in the MCR + FOOD treatment, toxicity was ameliorated (>90% survival), despite the fact that dissolved Cu (10 µg/L) was slightly greater than in the MCR treatment (5 µg/L; dissolved Zn <3 µg/L in MCR + FOOD). The significantly higher survival in the MCR + FOOD treatment most likely is due to organisms selectively feeding on added food rather than contaminated sediment particles, thereby reducing the effects otherwise observed via the dietary uptake of contaminants. In the MCR-top and MCR-top + FOOD treatments, survival was increased significantly to 49% and 95%, respectively, and dissolved Cu and Zn were <3 µg/L. In both the MCR and MCR-top treatments, dissolved Cu was below toxicity thresholds; however, survival was markedly higher in MCR-top. The major difference between these treatments was that, in MCR-top, the resin covered approximately 80% of the sediment surface, thereby restricting the organism's access to the sediment available for ingestion. Therefore, the markedly higher survival in MCR-top suggests a significant contribution of toxicity was due to the dietary exposure to contaminants. This was further demonstrated in MCR-top + FOOD, where toxicity was ameliorated. In this treatment, the concentration of dissolved contaminants and organism's exposure to the sediment surface were identical to MCR-top; however, the difference in survival between these 2 treatments was significant. Evidence suggested that the amphipods were able to feed selectively on the added clean food rather than contaminated sediment particles. The contribution of dietary toxicity also was demonstrated in MEC treatments. For MEC and MEC + FOOD, survival increased (42 ± 5% and 66 ± 11%, respectively) even though dissolved Cu and Zn concentrations were greater than in the baseline treatment.

The effectiveness of new WS-TIE methods for identifying dietary toxicity now has been demonstrated for a range of sediments containing toxic concentrations of metals and hydrophobic organic contaminants. These results suggest that a significant fraction of the toxicity occurs via dietary exposure to contaminants from sediment ingestion, in addition to exposure from dissolved contaminants. The findings also demonstrate the importance of dietary uptake pathways in whole-sediment toxicity tests, especially toxicant identification studies.

Whole-sediment TIE methods that include a combination of manipulations that identify the contribution of toxicity-causing contaminants occurring via both dissolved and dietary exposure pathways (new lines of evidence of causality) will be more successful in determining which of the chemical constituents are responsible for the observed toxicity. The proposed new WS-TIE methods are expected to be useful tools for future sediment quality assessment applications (Simpson et al. 2005).

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FISH CELL LINES AS RAPID AND INEXPENSIVE SCREENING AND SUPPLEMENTAL TOOLS FOR WHOLE EFFLUENT TESTING

Lucy EJ Lee,† Vivian R Dayeh,‡ Kristin Schirmer,§ and Niels C Bols‡
†*Wilfrid Laurier University, Waterloo, ON, Canada*
‡*University of Waterloo, Waterloo, ON, Canada*
§*Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switerland*
llee@wlu.ca

Background

General agreement exists as to the need to protect aquatic environments from the effects of toxic substances and discharges. Legislation designed to regulate effluent discharges in order to minimize the risk of harm to the environment is in place in most developed nations. For instance, whole effluent toxicity (WET) testing has been legislated with a set of consistent requirements where tests must be initiated within 36 h of effluent sampling. Required tests may be acute (endpoint being survival of test organism) or chronic (endpoints being survival and a sublethal measurement). Regardless of whether tests are acute or chronic, all dischargers would like their test results to be negative, showing no toxicity. As well, they would like the tests to be conducted correctly, under appropriate and consistent quality assurance/quality control guidelines. A detailed and efficient system of completing tests, tracking data, and ensuring quality results is costly and time demanding. Thus, although WET testing generally is useful, it is proving costly, ineffective, and impractical. Many disadvantages have been pointed out and supplemental tests would be desirable (Chapman 2000).

