American Petroleum Institute

STEP
Strategies for Today's Environmental Partnership

THE TOXICITY OF COMMON IONS TO FRESHWATER AND MARINE ORGANISMS

HEALTH AND ENVIRONMENTAL SCIENCES DEPARTMENT
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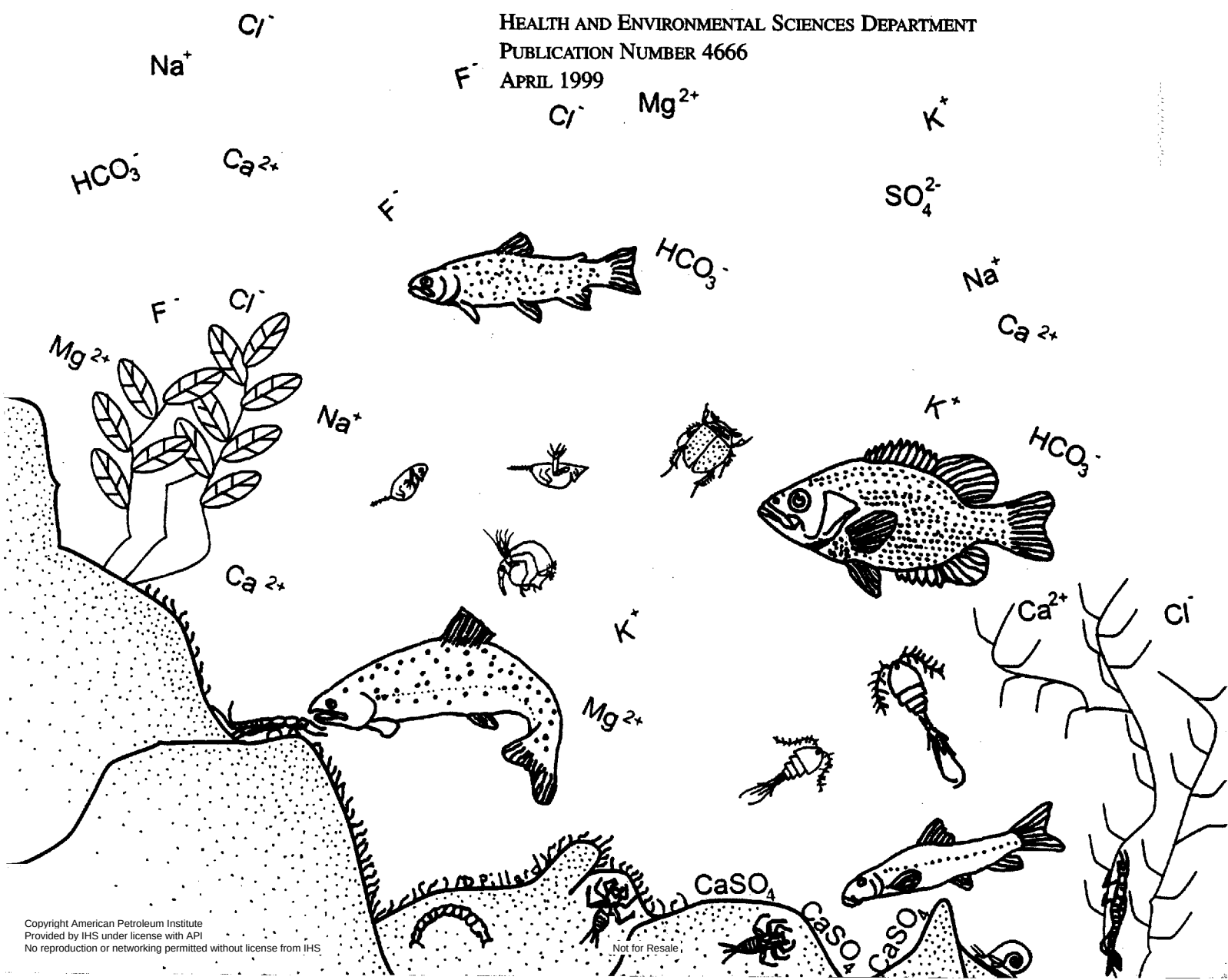

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STEP Strategies for Today's Environmental Partnership

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- To promote these principles and practices by sharing experiences and offering assistance to others who produce, handle, use, transport or dispose of similar raw materials, petroleum products and wastes.

