Early Evolution of the Toxicity Identification Evaluation Process: Contributions from the United States Environmental Protection Agency Effluent Testing Program

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Abstract During the 1980s, whole effluent toxicity testing was incorporated into the regulatory control program for municipal and industrial effluents in the USA, as a complement to chemical-specific limitations. While regulating effluent toxicity offered several advantages, it also required the development of means to identify and control sources of toxicity within effluents, which could include toxicants not previously monitored or even known. To meet this need, the US Environmental Protection Agency developed an effects-directed analysis procedure called "toxicity identification evaluation". This involved a suite of physical/chemical manipulations that are applied to aliquots of a toxic effluent sample, and the relative effects of these manipulations on effluent toxicity are used to infer the type of toxicant(s) responsible for toxicity, and to guide their isolation and analytical identification. This chapter provides an overview of these methods and their component phases: I – Characterization, II – Identification, and III – Confirmation. Case examples of toxicant identification in effluents from municipal and industrial sources are discussed, along with a broad summary of the types of toxicants identified, and the characteristics of those toxicants that helped guide their assessment.

Keywords Effluent, Toxicity, Toxicity identification evaluation

Contents

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1 Brief History of Effluent Regulation in the USA

Prior to 1970, most water pollution control efforts in the USA were focused on sanitation and human health; there was little coordinated regulation of the discharge of toxic chemicals to surface waters, particularly for the purpose of protecting aquatic organisms. Many lakes and rivers in industrialized areas were extremely polluted. In the 1960s, the Federal Water Pollution Control Administration issued annual reports chronicling pollution-induced fish kills, and in 1966 listed industrial pollution and municipal wastewater as the two most important causes among the reported 436 pollution-related fish kills spread across 46 states [1]. Of the Cuyahoga River in Ohio, the August 1, 1969 issue of TIME magazine said:

> Chocolate-brown, oily, bubbling with subsurface gasses, it oozes rather than flows. "Anyone who falls into the Cuyahoga does not drown," Cleveland's citizens joke grimly. "He decays." The Federal Water Pollution Control Administration dryly notes: "The lower Cuyahoga has no visible signs of life, not even low forms such as leeches and sludge worms that usually thrive on wastes." It is also – literally – a fire hazard. A few weeks ago, the oil-slicked river burst into flames and burned with such intensity that two railroad bridges spanning it were nearly destroyed.

Through the Federal Water Pollution Control Amendments of 1972, the Clean Water Act of 1977, and other related legislation, the US Environmental Protection Agency (EPA) was charged with developing and implementing approaches and requirements to control the release of pollutants to the waters of the USA. Early efforts focused primarily on so-called conventional pollutants, such as biochemical oxygen demand, fecal coliform bacteria, and suspended solids, with later expansion to a set of "priority pollutants," which included several metals and common industrial chemicals. The approach to controlling these pollutants relied on "technology-based" treatment standards, which established the allowable amounts that could be discharged based on the category of industry and the degree that existing technology could remove those pollutants with reasonable expense.

While these early efforts were effective at reducing some important sources of aquatic pollution, their scope and structure was found to be insufficient to adequately protect surface waters from chemical pollution. Because discharge limits were based on the ability of treatment technology to remove chemicals rather than on maintaining adequate water quality in the water body receiving the

discharge, there was no assurance that treatment would render a discharge nontoxic to aquatic life. Further, these technology-based limits focused on a limited number of pollutants rather than the much larger range of chemicals potentially present in industrial and municipal effluents. In 1984, a major step forward was taken with the EPAs issuance of a new national policy, *Development of Water Quality-Based Permit Limitations for Toxic Pollutants* (US Federal Register, 49:9016, 1984). This policy included two very important new directions for effluent control: (a) acceptable discharges of individual toxic chemicals would be based on maintaining safe concentrations in the receiving stream; and (b) biological methods (e.g., toxicity tests) would be used, in addition to chemical-specific limitations, to establish limits on the release of toxic chemicals. Toxicity tests were an important addition to the regulatory approach because they address several shortcomings in a chemical-specific approach. As outlined in that policy, these shortcomings include (a) the great number of toxic chemicals that may potentially be discharged into receiving waters and the difficulty in their analysis, (b) the changes in toxic effects of a chemical resulting from reactions with the matrix in which it exists, and (c) the inability to predict the effects of exposure to combinations of chemicals.

Measuring the aggregate effects of all effluent constituents in what became known as "whole effluent toxicity" (WET) tests overcame these weaknesses, because a toxicity test measures the combined effects of all chemicals in the effluent, known and unknown, in the context of the water chemistry of the effluent and/or receiving water. This provides a critical backstop to chemical-specific approaches, because one does not have to know anything about the presence or toxicology of a chemical in order to monitor its potential effect on aquatic organisms.[1]

While the concept of WET testing as a monitoring tool was (and remains) very appealing, its comprehensiveness could be simultaneously its greatest strength and greatest weakness. Even though a WET test can detect an effect from virtually any chemical, the finding of an effect in a WET test is essentially generic with respect to its cause – almost any chemical, including unknown chemicals, could be the cause of an observed effect. Because treatment technologies are generally designed around knowledge of the type of pollutant to be controlled, simply knowing that an effluent was toxic provided very little direction toward the means by which toxicity could be controlled. To make control of effluent toxicity practical it was highly desirable, if not essential, to develop a means by which the specific cause of toxicity could be identified, so that targeted and cost effective means could be found to control that cause.

