>
<td>5 minutes and 15 minutes</td>
<td>EPS 1/RM/24, November 1992</td>
</tr>
</tbody>
</table>

*LC50 is the median lethal concentration, i.e., the concentration of material that is estimated to be lethal to 50% of the test organisms. ICP is the inhibiting concentration for a specified percent effect, e.g., IC25 for weight would be the concentration estimated to result in organisms having a 25% lower weight than that attained by control organisms. EC25 is the concentration of material that is estimated to cause a specified toxic effect to 25% of the test organisms. EC50 is the median effective concentration, i.e., the concentration of material that is estimated to cause a specified toxic effect to 50% of the test organisms.

**E-test is embryo test, EA is embryo/alevin, and EAF is embryo/alevin/fry.**

### Table 2

<table>
<thead>
<tr>
<th>Title of biological test method or guidance document</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Reference method for determining acute lethality of effluents to <em>Daphnia magna</em></td>
<td>Aqueous</td>
<td>LC50 or % mortality</td>
<td>48 hours</td>
<td>EPS 1/RM/14, 2nd Edition, December 2000</td>
</tr>
<tr>
<td>Reference method for determining acute lethality of sediment to marine or Estuarine Amphipods</td>
<td>Sediment</td>
<td>% mortality</td>
<td>10 days</td>
<td>EPS 1/RM/35, December 1998</td>
</tr>
<tr>
<td>Reference method for determining the toxicity of sediment using luminescent bacteria in a solid-phase test</td>
<td>Sediment</td>
<td>IC50</td>
<td>20 minutes</td>
<td>EPS 1/RM/42, April 2002</td>
</tr>
<tr>
<td>Procedure for pH stabilization during the testing of acute lethality of wastewater effluent to Rainbow Trout</td>
<td>Aqueous</td>
<td>LC50 or % mortality</td>
<td>96 hours</td>
<td>EPS 1/RM/50, March 2008 (add-on procedure to EPS 1/RM/13)</td>
</tr>
</tbody>
</table>
5. To assist with the implementation of quality assurance and quality control (QA/QC) practices in Canadian ecotoxicology laboratories.

This paper discusses IGETG’s role in the establishment of ecotoxicity testing methods in Canada, its influence on current test method development and applications, and its pivotal role in bringing standardized ecotoxicological testing to federal, provincial and private Canadian laboratories. As well, IGETG’s vision is presented for the future of ecotoxicological testing in Canada in support of environmental protection and conservation goals. The paper updates an unpublished Environment Canada report on this topic (IGETG 2011).

**HISTORY OF IGETG**

In 1972, the Canadian federal Department of the Environment (now Environment Canada) planned for toxicity testing to be included in new water pollution regulations promulgated under the Fisheries Act. The Act provided the legal basis for the protection of fish and fish habitat from harm caused by physical alterations or pollution. Standardized toxicity testing methods, with defined quality control procedures, were required for scientific credibility and defensibility of the data. An acute lethality test using rainbow trout (then *Salmo gairdneri*, now *Oncorhynchus mykiss*) was developed for the testing of industrial effluents and oil spill dispersants. This test procedure was published as the Department’s first standard method (EC 1980; EC 1984; see also Sprague 1973; Doe & Wells 1978; Pessah & Cornwall 1980).

To provide the scientific and technical support for the development of the method and other anticipated methods (Pessah & Cornwall 1980), a technical sub-committee was established in 1976 within EC’s Environmental Protection Service (EPS). This committee, known as the Inter-Governmental Aquatic Toxicity Group (IGATG), eventually drew on expertise from many other federal and provincial agencies. Over the years, IGATG grew and by the 1990s consisted of representatives from all federal and provincial toxicity laboratories, the National Water Research Institute, various EC headquarters groups, Fisheries and Oceans Canada, and Natural Resources Canada. Representatives from private sector laboratories sometimes were observers at its annual meetings, held at the same time and place as the Canadian annual Aquatic Toxicity Workshops (ATW, more details at: www.atw.ca).

Up to the early 1980s, Canadian environmental assessment and monitoring programs were dominated by effluent and chemical testing. MacGregor & Wells (1984) provided the rationale and impetus for continuing to use toxicity tests for effluent monitoring and chemical substance effects assessment in Canada. However, there was continued debate about the relative costs and benefits of conducting field-oriented, biological effects monitoring, chemical analysis alone on organic and inorganic substances, and toxicity testing (i.e., bioassays) within various programs (e.g., Fisheries Act Regulations, New Substance Assessments). Ecological risk assessment was also being introduced as an approach that evaluates the potential for adverse effects on the organisms that make up ecosystems as a result of human activities. This history and the various methods available at the time are described in American Society for Testing and Materials Special Technical publications (e.g., Cairns & Dickson 1973; Cairns et al. 1978; Buikema & Cairns 1980; Eaton et al. 1980), as well as by Blaise et al. (1988) and Rand et al. (1995), all of which are still a rich source of useful information on methods in ecotoxicology.