The Toxicity of Common Ions to Freshwater and Marine Organisms

Health and Environmental Sciences Department

API PUBLICATION NUMBER 4666

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ABSTRACT

Whole effluent toxicity (WET) tests have become a common tool in the evaluation of effluent for discharge acceptability. The majority of toxicants identified in effluents are either inorganic trace metals (e.g., cadmium, copper, etc.) or organic compounds (e.g., diazinon, surfactants). Others, however, are inorganic ions that nearly always are present in most aquatic systems and, in most cases, are present at nontoxic concentrations. These ions include bicarbonate, calcium, chloride, magnesium, potassium, sodium, sulfate, and others. Recent investigations have indicated that, in certain effluents, deficiencies or excesses of these "common" ions can cause significant acute or chronic toxicity in WET tests. This report presents the results of a review of toxicological and physiological data on inorganic ions that have been implicated in causing significant toxicity.

The scientific literature was searched for freshwater and marine toxicity data on bicarbonate (HCO_3^-), borate ($B_4O_7^{2-}$), bromide (Br^-), calcium (Ca^{2+}), chloride (Cl^-), fluoride (F^-), magnesium (Mg^{2+}), potassium (K^+), strontium (Sr^{2+}), and sulfate (SO_4^{2-}). A review also was completed on the roles that several common ions play in normal physiological functions and the impacts of abnormal levels of these ions. All states and EPA regions were surveyed to determine what, if any, guidelines currently are in place to address the question of common ion toxicity.

The impact of aberrant levels of ions differs markedly with the ion in question as well as the organism being tested. Some ions, Ca^{2+} and K^+ for example, cause significant acute toxicity when they are deficient in the exposure media, while other ions appear to have demonstrable effects only at excess levels. Whole effluent toxicity due to the common ions can pose a problem for some dischargers who must identify and/or eliminate toxicity in their effluent. This problem arises because standard Toxicity Identification Evaluation (TIE) manipulations often are ineffective in separating ion toxicity from other potential candidates. However, techniques such as mock effluent studies and computer models can be used in conjunction with traditional TIE methods to provide definitive identification of ion toxicity.

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EXECUTIVE SUMMARY

This report presents and discusses the results of a review of the scientific literature pertaining to the toxicity of common inorganic ions (e.g., calcium, potassium, chloride) to freshwater and marine organisms. Also examined were 1) important physiological functions of the ions, 2) how these ions can affect whole effluent toxicity (WET) tests, and 3) what methods may be used to identify ion toxicity and discern it from toxicity due to other chemicals.

WET testing has become a common parameter evaluated as part of National Pollutant Discharge Elimination System (NPDES) permits. The use of WET tests brings such permits closer to the fulfillment of one of the major goals of the Clean Water Act, to prevent the discharge of toxic materials in toxic amounts. One of the problems that has arisen in the WET testing program has been associated with the confounding effects of ions typically associated with total dissolved solids (TDS). It is well known that elevated TDS or salinity will cause adverse effects to some species, and the salinity of an effluent (or of the receiving environment) will often dictate the test organisms used for WET testing. However, more recent data have shown that the individual ions that comprise TDS may have more influence on toxicity than can be estimated through gross measurements such as TDS, salinity, conductance, or even chloride concentration. In addition, toxicity caused by TDS ions often is difficult to identify through traditional techniques and thus may be difficult to separate from toxicity due to other materials.