Whole organismal testing in WET tests

Analytical chemistry cannot tell us whether a sample is toxic or not. Living entities are needed for this. The standard WET vertebrate tests, acute 96-h 50% lethal concentration or 7-d chronic fish WET tests, are relatively expensive because of their reliance on living whole organisms and associated costs of maintenance. The expense of the tests discourages realistic monitoring frequencies and reduces the cost-effectiveness of toxicity detection. In order to allow adequate effluent-monitoring frequencies and improve the cost-effectiveness and efficiency of the regulation of effluent toxicity, a selection of rapid screening toxicity tests that are quicker and cheaper than standard toxicity tests need to be established. Alternatives are being sought to the acute toxicity tests with live vertebrates such as fathead minnows (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), or even invertebrates such as *Daphnia magna*.

Improvements in cost and turn-around times are needed. Sample collection, shipping, testing, and report preparation can be lengthy, delaying any appropriate remedial action to deal with toxic effluent test results. Estimated costs for a trout bioassay, including laboratory fees and the costs associated with sample collection and shipping, can be around US$1000 to US$2000 per sample. Such costs can escalate when weekly or monthly sampling must be performed for numerous effluent streams or receiving environments. Commercially available toxicity test kits based on bacteria and/or invertebrates, such as Microtox® (luminescent bacteria), Biohidrica®'s Toxi-Chromotest (bacteria), Rotoxkit F (rotifer) and Thamnotoxkit F (crustacean), and

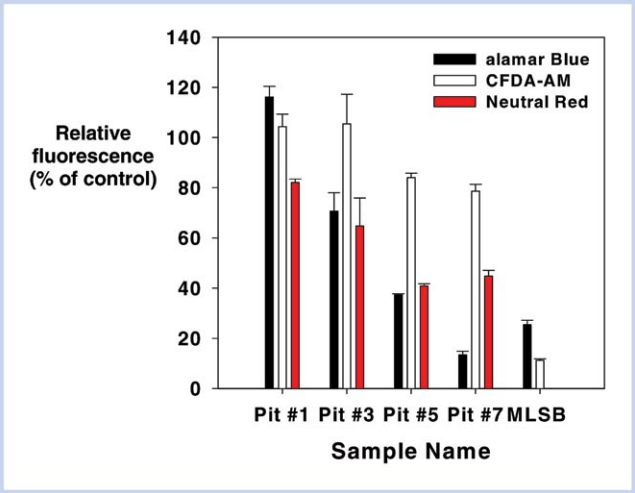

Figure 1. Comparison of the toxicity of whole water samples from various oil-sands process–affected waters to RTgill-W1. Cells were exposed to water samples from various sources. After the cultures had been exposed for 24 h, cell viability was assessed with alamar blue (black bars), 5-carboxyfluorescein diacetate-acetoxymethyl ester (CFDA-AM) (white bars), and neutral red (red bars). Results were expressed as a percentage of the readings in control wells exposed to the basal media (L-15/ex) alone. The data points represent the mean of 5 culture wells with standard deviation.

Daphnia IQ™ (*D. magna*), have been developed which, although quick and relatively inexpensive, do not necessarily reflect the impact on vertebrate species. Vertebrate cell cultures, especially those derived from aquatic species, could become a useful replacement for animals in regulatory tests. In the case of human and veterinary health, several mammalian cell models are accepted for regulatory purposes in drug testing, pharmacology, and medicine, as well as in some toxicity evaluations such as the test for phototoxicity with the mouse 3T3 cell line.

