this issue:

Whole Effluent Toxicity Testing: Ion Imbalance

SETAC**TIP***

*technical issue paper

Purpose: SETAC is a professional society with worldwide membership from academia, government, business, and nongovernmental organizations. TIPs provide a credible and balanced scientific discussion of important environmental issues.

What is Whole Effluent Toxicity (WET) testing?

Whole Effluent Toxicity (WET) testing is an important component of the U.S. Environmental Protection Agency's (USEPA's) integrated approach for assessing the potential for toxicity to the nation's waters. WET testing is used in USEPA's National Pollutant Discharge Elimination System (NPDES) to regulate industrial and municipal point source wastewater discharges. However, most non-point source discharges are not regulated by USEPA under the NPDES permit system.

The primary objective of WET testing is to ensure that treated effluent released from industrial and municipal facilities into the nation's waters does not cause unacceptable levels of instream toxicity to aquatic life. To determine whether an effluent has the potential to be toxic, WET tests are performed on various aquatic test species. Depending on the regulatory goal of the test, the test may be short term (acute) or long term (chronic). Acute tests are usually performed to determine the survival of organisms exposed to various concentrations of effluent. Chronic tests are generally conducted to assess survival, growth, and reproduction of organisms exposed to various concentrations of effluent. Another SETAC Technical Issue Paper (TIP), Whole Effluent Toxicity Testing, gives additional details.

By nature and definition, toxicity cannot be measured analytically. Chemical analyses are practical only when one knows what potential constituents are present in an effluent. WET testing is capable of assessing the combined toxic effects of all constituents of an effluent, known or unknown. By bringing together information from all constituents, a clearer picture can be developed of the overall potential effects of a wastewater discharge on the aquatic environment.

Toxicity from effluent discharges has classically been associated with chlorine, ammonia, heavy metals, and/or synthetic organic compounds. Recently, it has been established that many other elements and compounds, including several ions commonly found in aquatic ecosystems, can also be toxic to aquatic organisms when present in concentrations above or below biologically tolerable levels. The issue of ion imbalance has caused confusion in technical assessments of effluents and in permitting or compliance because dischargers and regulators often are not aware of the problem and/or it is difficult to identify ion imbalance through traditional toxicity testing procedures.

What is an ion?

An ion is an atom or a group of atoms whose negative or positive electric charge results from having lost or gained one or more electrons. When an acid, base, or salt dissolves in water, some of its atoms or elements separate into positive and negative ions. Cations are positive ions formed by the loss of electrons; anions are negative ions formed by the gain of electrons. The number of electrons lost or gained is denoted by a positive sign for cations (e.g., Mg^{2+} for magnesium) or a minus sign for an anion (e.g., F^{-} for fluoride).

What is ion imbalance?

Adverse effects can occur in aquatic organisms when common ions exceed a certain concentration, when the normal composition (ratio) of ions is not correct, or in some cases, when ion concentrations are too low. Common chemical constituents normally found in aquatic habitats can also be toxic to aquatic organisms when the chemicals occur in concentrations or ratios outside an organism's normal tolerance range. A number of inorganic ions (e.g., calcium [Ca^{2+}]), magnesium [Mg^{2+}], and sodium [Na^{+}]) occur naturally in aquatic environments and are essential for the health of aquatic organisms. Ion imbalances can occur because of effluents and can cause a toxic response in a WET test. An ion imbalance that causes a toxic response in a WET test may cause a water sample to appear to be more toxic than is acceptable according to water-quality standards. There is currently a debate regarding whether ion imbalance causing toxicity in a test should be considered a toxicant instream, the receiving water that collects the effluent.

What is the ionic composition of water?

Salinity is a measure of the weight of dissolved salts in one kilogram of seawater and reflects ionic composition. Seawater salinity is very consistent throughout the oceans of the world and ranges between 33 and 37 g/L (or parts per thousand [ppt]). The average salinity of oceanic seawater is approximately 35 ppt. Sodium and chloride account for more than 85% of the total dissolved ions in natural seawater. Other major seawater constituents include the cations manganese (Mn^{2+}), potassium (K^+), and calcium (Ca^{2+}) and the anions bromide (Br^-), sulfate (SO_4^{2-}), and bicarbonate (HCO_3^-). Salinity can vary significantly in estuarine or brackish waters that experience large tidal cycles and high freshwater flows.

Calcium and, to a lesser extent, magnesium are typically the predominant dissolved cations in freshwater. These cations are largely responsible for the characteristic of freshwater referred to as "hardness." The hardness values of most freshwater systems of the world range from about 50 to 90 mg/L of calcium carbonate. A wide range of hardness can be found in the continental United States. Streams, rivers, lakes, and reservoirs in igneous, siliceous, or forested topography may consistently have 20 mg/L of calcium carbonate or less hardness. Hard water streams and lakes may have hardness values ranging from 350 to 380 mg/L of calcium carbonate.