Several literature databases were searched to obtain information for this review, including AQUIRE, Enviroline, WATERNET, and others. Additional literature was collected through searches of specific journals, such as *Environmental Toxicology and Chemistry*, and by cross-referencing from in-hand articles. The physiology section of this report provides a review of how essential ions interact and affect functions in living organisms, primarily animals. The most current information is reviewed and discussed, recognizing that ongoing investigations into physiological aspects of ion toxicity may yield new information. Information on Toxicity Identification Evaluations (TIEs) was obtained through recent literature reviews and discussions with laboratory technicians and scientists currently involved in TIE studies where ion toxicity is suspected.

ION TOXICITY

Bicarbonate (HCO_3^-), borate ($B_4O_7^{2-}$), bromide (Br^-), calcium (Ca^{2+}), chloride (Cl^-), fluoride (F^-), magnesium (Mg^{2+}), potassium (K^+), strontium (Sr^{2+}), and sulfate (SO_4^{2-}) toxicity were evaluated. These ions have demonstrated substantially different toxicity to freshwater and marine organisms. There are also differences in sensitivity to a single ion among species. For example, to *Ceriodaphnia dubia* and *Mysidopsis bahia*, two common invertebrate organisms used in WET tests, the approximate relative acute toxicity (as measured by mass concentration) of some ions is:

C. dubia (More Toxic) $K^+ > HCO_3^- > Mg^{2+} > Cl^- > SO_4^{2-} > Br^-$ (Less Toxic)

M. bahia (More Toxic) $F^- > B_4O_7^{2-} > K^+ > HCO_3^- > Ca^{2+} > Mg^{2+} > Br^- > SO_4^{2-}$ (Less Toxic)

To freshwater organisms, Mg^{2+}, HCO_3^-, and K^+ were the most toxic, generally causing acute toxicity at less than 1,000 mg/L. While Br^- was one of the least acutely toxic ions to freshwater organisms, it had apparent chronic effects at much lower concentrations. To marine test organisms, HCO_3^-, K^+, $B_4O_7^{2-}$, and F^- caused acute toxicity at lower concentrations than the other ions evaluated; Sr^{2+} may also cause toxicity to *Menidia beryllina* at approximately 200 mg/L.

As with many toxicants, the complexity of common ion toxicity is associated with the chemistry of effluents and the interactions of all the chemicals within that effluent. This relationship is especially true in waters of high ionic strength such as those discharged to marine environments. Because some ions may be near saturation and can form strong bonds with other materials, toxicity may be reduced through complexation and precipitation of salts. Toxicity, therefore, cannot always be defined in terms of the concentration of one or more ions, as measured in an analytical laboratory; rather, the chemistry of the whole effluent, including such modifying factors as temperature, atmospheric pressure, carbon dioxide concentration, and pH may be considered. Isolation of the causative toxicant(s) in an effluent may require investigations along several lines in a toxicity identification evaluation. In addition to comparing measured ion concentrations with historic literature, the use of synthetic or "mock" effluents and computer models can prove useful. Even these multiple lines of evidence may prove inconclusive in some cases where toxicity is associated with common ions and other organic or inorganic compounds.

PHYSIOLOGICAL ROLE OF COMMON IONS

Several of the ions reviewed in this report are essential to aquatic organisms in various metabolic activities, as well as to maintain a favorable intra- and extracellular environment in which those activities occur. Calcium, for example, in addition to being critical in building skeletal structures, also contributes significantly to the regulation of membrane permeability and control of the gating of Na^+-fluxes in the nerve membrane, and is also an essential cofactor in blood clotting and for digestion. Because of the importance of Ca^{2+} and other ions to physiological processes, organisms have developed mechanisms for maintaining intra- and extracellular ion concentrations within the favorable ranges that individual species can tolerate. Mechanisms include active excretion or absorption of ions through gills or other structures and adjustments in the permeability of cellular tight junctions.

CONCLUSIONS

Common ions have been found to cause toxicity in effluents from several different sources, including gas and oil production, chemical manufacturing, refining, agriculture, and seawater desalination. In a large number of studies, the concentrations of ions that are likely to cause adverse effects on aquatic organisms have been identified. While most of these studies have addressed acute toxicity, chronic effects have also been investigated and may become increasingly important as the inclusion of short-term chronic studies becomes more commonplace in NPDES permits. Organisms that are commonly used in NPDES WET tests differ in their responses to these ions, with some, such as *Cyprinodon variegatus*, being much more tolerant to low and high ion concentrations than others.