Fish cell lines in WET testing

As vertebrate tissues and organs perform specific tasks and physiological functions, organ-specific tests have been sought to elucidate mechanisms of toxicity. For example, the gills of aquatic organisms are the primary target and uptake sites of water contaminants (Evans 1987). As such, gills are appropriate organs for the study of aquatic toxicant effects. However, gills in vivo are difficult to evaluate or manipulate; thus, gill cells in vitro could represent ideal systems for the study of aquatic contaminants. Although primary gill cultures have been used for the evaluation of aquatic toxicants, the difficulty of their isolation, maintenance, and reproducibility makes them cumbersome to use. Continuous or permanent cell lines, on the other hand, are easy to maintain and manipulate, and yield highly reproducible results. Continuous cell lines do have significant advantages over the use of primary cultured cells in that they could eliminate the need for animals in chemical and water testing and demonstrate ease of culture, availability, and reproducibility (Bols et al. 2005). Societal pressure also favors use of animal alternatives. Specifically, in Europe the European Commission encourages the development and application of alternatives to animal tests in order to allow the new European legislation on the Registration, Evaluation and Authorization of Chemicals to be executed in an ethically and financially acceptable manner (Castano et al. 2003; Schirmer 2006). Thus, fish cell lines could be very useful in testing the toxicity of effluents.

Table 1. Oil-sands process–affected water samples and naphthenic acid (NA) content

Water samples	Total NA (mg/L)
Pit 1	1.4
Pit 3	3.6 ± 0.34
Pit 5	24.1 ± 1.00
Pit 7	21.5
MLSB[a]	88.0 ± 8.00

[a] MLSB = Mildred Lake Settling Basin

RTgill-W1 is a rainbow trout–derived gill cell line that shows characteristics of gill epithelial cells (Bols et al. 1994). This cell line is available from the American Type Culture Collection (number CRL 2523). These cells have been used to evaluate the toxicity of industrial effluents, including petroleum refinery effluents, and the toxicity of several compounds (reviewed in Dayeh et al. 2005). RTgill-W1 also was used to evaluate the toxicity of oil-sands process–affected waters. Whole water samples were evaluated on RTgill-W1 by direct exposure without extraction or concentration steps. The environmental water samples, in this case oil-sands process–affected waters, were mixed with basic medium components for cells and applied to RTgill-W1 for toxicity evaluation. The endpoints were 3 viability tests: Alamar blue, 5-carboxyfluorescein diacetate-acetoxymethyl ester (CFDA-AM), and neutral red. These endpoints measure effects on 3 cellular parameters that provide information on the mechanisms of toxicant action at the cellular level: Impairment in cellular metabolism, plasma membrane integrity, and lysosomal function, respectively (Dayeh et al. 2005). The evaluation was done blindly without knowledge of sample content. The samples were oil-sands process–affected waters. The toxicity of the samples (Figure 1) correlated with their naphthenic acid content (Table 1), which was revealed after the tests were completed. Naphthenic acids are a mixture of carboxylic acids whose toxicity to live organisms has been widely reported.

Conclusion

For approximately a century, cell cultures have proven invaluable for understanding a multitude of biological processes in vertebrates, primarily mammals. On the other hand, fish cell cultures have been in use for just over 3 decades (Schirmer 2006) and, although various applications have been reported, research with fish cell lines lags far behind that for mammalian cells. The potential roles of fish cell cultures in science and technology have just begun to be tapped and, although most fish cell lines are used in virology, their functions in toxicology and ecotoxicology are only beginning to be exploited (Bols et al. 2005). Studies with cultured cells permit the determination of molecular and cellular mechanisms by which contaminants lead to toxic effects in organisms at sublethal and chronic levels. The RTgill-W1 cells are proving useful for evaluating the effects of aquatic samples on cellular functions. A preliminary blind evaluation with RTgill-W1 cells of oil-sands process–affected waters from experimental ponds revealed a good correlation between acute cellular toxicity and naphthenic acid content. Many other fish cell lines are available that could be used for toxicity testing, including a rainbow trout cell line (RTL-W1) that can be used to rank the potency of dioxin-like compounds

(Bols et al. 2005). Furthermore, cell lines are more amenable to toxicogenomic technologies and up or down regulation of genes or proteins. The homogeneity of cell lines makes these responses to toxicants easier to detect and with less variability than whole organisms (Castano et al. 2003; Schirmer 2006). However, a lot of research still is needed to optimize fish cell cultures and assays (Castano et al. 2003) for evaluating WET in the water industry.