How do animals live in water with different ion concentrations?

The internal fluids of most freshwater animals have higher ion concentrations than the external media. Thus, there is a tendency for water to move continually into the body tissues and ions to move out of the body. Freshwater invertebrates eliminate water through a number of highly specialized excretory structures. Many invertebrates have hard outer bodies (exoskeletons) that reduce the external flow of ions. The majority of freshwater fish drink very little because water moves into their bodies. Despite this physiological challenge, freshwater fish maintain the proper balance of internal ions by active uptake of ions and by renal mechanisms for ion retention.

Most marine invertebrates possess body fluids that have approximately the same ion concentration as that of the seawater in which they live. When a small change in the concentrations of ions in the external medium occurs, there is an equivalent change in the concentration of the internal fluids. In contrast, most marine fish have physiological mechanisms that allow them to live in seawater but maintain the ionic balance of their internal body fluids similar to that of freshwater fish. Marine fish are faced with the problem of losing large quantities of water from their bodies and gaining large quantities of salts into their bodies. Marine fish therefore drink large amounts of water to replace the water they lose, and they excrete the salts they take in primarily via the gills and kidneys.

Why do changes in ionic composition affect aquatic organisms?

Aquatic organisms have developed a number of physiological mechanisms to balance water and ion concentrations in their body fluids. A great deal of metabolic energy is spent by most aquatic animals trying to regulate water and ions. Changes in the concentration or composition of ions in the external medium, particularly over long periods of time, can cause an organism to expend too much energy trying to regulate water and ions. This may result in chronic stress affecting important functions such as growth and reproduction. Sudden changes in ion concentration or composition can result in death.

What common inorganic ions are toxic?

Total dissolved solids (TDS) and conductivity are often used as surrogate measures of the collective concentration of the common ions in freshwater. The correlation between increasing TDS and toxicity is not always caused by the same ions and therefore is not the best predictor of toxicity due to an effluent. Because cations and anions are not present as individual constituents but rather are in combination with other ions, the individual toxicity of a cation or anion may be masked or confounded by the associated anion or cation of the compound. Thus, ion imbalance toxicity must be considered as combinations of ions, with an understanding of the effects of the various ions. In addition, the chronic deficiency of common ions can be as detrimental to aquatic organisms as excessive ions. This is most common in marine systems. For example, manganese deficiency can result in toxicity to the saltwater mysid. Ion deficiency can also occur in freshwater systems where extremely low ionic water (e.g., distilled water) is discharged to the aquatic environment.

For some ions, either their excess or deficiency has been found to be toxic to freshwater and marine organisms. In a recent evaluation of two common invertebrates used in WET testing, the relative acute toxicity (most toxic to least toxic) to the freshwater cladoceran (*Ceriodaphnia dubia*) was

Potassium (K^+) > bicarbonate (HCO_3^-) > magnesium (Mg^{2+}) > chloride (Cl^-) > sulfate (SO_4^{2-}) > bromide (Br^-).

The relative toxicity to the marine mysid (*Americamysis bahia;* formerly *Mysidopsis bahia*) was

Fluoride (F^-) > potassium (K^+) > bicarbonate (HCO_3^-) > calcium (Ca^{2+}) > magnesium (Mg^{2+}) > bromide (Br^-) > sulfate (SO_4^{2-}).

In general, the most toxic ions to freshwater organisms are potassium, bicarbonate, and magnesium. The most toxic ions to marine organisms are potassium and bicarbonate. A number of aquatic toxicologists have studied the toxicity of borax ($Na_2B_4O_7 \cdot 10\ H_2O$) to both freshwater and marine organisms and concluded that it was particularly toxic to marine organisms. The borax unit $B_4O_7^{2-}$ was thought to be an ion in these studies; however, borax is a solid that dissolves in water to form boric acid ($B[OH]_3$) and the borate ion ($B[OH]_4^-$). The dominant form in seawater is boric acid, which is a neutral molecule and not an ion.

What types of effluents may produce ion imbalance problems?

A variety of water treatment processes produce effluent that is ionically imbalanced relative to the molar ratios or concentrations of ions that naturally occur in the water into which the effluent is discharged. The imbalance may be due to an excess or a deficiency of ions. Extremes in ion concentrations in effluents generally arise from one or more of the following:

- Direct addition of chemicals to water (e.g., salt, lime, alum) in production treatment processes
- Change of ion concentrations by chemical or physical manipulation (e.g., pH modification, reverse osmosis, distillation or evaporation)
- Discharge of wastes initially high in ion content (e.g., seawater, co-produced groundwater or mine de-watering, contaminated groundwater remediation)
- Discharges of extremely low ion effluent (e.g., distilled and reverse osmosis permeate waters, remediated petroleum-contaminated groundwater).