While in many cases toxicity can be associated with specific ions, adverse effects often are difficult to quantify, particularly in high ionic strength solutions, due to the interactions that common ions have with each other and with other organic and inorganic constituents. The identification of ion toxicity, therefore, often involves using not only historical toxicity data but also traditional TIE methods and computer modeling to provide a weight of evidence approach to toxicity identification. Because many of these ions are essential nutrients to aquatic organisms and may normally be present in source and receiving water, it may be appropriate to evaluate the potential impacts of ion toxicity, as found in laboratory studies, in light of the ecology of the receiving environment.

Section 1
INTRODUCTION

It has long been recognized that some chemical constituents, when present in the aquatic environment above certain levels, may be toxic to organisms. Aquatic toxicology can, in fact, be defined as "the qualitative and quantitative study of the adverse or toxic effects of chemicals and other anthropogenic materials or xenobiotics on aquatic organisms" (Rand and Petrocelli, 1985). Typically, any reference to "toxic materials" usually is associated with complex synthetic chemicals or heavy metals. However, common constituents found in aquatic environments can also be toxic to aquatic organisms when present in sufficient quantities. Ions such as potassium, magnesium, and calcium are present naturally in water and are part of a group of elements that are essential to proper organism function. When concentrations of these common ions exceed a certain level or, in the case of some essential ions, are below a certain level, adverse effects can occur.

The issue of ion imbalance in effluents recently has been highlighted in a re-evaluation of EPA's whole effluent toxicity (WET) testing program. Waters with substantially elevated salinity or total dissolved solids (TDS) have been shown to be toxic when ionic constituents are not in the same proportions as in natural saline waters. High-TDS effluents from operations utilizing water conservation have also shown toxicity. Many processes in manufacturing plants result in a high-TDS effluent with disproportionate ionic ratios. Examples of effluent that may have ion imbalances include those from oil and gas production, water conservation or recycled process waters, and caustic/basic treatment processes using $CaCO_3$ neutralization. The process of increasing effluent salinity ("salting-up") to accommodate marine/estuarine organism tolerances also can result in toxicity.

SCOPE OF REVIEW

This review focuses on laboratory data regarding the effects of common cations and anions on both freshwater and marine organisms. While a given water can have a variety of constituents, only a few are considered to be common. The major cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}), and strontium (Sr^{2+}), and the major anions are bicarbonate (HCO_3^{-}), borate ($B_4O_7^{2-}$), bromide (Br^{-}), chloride (Cl^{-}), fluoride (F^{-}), and sulfate (SO_4^{2-}). This document provides a general summary of the results of toxicity studies on ions and explores the physiological effects of those ions on a tissue and cellular basis.

Section 1 describes some of the current regulatory schemes concerned about ion toxicity. Section 2 summarizes the ionic composition of natural waters in the world, both fresh and saline. A review of some of the sources of high TDS waters is provided in Section 3, along with a discussion of Toxicity Identification Methods (TIE) and what techniques are effective in separating toxicity related to common ions from toxicity due to other constituents. Section 4 is a review of the toxicological data for different ions. Section 5 explores the physiological function of ions and the various models of ion regulation that exist in different taxa. A summary is included in Section 6. References, a Glossary, and a Bibliography follow the summary.

Information for this review was gathered in three ways. First, several computer databases were searched for information related to the toxicity of the common ions (listed above) to aquatic organisms. Those databases included AQUIRE, Biosis Previews®, Compendex®, Oceanic Abstracts, Aquatic Science Abstracts, CAB Abstracts, Inside Conferences, Wilson Applied Science and Technology Abstracts, Water Resources Abstracts, WATERNET™, GEOBASE™, IAC Newsletter DB™, Enviroline®, Pollution Abstracts, Environmental Bibliography, and SciSearch®. Second, a manual literature search was conducted to gather information that might not be found in the databases. Finally, there was direct communication with researchers involved in ion toxicity studies.