The tension between the different approaches to controlling and regulating pollutants, the urgent need for improved surface water quality and effective pollutant control, and the increasingly recognized applications of toxicity tests resulted in the OECD (the Organization for Economic Co-operation and Development), led by the United States Environmental Protection Agency (USEPA) and Environment Canada, to hold an expert workshop on biological testing of effluents in 1984 (USEPA & EC 1984). Participants at the workshop presented papers (e.g., Dafoe et al. 1984, 1987), discussed and reviewed the various types and uses of biological tests for effluent and chemical control, and reached a consensus on their primary application for control of pollution at source, supported by chemical analysis and field effects monitoring. Certain methods (e.g., fish, crustaceans, algae) were given high priority for further development and standardization. The criteria for
determining which types of biological tests were believed to be useful as routine procedures were level of difficulty, cost and amount of inter-laboratory calibration completed. There was recognition that standard organizations such as the American Society for Testing and Materials E-47 Biological Effects and Environmental Fate committee and working groups within the OECD and its other member countries would have to make development and validation of standardized toxicological method a priority of their work programs. Of special note was the formal acceptance of end-of-pipe effluent toxicity testing by the USEPA and its incorporation into their National Pollutant Discharge Elimination System program.

In 1987, EPS, with contributions from IGATG members, published a second report to senior management advocating the use of toxicity tests as practical tools to ‘predict, prevent and control’ the adverse effects of chemicals (Sergy 1987). Specifically, the report recommended that a core aquatic toxicity testing capability be established in Environment Canada’s laboratories across the country; that a national program be developed for the standardization of the toxicity methodologies for testing contaminants and chemicals in marine and freshwater samples (water and sediment); and that a quality assurance program be implemented in Environment Canada’s toxicology laboratories. IGATG became active in the implementation of these recommendations by developing test methods, assisting with guidance document development and training materials, and by providing expert advice for incorporating toxicity tests into policy and regulations. The group also participated in inter-laboratory calibration and QA/QC of the test methods and endpoints. In the late 1980s, the assessment needs of dredging and disposal at sea broadened the group’s scope to include sediment toxicity testing and, by the late 1990s, terrestrial testing was also added. In recognition of the importance of sediment and terrestrial ecotoxicity tests, the group changed its name to the Inter-Governmental Ecotoxicological Testing Group in 1999.

Initially, IGETG members prepared the standardized acute lethality test methods with funding solely from EPS programs. However, through funding from the federal ‘Green Plan’ initiative of the early 1990s, Environment Canada funded the biological methods program, which led to the establishment of the Method Development and Applications Division (now, the Method Development and Applications Unit, MDAU) within EPS in 1991. The MDAU’s mandate is to develop and validate toxicological test methods as part of the standardization process and to develop and maintain a quality assurance program to ensure that data generated by private and government laboratories were of acceptable quality. MDAU continues in this role today, with the support of IGETG.

At the 1991 annual ATW in Ottawa, an independent accreditation program for toxicology laboratories was initiated by IGETG members and representatives of private sector laboratories to ensure that Canadian environmental toxicological testing services produced high quality data. In 1993, the toxicology accreditation program was integrated into the existing chemistry accreditation program managed by the Canadian Association for Environmental Analytical Laboratories (CAEAL). CAEAL struck a partnership with the Standards Council of Canada (SCC) the following year through which CAEAL conducted site visits and SCC granted accreditation based on CAEAL’s recommendations. IGETG strongly supported the toxicity laboratory component of this national laboratory accreditation program by providing trained ecotoxicologists as volunteer laboratory assessors for biennial CAEAL laboratory assessments.

In 2005, the International Laboratory Accreditation Cooperation recognized CAEAL as an independent accrediting body. Standards established for toxicity laboratories were consistent with those of the International Organization for Standardization, specifically ISO/IEC 17025 (2005). In June 2008, CAEAL changed its name to the Canadian Association for Laboratory Accreditation (CALA), in response to member desires to deliver accreditation, proficiency testing and training to more than just environmental laboratories.