How is ion toxicity identified?

A variety of approaches can be used to determine if ion imbalance is responsible for observed toxicity. Initial insight can be obtained by determining the salinity or conductivity of the effluent. If the TDS of a freshwater effluent is above approximately 1340 mg/L, the concentration of dissolved salts can be high enough to adversely affect freshwater organisms. Correlations between organism response and TDS concentrations may indicate that ion imbalance is responsible for WET toxicity. Additional information can be obtained by following USEPA's Phase I Toxicity Identification Evaluation (TIE) protocols. If the results of the Phase I TIE manipulations on the effluent indicate that toxicity cannot be eliminated or significantly reduced by any of the treatment steps, ion imbalance may be responsible for toxicity and should be further evaluated. Further evaluations may include such procedures as the following:

- Using mathematical models to analyze ion concentrations to determine if they approach or exceed those found to be toxic to test organisms
- Using synthetic effluents that mimic the major ions in the effluent under evaluation
- Identifying ion-specific toxicity
- Restoring ionic balance.

What regulatory approaches are available to help resolve the problem?

Consideration of ion toxicity and management of effluent are important because of the ubiquitous presence of effluents with various ion strengths and compositions. The significance of these issues could be minimal if balancing, removal, or treatment of ions was an inexpensive undertaking. Unfortunately, cost-effective waste treatment control options for a facility whose effluent is toxic because of TDS or specific ions are scarce, if available at all. Regulatory solutions to ion imbalance toxicity when no other toxicants are present may include

- modifications to the site-specific exposure through discharge,
- use of dynamic models to predict toxicity in a body of water,
- use of exposure-specific WET toxicity tests, or
- use of alternate mixing zones for TDS or specific ions.

These solutions could help avoid costly or energy-consuming treatment options that are often ineffective treatment controls for the removal or addition of ions.

Additional regulatory or technical solutions may be possible if ions are identified as the only responsible toxicant. Some realistic options are

- consideration of waste reduction and pretreatment options,
- use of appropriate or alternative mixing zones and alternative dilution models (e.g., dynamic modeling),
- co-mingling with receiving waters or other plant process waters prior to final discharge,
- development of site-specific ions or TDS limits, and
- use of bioassessment techniques to assess instream effects.

Where is there more information about ion imbalance?

While there are a number of excellent sources on ion imbalance, the following resources will help in further understanding the issue:

[API] American Petroleum Institute. 1999. The toxicity of common ions to freshwater and marine organisms. Washington DC, USA: API. API Publication 4666. 75 p.

Douglas WS, Grasso SS, Hutton DG, Schroeder KR. 1996. Ionic imbalance as a source of toxicity in an estuarine effluent. *Arch Environ Contam Toxicol* 31:426-432.

[FDEP] Florida Department of Environmental Protection. 1995. Protocols for determining major seawater ion toxicity in membrane technology water treatment concentrate [unpublished report]. Tallahassee FL, USA: FDEP. 26 p. Available from: http://www.dep.state.fl.us/labs/library/methods.htm. Accessed 11 Dec 2003.

Goodfellow WL, Ausley LW, Burton DT, Denton DL, Dorn PB, Grothe DR, Heber MA, Norberg-King TJ, Rodgers JH. 2000. Major ion toxicity in effluents: A review with permitting recommendations. *Environ Toxicol Chem* 19:175-182.

Mount DI, Gulley DD. 1992. Development of a salinity/toxicity relationship to predict acute toxicity of saline waters to freshwater organisms. Chicago IL, USA: Gas Research Institute (GRI). GRI-92-0301. 158 p.

Mount DI, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna*, and fathead minnows (*Pimephales promelas*). *Environ Toxicol Chem* 16:2009-2019.

[SETAC] Society of Environmental Toxicology and Chemistry. 2004. Technical issue paper: Whole effluent toxicity testing. Pensacola FL, USA: SETAC. 4 p.

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Society of Environmental Toxicology and Chemistry

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists—biologists, chemists, toxicologists—and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on the growth in membership, annual meeting attendance, and publications, the forum was needed.

Like many other professional societies, SETAC publishes an esteemed scientific journal (*Environmental Toxicology & Chemistry*) and convenes an annual meeting replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of many other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Sister organizations in Europe (1989), Asia/Pacific (1997), and Latin America (1999) have been formed, and the nonprofit SETAC Foundation for Environmental Education was founded in North America in 1990. International acceptance of the SETAC model continues with widespread interest in Russia and Africa.

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