TOTAL DISSOLVED SOLIDS IN WET TESTS

Recent studies have shown that toxicity in effluents from many different sources can be attributed to major ions. Many of these ions occur naturally in receiving streams and do not pose the bioaccumulative risk that some other toxicants do. Elevated ion levels occur in some industry source waters and WET toxicity may therefore be artifactual and not a true reflection of effluent toxicity resulting from a manufacturing or treatment process. Nevertheless, there are few regulatory guidelines specifically designed to address TDS ion toxicity. Many states have limits on TDS or a few TDS ions (principally Cl^- and SO_4^{2-}). But compliance with existing water quality discharge criteria or state standards does not guarantee that an effluent will not be toxic. In addition, few permits require analysis of a full suite of ions. Measurement of limited parameters such as TDS, Cl^-, and SO_4^{2-} would be insufficient to determine if toxicity were due to an unmeasured single ion.

There is no national policy for addressing TDS toxicity issues. A survey conducted of all state and USEPA regions indicated that many states have not experienced problems with TDS, although unexplained episodes of toxicity might be attributable to TDS. Only three states and two USEPA regions were identified that have established some current or proposed procedural

guidance for dealing with TDS toxicity; those are described in the following sections. Table 1-1 provides a list of individuals who can provide information from states and EPA regions about TDS and toxicity related to TDS. Additional information on the role of TDS and ion imbalance in toxicity testing may be found in Goodfellow *et al.* (In Preparation).

USEPA Regions 9 and 10

USEPA Regions 9 and 10 recognized that TDS ions in effluent can cause toxicity and confound efforts to identify the causative toxicant(s). As a general guide, it is suggested that if conductivity exceeds 3,000 and 6,000 μmhos/cm at the LC_{50} for *Ceriodaphnia dubia* and *Pimephales promelas* (fathead minnow), respectively, then TDS toxicity should be considered (USEPA, 1996). In order to quantify the impacts of TDS, an effluent sample should be thoroughly characterized relative to the ions in the sample. Once this characterization is completed, a computer model (the ***GRI-FW STR***™ program, Tietge *et al.*, 1994) can be used to predict toxicity. Mock effluent tests are also an important part of the confirmation process.

Colorado

The Colorado Department of Public Health and Environment (CDPHE) Water Quality Control Division (Division) has prepared a draft revision of its "Whole Effluent Toxicity Permit Implementation Guidance Document" that specifically addresses TDS as a toxicant. Although this document remains in draft form (as of this writing), permittees can follow the procedures to identify and address toxicity due to TDS ions. The guidelines state that, if a TIE rules out other toxicants, except TDS, then the permittee can provide the Division with 1) effluent analytical chemistry, 2) results of an effluent WET test, and 3) results of a mock effluent WET test. If acute toxicity is of concern, then the Division will use a computer program (the ***GRI-FW STR***™ program, Tietge *et al.*, 1994) to complement existing WET data. If the acute WET test is passed using *Daphnia magna* (which is more tolerant than *C. dubia* to TDS ions), then the permittee may request a permit amendment to change WET test species.

If *D. magna* cannot tolerate the elevated TDS, or if the required test is chronic, permittees may be required to conduct an Aquatic Impairment Study (AIS) of the receiving stream. A CDPHE AIS includes the collection of *in situ* biological, chemical, and physical data and incorporates some of the methods described in the USEPA's Rapid Bioassessment Protocols (Plafkin *et al.*, 1989). Following the AIS, WET tests may be modified to switch species or remove TDS (if possible). Additional mitigation measures also may be needed.

Table 1-1. State and Regional Contacts Regarding TDS Toxicity Questions.