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A WEIGHT-OF-EVIDENCE (WOE) APPROACH FOR DETERMINING MODE OF ACTION: AN ECETOC CASE STUDY

Paul Thomas,† Martin Holt,‡ Philippe Lemaire,§ Ian Malcomber,‖ Dan Salvito,# and Roy Thompson††

†*AkzoNobel, Arnhem, The Netherlands*

‡*ECETOC, Brussels, Belgium*

§*Arkema, Paris, France*

‖*Unilever, Sharnbrook, United Kingdom*

#*RIFM, Hackensack, NJ, USA*

††*AstraZeneca, Brixham, United Kingdom*

paul.thomas@akzonobel.com

This work was carried out by a task force from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Brussels.

Introduction

In principle, environmental risk assessment (ERA) is possible for all substances. However, there is increased uncertainty in prediction of environmental fate and effects for certain substances (e.g., persistent, bioaccumulative, and toxic [PBT] and very persistent very bioaccumulative [vPvB] chemicals under the European Union Registration, Evaluation, Authorization, and Restriction of Chemicals legislation). Up to now, this has prevented unequivocal assessment of risk reduction measures to adequately control safe use for such substances. This has made it difficult for some authorities to embrace ERA for substances with such properties.

Risk assessment is a tiered process and the level of work required is proportional to the concern determined in the lower tiers. For example, using the European Union Technical Guidance Document in its current form (EC 2003) may lead to high uncertainty when risk assessing certain difficult chemicals such as those categorized as PBT and vPvB. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 2005) has proposed a scheme that may reduce uncertainty in the ERA of some PBT/vPvB-like chemicals.

Key to the scheme proposed by ECETOC is an understanding of the mode of action (MOA) of a chemical. Currently, risk assessment methodologies are in place and have been accepted for substances not classified as PBT regardless of MOA (EC 2003) and for substances with a specific MOA (e.g., pesticide, biocidal product, and pharmaceuticals legislation worldwide). Since the 1990s it has been widely recognized (Verhaar et al. 1992; Russom et al. 1997; Di Toro et al. 2000) that for chemicals with a narcotic (also known as Baseline) MOA, the levels of toxicity observed in ecotoxicological studies can be predicted based on the knowledge that the effects are not specific to a target species, organ, or cell. This level of predictability can play a role in improving the risk assessment of narcotic PBT-like substances when used within an approach that incorporates internal body concentrations to measure environmental effects.

Currently, no single, simple definitive method exists that allows us to guarantee the MOA of a chemical. A method is described below to determine MOA using a battery of predictors built into a weight-of-evidence (WoE) approach. Such an approach helps to reduce the level of uncertainty in predicting the toxicity of chemicals and allows their risk assessment by increasing the confidence of correctly assigning an MOA.

Outline of methods used

Data sets were prepared for 38 chemicals from data reported in either risk assessments conducted under the European Union Existing Substances Regulation or in the ECETOC EAT3 database (ECETOC 2003). These sources were chosen due to the relative richness of data (quantity) and the high level of validation applied (quality), and not because they are PBTs or vPvBs. Each chemical was subjected to the property assessment outlined in Table 1.

Results of mode-of-action assessment

In assessing the 38 chemicals in this preliminary study, a binary yes/no approach was taken in developing the WoE approach. A 'Yes' indicates that either the structural alert, measured endpoint, or calculated ratio (acute to chronic ratio or critical body burden) is indicative of what is expected for a narcotic chemical. A 'No' indicates that the value in Table 1 does not support the finding for that method that the chemical presents a narcosis mode of action.

In our evaluation, a sum of the battery of methods (i.e., the WoE), is considered to indicate a narcotic MOA if for the total score >70% of the answers are positive. No individual factor is considered to express the true MOA against which to judge the results of the WoE approach because each method contains flaws or weak points (e.g., the Verhaar et al. 1992 method has not identified all structural alerts known today).

In many cases the predictions of a substance as a narcotic chemical fit with conventional expectations. Using the WoE