ACCOMPLISHMENTS

The IGETG meets annually at the ATW, and on an as-needed basis, and has been instrumental in:

- implementing a biological test method standardization program;
• initiating inter-laboratory round-robin studies for method validation to support the method standardization program;
• establishing an EC capability for initial core aquatic, sediment and terrestrial toxicity testing;
• the application of standardized toxicity tests for regulatory use in Canada (i.e., reviewing, summarizing and interpreting toxicity data);
• acting as a forum for cooperation and technical guidance to expand member laboratory’s expertise and capability; and
• promoting and participating in a national program for QA accreditation program for ecotoxicological testing services.

STANDARD TOXICITY TEST METHODS

Since 1990, a total of 22 standardized aquatic (i.e., five marine and 11 freshwater), sediment and soil toxicity methods and eight supporting guidance documents have been published and distributed both nationally and internationally by Environment Canada’s MDAU (e.g., EC 1992; EC 1997; EC 2001; EC 2005a; EC 2008a; EC 2008b; Table 1), and four of these test methods are highly standardized reference methods (e.g., EC 2000a; EC 2000b; Table 2) which are included in Regulations under various Federal Acts (Fisheries Act and Canadian Environmental Protection Act (CEPA)). The basic characteristics of each of the 22 test methods are provided in Tables 1 and 2 and all are considered single-species type tests (as opposed to multiple species or mesocosm type tests which have proven difficult from a standardization point of view given their complexity and cost). Most recently, procedures for measuring effects on terrestrial plants and invertebrates have been developed and published as standard methods (EC 2006a; EC 2006b; Table 2) which are included in Regulations under various Federal Acts (Fisheries Act and Canadian Environmental Protection Act (CEPA)). The basic characteristics of each of the 22 test methods are provided in Tables 1 and 2 and all are considered single-species type tests (as opposed to multiple species or mesocosm type tests which have proven difficult from a standardization point of view given their complexity and cost). Most recently, procedures for measuring effects on terrestrial plants and invertebrates have been developed and published as standard methods (EC 2004; EC 2005b; EC 2007). A large part of the MDAU’s success in publishing test methods and guidance documents is attributable to IGETG member laboratories; they carry out much of the method development, participate in inter-laboratory validation (round-robin testing), review draft methods and recommend improvements for all aspects of culturing, testing, data analysis and quality assurance. Figure 1 outlines the process that is followed in the development and standardization of EC test methods. Ongoing co-operation on national method development exists between the MDAU and EC’s toxicological laboratories in Moncton, Montréal, Edmonton, and North Vancouver, as well as the Ontario Ministry of the Environment’s Aquatic Toxicology Unit at Rexdale. The group’s work on standardized ecotoxicity test methods has both national and international recognition (e.g., Thompson et al. 2005).

Member laboratories generally work through the MDAU in consultation with the IGETG and often directly with the departmental program staff who require the method. After an initial draft is prepared, method validation and round-robin studies with both government and private sector laboratories are carried out to determine the expected level of intra- and inter-laboratory variability. With the results of the round-robin studies and further feedback from a second international peer review, the final method is translated into both official languages and published as a formal Environment Canada document. The MDAU makes available printed copies of the methods and guidance documents and also provides freely downloadable copies on their website. Electronic copies of the amended methods are posted with the colour highlighted changes. All test methods can be downloaded from the following web sites: English: http://www.ec.gc.

Standard toxicity test methods and advice from IGETG members support a number of environmental protection programs. Specific examples of applications of standard toxicity test methods are as follows.

**Enforcement and compliance**

Federal and provincial water pollution control regulations, such as the Pulp and Paper Effluent and Metal Mining Effluent regulations under the Fisheries Act, and Ontario industrial sector effluent regulations under the Ontario Environmental Protection Act, require toxicity testing for compliance monitoring according to Environment Canada standard reference methods. The consistency of the federal and the provincial requirements, achieved through co-operation of IGETG members, allows industry to comply with regulations of both jurisdictions by conducting a single set of rainbow trout and *Daphnia magna* acute lethality tests. Enforcement of the acute lethality provisions of the effluent regulations has been an important factor in the improvement of industrial effluent quality, reflected by the dramatic increase in compliance over the past several years. For example, compliance with Ontario’s Municipal/Industrial Strategy for Abatement (MISA) pulp and paper toxicity regulations was approximately 50% in 1990 but rose to approximately 90% for both *D. magna* and rainbow trout toxicity at mills with secondary treatment in 1996 (O’Connor & Voss 1998). Additionally, over 50% of pulp and paper mills across Canada routinely had an acutely lethal effluent until the amended Pulp and Paper Effluent Regulations came into force under the Fisheries Act in 1992, and further enforcement actions were undertaken. By 2007, 98% of the Canadian pulp and paper mills were in compliance with the rainbow trout acute lethality requirement. When toxicity test results have been challenged in court, many IGETG members have provided direct testimony or acted as expert witnesses, defending the standard methods and supporting the Crown’s environmental case. Recent amendments to the acute lethality reference methods for trout and *Daphnia* have further standardized the methods, reducing the likelihood of bias or manipulation of test results. A number of the amendments to the reference methods were prompted by court cases, or advances in scientific knowledge. Amendments will continue to be made as the need arises.