State or Region	Name	Phone	Affiliation
AK	Madonna Narvaez	(206) 553-1774	USEPA Regional Office (Region 10) in Seattle
AL	Marion Bertolotti	(334) 260-2748	Alabama Department of Environmental Management
AR	Bernie Finch	(501) 682-0744	Arkansas Department of Pollution Control and Ecology, NPDES Branch
	Nat Nehus	(501) 682-0663	Biomonitoring Branch
AZ	Linda Taunt	(602) 207-4665	Arizona Department of Environmental Quality, Water Permits
CA	Victor de Vlaming	(916) 657-0795	California State Water Resource Control Board, Division of Water Quality
Reg 1-	Bruce Gwynne	(707) 576-2661	California Regional Water Quality Control Board, North Coast Region
	William Rodriguez	(707) 576-2863	California Regional Water Quality Control Board, North Coast Region
	Tuck Vath	(707) 576-2699	California Regional Water Quality Control Board, North Coast Region
	Peter Otis	(707) 576-2662	California Regional Water Quality Control Board, North Coast Region
Reg 2-	Lila Tang	(510) 622-2300	California Regional Water Quality Control Board, San Francisco Bay Region
Reg 3-	Brad Hagemann	(805) 549-3697	California Regional Water Quality Control Board, Central Coast Region
Reg 4-	Dennis Dasker	(323) 266-7518	California Regional Water Quality Control Board, Los Angeles Region
Reg 5-	Valerie Connor	(916) 255-3111	California Regional Water Quality Control Board, Central Valley Region
Reg 6-	Bruce Warden	(530) 542-5416	California Regional Water Quality Control Board, Lahontan Region
Reg 7-	Orlando Gonzalez	(760) 776-8962	California Regional Water Quality Control Board, Colorado Basin
Reg 8-	Hope Smythe	(909) 782-4493	California Regional Water Quality Control Board, Santa Anna Region
Reg 9-	Bruce Posthumus	(619) 467-2964	California Regional Water Quality Control Board, San Diego Region
CO	Robert McConnell	(303) 692-3578	Colorado Department of Public Health and Environment
CT	Lee Dunbar	(860) 424-3731	Connecticut Department of Environmental Protection
DE	Rick Greene	(302) 739-4590	Delaware Department of Environmental Quality, Office of Water Resources
FL	Steve Wolfe	(850) 921-9830	Florida Department of Environmental Protection, Biology Section

Table 1-1. Continued.

State or Region	Name	Phone	Affiliation
GA	Susan Salter	(404) 362-2680	Georgia Environmental Protection Division
HI	Alec Wong	(808) 586-4309	Hawaii Department of Health, Environmental Management Division, Clean Water Branch
IA	Charles Furrey Steve Williams	(515) 281-4067 (515) 281-8884	Iowa Department of Natural Resources, Municipal Permits Industrial Permits
ID	Rick Huddleston	(208) 373-0502	Idaho Department of Environmental Quality
IL	Bob Mosher Mike Henebry	(217) 782-3362	Illinois Environmental Protection Agency, Planning Division
IN	Catherine Hess Steve Roush	(317) 232-8704 (317) 232-8706	Indiana Department of Environmental Management, Office of Water
KS	Mike Tate	(785) 296-5504	Kansas Department of Health and Environment, Division of Environment, Bureau of Water
KY	Charlie Roth	(502) 564-3410	Kentucky Department of Environmental Protection, Division of Water
LA	Ronnie Bean	(504) 765-2779	Louisiana Department of Environmental Quality
MA	Arthur Johnson	(508) 767-2873	Massachusetts Department of Environmental Protection, Office of Watershed Management
MD	Melvin Knott	(410) 631-3906	Maryland Department of the Environment
ME	Dennis Merrill	(207) 287-7788	Maine Department of Environmental Protection, Bureau of Land and Water Quality
MI	Phil Diamond	(517) 323-0880 F (517) 323-9084	Michigan Department of Environmental Quality, Great Lakes and Environmental Section, Surface Water Quality Division,
MN	Gary Kimball	(612) 297-8221	Minnesota Pollution Control Agency
MO	Ranall Crawford	(573) 526-3353	Missouri Department of Natural Resources
MS	Phil Bass	(601) 961-5143	Mississippi Department of Environmental Quality, Office of Pollution Control
MT	Mike Pasichnyk	(406) 444-5326	Montana Department of Environmental Quality, Water Protection Bureau
NC	Matt Matthews	(919) 733 2136	North Carolina Department of Environment and Natural Resources
ND	Gary Bracht	(701) 328-5227	North Dakota Health Department, Division of Water Quality
NE	Ron Asch	(402) 471-2188	Nebraska Department of Environmental Quality
NH	Jeff Andrews	(603) 271-3503	New Hampshire Department of Environmental Services, Surface Water Quality Bureau
NJ	Betty Boros-Russo	(609) 292-3950	New Jersey Department of Environmental Protection
NM	Glenn Saumes	(505) 827-0596	New Mexico Environment Department, Surface Water Quality Bureau