[1]While WET testing is a powerful tool, it is important to note that its ability to detect the effects of toxic chemicals is limited to the types of effects that can be measured in the toxicity test procedures used. As such, effects such as those on secondary consumers mediated through food chain transfer, on life stages not tested, or on organisms more sensitive than those tested, may not be detected and must be evaluated by other means.

It was in response to this challenge that the EPA developed an effects-directed analysis (EDA) approach to identifying the cause of toxicity in toxic effluents or ambient waters. This EDA process, termed a "toxicity identification evaluation (TIE)", is the focus of the remainder of this chapter. In the following sections, we provide a brief overview of the original TIE procedures developed, followed by a more detailed discussion of the philosophical basis of the approach relative to choice of biological system/endpoints, sample manipulations and interpretation of test results. Finally, to illustrate the TIE process, including the logic involved in data collection and interpretation, we describe several case examples in which specific chemicals/classes of chemicals were successfully identified as causative of toxicity in effluents.

2 Overview of the EPA Toxicity Identification Evaluation Process

The EPA toxicity-based approach to effluent regulation necessitated the development of EDA techniques that fundamentally differed from more limited methods used to that point in the field of environmental toxicology. Previously, EDA approaches had been applied with some success to simplifying complex environmental mixtures that caused mutagenicity in bacteria (e.g., [2–4]). However, application of these approaches to more complex responses associated with acute or chronic toxicity in higher organisms was less successful (e.g., [5–8]). There were multiple reasons for this lack of success, but the most important involved how the test samples (typically surface water, effluent, or sediment) were handled. Specifically, many of the manipulations involved extraction and/or fractionation using relatively harsh techniques, often with strong solvents. This confounded the EDA process from several perspectives, including (a) altering the bioavailability of contaminants in the original samples (e.g., extracting/concentrating compounds that did not contribute to toxicity in the intact sample), (b) loss of some classes of possible toxicants (e.g., labile or volatile compounds), and (c) production of artifactual toxicity emanating from the treatments themselves (e.g., residual solvent). The net result of these problems was loss of a linkage between the initial sample (and the chemicals responsible for toxicity) and the biological endpoint(s) of concern. To address this, researchers supporting the EPA effluent testing program developed EDA/TIE techniques designed to preserve, as much as possible, linkages between the original test sample and observed toxicity [9]. This work involved development not only of novel physical/chemical manipulations and test approaches, but a logic framework for conducting EDA/TIE analyses to support ecological assessments.

The TIE process developed by the EPA consists of three phases: characterization, identification, and confirmation [10–15]. Although Phases I, II, and III are described as discrete activities and logically proceed from one another in a linear

fashion, in reality they often are iterative and, depending on the nature of the toxicants, may occur simultaneously. Phase I characterization is conducted in response to an effluent being identified as toxic to one (or more) of the test species required for discharge monitoring (typically the cladoceran *Ceriodaphnia* or the fathead minnow, *Pimephales promelas*). Phase I consists of a variety of sample manipulations conducted in conjunction with toxicity testing (with the species/endpoint that triggered the TIE) to characterize the general physicochemical properties of the causative toxicants(s) [10, 13]. Sample manipulations include aeration and filtration of the sample at low, neutral, and high pH values, solid-phase extraction with a nonpolar (C18) resin, addition of substances (e.g., sodium thiosulfate, EDTA) designed to mitigate the toxicity of different classes of chemicals, and testing at a graduated range of (physiologically tolerable) pH values. At conclusion of a successful Phase I study, causative toxicants can be broadly classified with respect to polarity, volatility, and stability (all as a function of pH), reactivity with thiosulfate (oxidants) or EDTA (cationic metals), and ability to exert differential toxicity at different pH values (e.g., ammonia).

Observed physicochemical characteristics from Phase I dictate approaches taken in the Phase II identification portion of the TIE [11, 14]. For example, if EDTA removed sample toxicity, toxicant identification would focus on measurement of cationic metals and comparison of measured concentrations to existing (or generated) toxicity data for the species/endpoint that triggered the TIE. Phase II identification also could involve further sample manipulation and toxicity testing in the context of fractionation. For example, if Phase I indicated the presence of a nonionic organic toxicant (toxicity removal by the nonpolar resin), Phase II would consist of fractionation of the sample via reverse-phase, low- and/or high-pressure liquid chromatography (L/HPLC). To decrease potential for artifactual toxicity and/or loss of toxicants from solution, Phase II TIE methods advocated use of low-toxicity solvents with some degree of miscibility in water (e.g., methanol, acetone), thereby ensuring that toxicity tests could be conducted directly on test fractions from the chromatography steps. Concurrent instrumental analysis of toxic fractions, using techniques such as gas chromatography–mass spectroscopy (GC–MS), is used to identify discrete chemicals, which then can be evaluated with respect to known or measured toxicity.

Once potential toxicants have been identified, Phase III analyses are conducted to confirm that the suspect chemicals are indeed responsible for sample toxicity [13, 15]. Because even minimal handling/manipulation of test samples can cause unanticipated changes in toxicity, and because complex environmental samples (and effluents in particular) can exhibit considerable temporal variability (i.e., chemicals responsible for toxicity may change over time), confirmation is a critical step, especially if substantial resource commitments to mitigation are to be made based on TIE results. The tools of Phase III include correlation of sample toxicity with concentrations of suspect toxicants over some gradient of toxicity/time, evaluation of relative species sensitivity, observation of signs of toxicity in test animals, and addition to, or deletion of, suspect toxicants from the test sample.

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