**Environmental assessments and monitoring**

Toxicity tests are used in environmental assessments to predict effects on the environment, quantify changes in effluent or ambient water quality, and for monitoring sites once a management decision has been implemented. EC’s standard methods use species relevant to the Canadian environment, with endpoints that allow determination of short-term, lethal, as well as longer-term, sublethal or chronic effects.

In EC’s Disposal at Sea Program, a battery of standard acute and sublethal tests is conducted as part of a tiered regulatory approach for assessing the acceptability of contaminated sediment for disposal at sea, based on comparison to ‘pass/fail’ toxicity criteria (Porebski & Osborne 1998). Toxicity tests used in the assessment were developed through the efforts of the MDAU and IGETG laboratory staff with overall direction from the various members of the Disposal at Sea Program at headquarters (Gatineau) and regional offices in Halifax and Vancouver. Monitoring of the impacts of sediment after disposal at registered dumpsites ensures that the initial decision to grant an ocean disposal permit was correct and that the disposal operations are causing minimal environmental harm.

**Environmental effects monitoring (EEM) programs**

Underway for the pulp and paper and metal mining effluent regulations, the EEM studies assess effluent quality and potential environmental effects based on laboratory sublethal toxicity tests that report effects on growth and reproduction in combination with wild fish surveys and benthic monitoring in the field. A number of IGETG members were involved in the multi-stakeholder consultations, which led to the setting of a minimum level of monitoring and testing in 1992. Seven years of sublethal toxicity data using a fish, invertebrate, plant and algal species in the pulp and paper EEM program have shown a strong correlation to environmental impacts observed in the field.
Scroggins et al. 2002, 2005). In addition, the national data set for the different sublethal toxicity tests generated under the EEM required monitoring has shown a continuous improvement in the quality of pulp and paper mill effluents, largely due to improvement in effluent treatment in the 1990s and continued improvement in mill operations. Results of sublethal toxicity tests during the second 3-year cycle of EEM (1997–1999) captured the dramatic improvement in effluent quality due to the installation of secondary biological treatment at many mills. Generally lower sublethal toxicity was observed when aerated stabilization basins’ treatment was in place as opposed to high-rate activated sludge treatment (EC 2002). In 2008, the Pulp and Paper Effluent Regulation was amended to remove the sublethal toxicity test with fathead minnows (EPS 1/ RM/22, Table 1) from EEM because it was no longer responsive to mill effluents.

By 2009, the Canadian mining sector had just completed two 3-year phases of sublethal toxicity testing monitoring which included the following freshwater species: Pimephales promelas, O. mykiss, Lemna minor, Pseudokirchneriella subcapitata, and Ceriodaphnia dubia (i.e., EPS 1/ RM/22, EPS 1/ RM/28, EPS 1/ RM/37, EPS 1/ RM/25, EPS 1/ RM/21 (Table 1)). A review of the sublethal toxicity data collected during these phases concluded that most of the sublethal toxicity tests were able to detect significant changes in mine effluent quality over time, that the tests detected differences in effluent toxicity related to the type of mine (i.e., base metal, uranium, precious metals, etc.) and that the effluent quality at a number of mines could vary dramatically from sample to sample at specific sites (Taylor et al. 2010).

General environmental monitoring and substance testing

IGETG members have been called upon to provide advice in the development of environmental monitoring programs for municipal sewage treatment plants, as well as in environmental management of non-regulated industrial sectors such as the oil and gas and diamond mines, north of the 60th parallel (a circle of latitude in the northern hemisphere). Toxicity testing in IGETG member laboratories has been part of larger non-target pesticide impact studies that have influenced the registration of certain pesticides (Ernst et al. 2001). A number of IGETG members assisted the Biotechnology Division of EC’s New Substances Branch with the development of guidance for the testing of microbial substances for potential toxicity and pathogenicity under the CEPA New Substances Notification Regulations. In addition, members helped generate data on aquatic and soil toxicity, pathogenicity and persistence for microbial substances listed on the CEPA Domestic Substances List (DSL; note: in this context and to differentiate them from chemical substances on the DSL, a ‘microbial substance’ refers to a living microorganism, e.g., Pseudomonas aeruginosa, strain 31480).