Table 1-1. Continued.

State or Region	Name	Phone	Affiliation
NV	Jim Williams	(702) 687-4670 x 3140	Nevada Division of Environmental Protection
NY	Joe Demera Ed Kuzia	(518) 457-0657 (518) 457-5320	New York Department of Environmental Conservation, Division of Water
OH	Eric Nygaard	(614) 644-2024	Ohio Environmental Protection Agency, Division of Surface Water
OK	Mike Moe	(405) 702-8100	Oklahoma Department of Environmental Quality, Water Quality Division
OR	Judy Johndohl	(503) 229-6896	Oregon Department of Environmental Quality
PA	R. La Roue Wyrick	(717) 787-8184	Pennsylvania Department of Environmental Protection, Bureau of Water Quality Protection
RI	Bob Richardson	(401) 222-7400 x 7240	Rhode Island Department of Environmental Management, Water Resources
SC	Andy Yasinsac Vernon Beaty	(803) 734-5246 (803) 734-5396	South Carolina Department of Health and Environmental Control, Industrial Wastewater Permitting Section Aquatic Biology Section
SD	Stacy Reed	(605) 773-3351	South Dakota Department of Environment and Natural Resources
TN	Saya Ann Qualls	(615) 532-0652	Tennessee Department of Environment and Conservation
TX	Faith Hambleton	(512) 239-4600	Texas Natural Resources Conservation Commission, Research and Environmental Assessment Division
UT	Don Hilden Mike Herkimer	(801) 538-6146	Utah Department of Environmental Quality, Division of Water Quality
VA	Deborah DeBiasi	(804) 698-4028	Virginia Department of Environmental Quality, Permits Section
VT	Doug Burnham	(802) 241-3777	Vermont Department of Environmental Conservation, Water Quality Division
WA	Randall Marshall	(360) 407-6445	Washington Department of Ecology
WI	Diane Drinkman	(608) 264-8950	Wisconsin Department of Natural Resources
WV	Dave Montali	(304) 558-4086	West Virginia Department of Environmental Protection, Office of Water Resources
WY	Leah Krafft	(307) 777-7093	Wyoming Department of Environmental Protection
EPA Region 1	Bill Beckwirth	(617) 918-1544	Environmental Protection Agency, Region 1
EPA Region 2	Phil Sweeney	(212) 637-3873	Environmental Protection Agency, Region 2
EPA Region 3	Kristine Matzko	(215) 814-5719	Environmental Protection Agency, Region 3
EPA Region 4	Marshall Hyatt	(404) 562-9304	Environmental Protection Agency, Region 4
EPA Region 5	Steve Jann	(312) 886-2446	Environmental Protection Agency, Region 5

Table 1-1. Concluded.

State or Region	Name	Phone	Affiliation
EPA Region 6	Phil Jennings	(214) 665-7538	Environmental Protection Agency, Region 6
EPA Region 7	John Dunn	(913) 551-7594	Environmental Protection Agency, Region 7
EPA Region 8	Debbie Thomas Glenn Rodriguez	(303) 312-6373 (303) 312-6832	Environmental Protection Agency, Region 8
EPA Region 9	Debra Denton	(415) 744-1919	Environmental Protection Agency, Region 9
EPA Region 10	Madonna Narvaez	(206) 553-1774	Environmental Protection Agency, Region 10

Note: The telephone numbers and affiliations in this table were determined to the best of the authors' abilities at the time of publication. Errors in this information may exist.