Standards setting

Standardized toxicity tests, using species endemic to the Canadian environment, provide much of the data necessary to support the development of defensible water, sediment and soil standards and guidelines. Toxicity tests also support regulatory initiatives such as those of CEPA, under which standards and guidelines are developed (e.g., Canada Wide Standards for Petroleum Hydrocarbons (PHC) in Soil, National Environmental Quality Guidelines and Provincial Water Quality Objectives) and as data requirements under regulations as these instruments are developed and improved through formal amendment (e.g., Ocean Disposal Regulations, New Substances Notification Regulations, and the new Wastewater Systems Effluent Regulation). Additionally, sediment toxicity data are being generated by IGETG member laboratories to fill information gaps that will allow a change in the status of Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines (i.e., data needed to change the guideline from ‘interim’ to ‘final’ status; http://www.ccme.ca/publications/cegg_rceg.html). IGETG member laboratories have been actively involved in generating toxicity data for all of the above programs.

TECHNICAL GUIDANCE AND CO-OPERATION

Eight guidance documents have been prepared and published by MDAU with the participation of IGETG members (e.g., EC 2005a; EC 2008a; EC 2012). They are intended for use along with the methods to clarify issues such as reference toxicant testing as part of method quality control, sampling techniques, interpretation of results, as
well as methods for evaluating the accuracy and precision of data. Since the methods are externally peer-reviewed, published, and admissible in court, these supporting documents are powerful tools for method validation, standardization and research. Much needed guidance on the statistical analysis of toxicity test data has been developed as report EPS 1/RM/46 (EC 2005a) through the participation of several IGETG members and private consultants. This report is proving to be of great value to both generators and end users of toxicity data, and is also being used as a student textbook for an environmental toxicology course at the Western Washington University, USA (Wayne Landis, pers. comm.). The latest national guidance document to be released by the MDAU gives much needed information on soil collection, sampling design and detailed instructions on the handling, storage and preparation of soil samples for single-species testing and microbial community health assessments (EC 2012).

In addition to work specifically focused around the development of standard methods, IGETG members regularly work together on issues requiring technical or scientific input. As a recent example, IGETG members formed a subcommittee and conducted round-robin testing to demonstrate method improvement that has led to the amendment of the echinoid fertilization method, EPS 1/RM/27 (EC 2011). Another IGETG team is conducting method improvement testing in support of the amended survival and growth test with the amphipod *Hyalella azteca*, EPS 1/RM/53 (EC 1997). In another case, Environment Canada laboratories in Vancouver, Edmonton, Montréal, and Moncton plus the Ontario Ministry of Environment laboratory at Rexdale undertook research on techniques for maintaining pH control in rainbow trout acute lethality tests and participated in inter-laboratory validation of these pH stabilization procedures. In 2007, this research and the use criteria were discussed and key decisions were made at the annual IGETG meeting that took place in Halifax that year. In July 2008, the MDAU released the published procedure for pH stabilization as report EPS 1/RM/50 (EC 2008a), which was accompanied by a supplementary guidance document to support the use of the pH stabilization procedure (EC 2008b). Development of pH stabilization techniques is important in addressing ammonia toxicity due to effluent aeration and pH drift in rainbow trout acute lethality testing of municipal wastewater effluents (EC 2008a).

Important information exchanges among IGETG members take place on a continual basis to reduce inter-laboratory variability associated with standardized test methods, train new staff, improve existing methods, and advance the development of new methods. They include comparing notes on culturing techniques for invertebrates, fish and amphibians, evaluations of statistical software, establishing uniform positions on quality assurance, and distribution of noteworthy scientific reports and publications. Annual IGETG meetings, coinciding with the ATW, serve as an invaluable forum for reporting on initiatives at each organization, formation of research partnerships, and for scientific debate that supports a consensus-based approach to applied ecotoxicity testing in Canada.

### QUALITY ASSURANCE

Several IGETG members volunteer as laboratory assessors and/or participate on the CALA Advisory Panel. Staff from the MDAU have prepared detailed toxicity method checklists for laboratory assessments and have arranged six toxicology assessor training sessions. These sessions involve lectures and laboratory demonstrations of new toxicity test methods for which CALA accreditation will soon be offered and amended methods for which CALA already offers accreditation. The toxicology sections of the Atlantic and Pacific Laboratories for Environmental Testing, and the Science and Technology Laboratories (at River Road) in Ottawa, have hosted training sessions in the past. Several IGETG members cooperated with CALA and private sector laboratories to develop guidelines for measuring uncertainty in toxicity tests, which is now required under ISO/IEC 17025 (2005). IGETG participation in CALA is an important linkage between government agencies involved in method development and the continuous improvement mandate of the International Organization for Standardization quality system. An IGETG member laboratory currently designs the CALA Proficiency Testing program for ecotoxicity laboratories.

IGETG members have insisted on a higher level of quality control where reagents must be purchased from outside
suppliers. For example, in 1997, representatives of six IGETG laboratories that used the Microtox® bacterial luminescence inhibition test (EC 1992) wrote to the reagent supplier, Azur Environmental, and strongly requested that the company supply proof of product quality to be sent with each batch of testing reagent. The company agreed to change its practices and now sends quality assurance information with each batch reagent.

PRESENT-DAY ROLE FOR IGETG AND CURRENT CHALLENGES FOR ECOTOXICITY TESTING

IGETG continues to be active in promoting and expanding ecotoxicity testing in Canada. Some of the activities of IGETG (circa 2012) include:

• Initiating and participating in interlaboratory method validation studies for test methods currently under development (the echinoid sediment contact test is currently in the method validation phase);
• Providing guidance and data interpretation to EC departmental programs using toxicity testing on an ongoing basis (e.g., Priority Substances, EEM, Disposal at Sea, Environmental Enforcement);
• Improving the implementation and amendment of new and existing regulations by provision of expert advice (e.g., Wastewater Systems Effluent Regulation, Metal Mining Effluent Regulations amendments to toxicity provisions);
• Conducting industry-specific national reviews of toxicity data (e.g., pulp and paper and metal mining);
• Providing toxicity data for standards setting (e.g., sediment quality guidelines);
• Developing technical guidance (e.g., a soil sampling guidance document will be published and distributed in 2012);
• Providing ongoing technical support to the CALA (e.g., many IGETG members are volunteer assessors and advisory panel representatives).

There are many challenges in developing, interpreting and applying ecotoxicity tests for environmental conservation and protection in Canada. The IGETG acts as a forum for common problem solving. Problems are brought to the group and are addressed to the extent possible, typically with national consistency in mind. Some current examples follow.

Costs and time for test method development

Standard methodologies can take between 3 years (e.g., acute lethality methods) and 9 years (e.g., polychaete survival and growth test, EC 2001) to develop, from inception to a final published method. The cost of development and standardization varies from 200,000 Canadian dollars to more than 500,000 dollars. Producing the documentation and the expertise to support these tools for use at a regulatory level has also required significant investments of time and resources. A broader funding base with a wider network of potential users (including the private sector) and more substantial government backing would help to produce the standard toxicity test methods more quickly.

Costs and time for conducting tests

Once developed and standardized, the costs associated with carrying out many tests seem quite high. For example, a C. dubia survival and reproduction test is 7 days in duration and requires over 10 hours to set up, monitor and terminate. The cost for running the test at a private CALA-accredited laboratory can range from $800 to $1300 (Can.) per test. A long-term goal should be to develop tests with high ecological relevance, sensitivity, reproducibility, and that are cheaper to use and faster to complete.

Contaminant bioavailability

Standard tests conducted under laboratory conditions serve as screens for the presence of toxic substances of concern and potential effects in the environment but seldom account for site-specific conditions that may mitigate toxicity (e.g., suspended matter, pH, salinity, oxygen levels, ultraviolet radiation may increase or decrease toxicity). Many applicants for effluent certificates of approval or permits for disposal at sea have expressed desire for the option to conduct additional site-specific testing if the recommended laboratory tests show detectible toxicity. However, it must be noted that the opposite situation may occur, that is that...
standard laboratory tests show no toxicity for a substance that proves toxic in the field as a consequence of site conditions. The use of test batteries with different levels of biological organization, tiered testing (e.g., start screening with acute tests then proceed using tests with more sensitive sublethal endpoints), site-specific analytical chemistry and environmental conditions, plus scientific literature reviews can provide a weight of evidence for decision-making on contaminant bioavailability in this scenario. Another example of a site-specific application of toxicity testing is the new Alberta Environment Tier II Eco-Contact Protocol for PHC in soil. Under this protocol, a battery of soil toxicity tests is used to evaluate whether a PHC contaminated soil poses a risk to the natural environment. Site clean up or remediation decisions are made based on five toxicity-based regulatory criteria (Alberta Environment 2007).

**Statistics for ecotoxicology**

Guidance has been prepared for the application and interpretation of toxicology data as well as for the use of statistics in calculating toxicity test endpoints in report EPS 1/RM/46 (EC 2005a). A number of IGETG members were involved in this effort. However, until recently, there was no ‘one-stop-shop’, commercially available software that met the needs of Canadian ecotoxicity laboratory data managers to calculate test results following Environment Canada’s improved statistical guidance. In early 2009, Tidepool Scientific Software released an upgrade to their CETIS toxicity test analysis software which now meets the needs of Canadian ecotoxicology laboratories, and has agreed to include future changes as needs evolve. As well, new International Organization for Standardization requirements to include estimates of uncertainty in toxicity testing results have recently been developed for application in Canada and will assist in the improvement of the quality of test data and its interpretation (ISO 2005; CALA 2010).

**Industry-specific guidance**

Some pollution issues, which require the use of toxicity testing to resolve, may vary as they are specific to a particular industrial sector (e.g., bioavailability of metals in mining effluents and sediments downstream of mine operations, and weathered PHC residual in soil). Therefore, specific guidance on suitable toxicity testing is sometimes needed to deal with sector-specific environmental impacts.

**THE FUTURE OF APPLIED ECOTOXICITY IN CANADA**

As its mandate and the responsibilities of its member groups broaden, IGETG will strive to maintain the ‘hands-on’ approach which has earned it, and the governments it represents, credibility and respect within industry and among the international community (Thompson et al. 2005). In the years to come, the IGETG will continue its direct involvement in method development and amendments in support of federal and provincial environmental legislation. In light of compliance by most major industrial dischargers with acute toxicity regulations, IGETG will lead the focus of environmental management beyond compliance testing. This will include smaller scale tests used for screening, development of less resource intensive *in vitro* screening tools, as well as more sophisticated measures of the potential for longer-term, more subtle ecotoxicological effects (e.g., ‘omics’ tools to measure genetic level changes), which also have applications in screening toxicity and mechanisms of action of new chemicals. Review of the application and any further standardization of applied ecotoxicological tests for the assessment of effects from nanomaterials will also be considered by IGETG in the near future.

By forming solid communication links with government decision-makers, as well as with various research facilities and industry, IGETG will continue to promote sound scientific approaches in applications of ecotoxicity tests. At the same time, IGETG will respond to and influence environmental conservation and protection policy, and meet the needs of clients. QA/QC will continue to be an important aspect of any new test methodologies developed and used by IGETG members. IGETG will also continue its long-term collaboration with the CALA program, including training of potential assessor volunteers.

Examples of new methods, potential applications and other future initiatives of IGETG include the following.
Micro-scale tests (microbiotests)

To reduce the number and types of organisms that are sacrificed for toxicity testing and to increase the speed at which toxicity can be detected, smaller scale tests that measure genetic or cellular change in exposed organisms are under development. This work builds on the advances in micro-scale testing over the past 30 years, as shown by the success of the Microtox® and other automated and small scale test methods (e.g., Blaise 1991; Wells et al. 1998; Persoone et al. 2000; Blaise & Férard 2005). For example, primary hepatocytes extracted from fish or fish cell-lines may provide a means of screening industrial effluents if concordance with whole fish toxicity can be demonstrated (Gagné & Blaise 1997). It is noteworthy that European regulatory agencies have embraced microbiotests (Wadhia & Thompson 2007).

Toxicogenomics

Genomics, the study of gene expression, has been a major area of research by the scientific community to monitor changes in gene expression by xenobiotics or other environmental factors. This technology offers the capacity to follow changes in the expression of thousands of genes at the same time, and will potentially serve as an early measure for effects in whole organisms or communities. The use of gene chips (microarrays) to determine gene expression in both amphibians and salmonids is already well advanced in Canada (van Aggelen 2005) and gene arrays for marine amphipods, rainbow trout, mummichog and amphibia will likely become tools for evaluation by IGETG. Genomic tools have the potential to provide the toxicological investigator with the ability to determine deleterious effects of contaminants at the molecular level although government investment in associated bioinformatics capacity to store, manipulate, and interpret the huge amounts of data generated is lagging behind the private sector.

If used as a regulatory tool, this will greatly enhance protection of biological organisms by defining more precisely the mode of toxic action of contaminants, hence contributing to a better understanding of the risks and impacts of pollutants in natural settings. However, the toxicological significance of gene activation/suppression and relevance to the status of whole organism health remain to be demonstrated (Ankley et al. 2008).

Genetically modified organisms

In recent years, awareness of the increasing use of genetically modified organisms (GMOs, e.g., Bacillus thuringiensis) in commercial operations has raised major concerns in the public and scientific community about the dangers to both human health and ecosystem health. EC laboratories in Ottawa and Montréal have been addressing some of these issues in the detection and monitoring for pathogenic and toxic effects of GMOs on the environment.

Sediment tests

IGETG laboratories collaborate on projects to update existing procedures. For example, Ontario’s 1992 sediment testing procedures will not be updated to include a freshwater amphipod test. Rather, Ontario MOE will endorse an amended Environment Canada standard Hyalella sediment test method through the co-operative research efforts of the federal and provincial IGETG members. Through this effort, advances in sediment test designs have been validated and incorporated as amendments to the current Hyalella survival and growth standard method (EC 1997). Additionally, more sensitive measures of effects on sediment organisms (e.g., reproduction which includes measures of fecundity, embryo development, etc.) will continue to be investigated as many contaminants of concern persist in sediments.

Bioaccumulation

Bioaccumulation tests estimate the uptake of a contaminant from sediment, food and/or the water column and provide a link to potential toxicity (through comparison to critical body concentrations) or to food chain effects. A bioaccumulation method is needed for the Disposal at Sea Program and the current Ontario bioaccumulation method has been updated (Van Geest et al. 2011a, 2011b). IGETG will play an important role in the effort to develop a more standardized national bioaccumulation method and in making recommendations for its application in environmental management.
Amphibians

The worldwide phenomenon of declines in amphibian populations may have a number of potential causes, which include anthropogenic chemicals, UV light, habitat destruction, disease and parasites (Sparling 2003). IGETG scientists are positioned to study the effects of contaminants under controlled laboratory conditions, and can contribute greatly to the study of amphibian declines. Because their metamorphosis from an aquatic to a terrestrial phase involves complex hormonal interactions, amphibians are also ideal organisms for use in studying endocrine disruption. Standardized test methods are being developed currently using native frog species, in close collaboration with EC scientists at the National Wildlife Research Centre, and University of Ottawa researchers.

Terrestrial toxicity

Three standardized test methods for plants, earthworms and springtails were published by EC between 2004 and 2007 (EC 2004; EC 2005b; EC 2007). This test battery is used in CCME soil criteria derivation guidance, for setting eco-contact values for Canada-Wide Standards on PHC in soil and in provincial contaminated sites programs (i.e., those of Alberta Environment). Following the publication of three soil toxicity test methods, efforts continue to expand the testing battery (e.g., oribatid mites) and to develop new single-species methods and test species more relevant to non-agronomic eco-zones of Canada (e.g., the boreal and taiga zones). Test method research into the use of locally relevant and keystone test species (e.g., earthworms, springtails, mites and plants) will increase the relevance and applicability of soil toxicity testing to assessment of contaminated soils. Research efforts are currently directed towards species living in the boreal forest and northern eco-zones, which although covering the majority of Canada’s land mass, remain under-represented from a testing protocol standpoint. Tools to address these eco-zones will include relevant test design changes, such as the assessment of individual forest soil horizons, as well as a soil sampling guidance document to provide guidance on the sampling design, collection, transport, storage and preparation for soil toxicity testing and microbial community assessments.

At the EC laboratory in Ottawa, soil microbial communities are also being assessed for toxicological effects due to soil contamination. A test suite is currently being developed to determine the effects of contaminants on soil microbial activity, microbial biomass, and microbial community structure and diversity. Test results from the individual microbial assays will be integrated, utilizing a statistical ranking scheme that will categorize the soil’s microbial health studied on a scale from severely impacted to unimpacted by soil contamination.

Nanotechnology

Manufactured nanomaterials possess unique properties by virtue of their very specific sizes and serve as novel solutions to many problems. The prefix ‘nano’, when applied in the context of nanotechnology, is used to denote a size of less than 100 nm but greater than 1 nm. Attempts have been made to use existing ecotoxicity test methods to explore the toxicity of nanomaterials. While this approach appears to be sound in principle, many of the difficulties associated with the toxicity testing of nanomaterials revolve around the uncertainty as to what specifically causes the variation in toxicity seen between a nanomaterial and its ‘bulk-form’ counterpart. Unfortunately, it is currently unknown which physicochemical properties of any specific nanomaterial are related to toxicity and thereby require measurement. Current practice is to characterize as many properties as are practical in the hope that casting a wide net now will allow for retrospective analysis of the appropriate properties at a later time. A guidance document, to be used by Canadian toxicology laboratories, on sample preparation and dosimetry for the ecotoxicological testing of manufactured nanomaterials, is being developed with input from those IGETG members currently conducting toxicity tests with these materials.

CONCLUSION

The goal of this article was to outline the history of applied ecotoxicological testing in Canada and commemorate IGETG’s 35th anniversary. IGETG’s challenging work continues with a vision for the future of ecotoxicological testing, in support of environmental protection and conservation.
goals, that includes method standardization for: (1) rapid, economical, screening-type tests, (2) tests using ‘omic’ endpoints, (3) sentinel species (e.g., amphibians), (4) more sensitive endpoints (e.g., bioaccumulation, reproduction), and (5) emerging substances (e.g., nanomaterials).

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