Florida

Some drinking water facilities in Florida incorporate membrane technology in the production of drinking water. The membrane concentrate (reject water) from these facilities typically has common seawater ions present at proportions dissimilar to actual seawater. Because many of the concentrates failed toxicity tests under the National Pollutant Discharge Elimination System (NPDES) program, the Florida Department of Environmental Protection (FDEP) developed a series of protocols designed to address major seawater ion toxicity in membrane effluents (FDEP, 1995). The protocols are a result of studies conducted from December 1994 through June 1995. The protocols consist of nine tests, including:

Test 1 - Initial test on unaltered concentrate.

If Test 1 indicates the concentrate is nontoxic, no additional tests are needed.

Test 2 - Baseline test on unaltered concentrate.

If the concentrate in Test 2 is not toxic, no further tests are necessary. This might occur if the toxicant from Test 1 was labile and degraded during the period (typically < 24 hours) between Tests 1 and 2.

Test 3 - Mock concentrate tests.

Test 4 - Ion-adjusted concentrate.

Chemical salts are added to the concentrate sample to balance the major seawater ions to seawater proportions. The balancing is controlled by the concentrate ion that is in the highest proportion relative to seawater.

Test 4a - Ion-adjusted concentrate tests where major seawater ions are incompletely adjusted.

Major seawater ions present in concentrations below that of 35‰ (salinity symbol; equivalent to g/L or parts per thousand of dissolved solids in laboratory-prepared artificial seawater) are adjusted up to 35‰ seawater concentrations. Major seawater ions present in concentrations greater than that of 35‰ seawater are left unaltered.

Test 4b - Ion-adjusted concentrate tests where major seawater ions are completely adjusted then diluted to 35‰.

After adjustment of all ions to seawater proportions, the concentrate is then diluted with deionized water to 35‰ salinity.

Test 5 - Ion-adjusted concentrate (diluted with mock concentrate).

This is the same as Test 4, except the diluent is mock, ion-adjusted concentrate. Therefore, as test concentrations are prepared, the major seawater ions will remain the same in all concentrations; however, the concentration of any other toxicant(s) will change.

Test 5a - Ion-adjusted concentrate tests where major seawater ions are incompletely adjusted (diluted with mock concentrate).

This is the same as Test 4a, except the diluent is mock, ion-adjusted concentrate.

Test 5b - Ion-adjusted concentrate tests where major seawater ions are completely adjusted then diluted to 35‰ (diluted with mock concentrate).

This is the same as Test 4b, except the diluent is mock, ion-adjusted concentrate.

Texas

If a permittee can demonstrate that effluent toxicity is caused by dissolved salts, then the permittee may be exempt from the Total Toxicity provisions of the Texas Surface Water Quality Standards (TSWQS). The exemption applies to 1) 100% end-of-pipe acute toxicity (24-hour acutes) and 2) 48-hour and chronic tests when dissolved salts originate in a permittee's source water. To demonstrate that effluent toxicity (24-hour acute tests) is due to TDS ions, the Texas Natural Resources Conservation Commission (TNRCC) requires one set of TIE/TRE characterization tests, including an ion-exchange procedure. If the TIE/TRE tests indicate TDS ions are a cause of toxicity, the permittee must then prove that these ions are the primary cause of acute toxicity, using a combination of the following techniques (TNRCC, 1995):

1) toxicity tests using a more TDS-tolerant species (e.g., *D. magna* vs. *C. dubia*),
2) side-by-side toxicity tests of the effluent and a mock effluent,
3) analytical verification of major ion concentrations,
4) computer models to predict acute toxicity of saline waters, or
5) effluent toxicity tests using salts that are formulated to correct ion imbalance.

If these or other acceptable techniques fail to confirm toxicity due to common TDS ions, the permittee must continue with the TIE/TRE process to address toxicity. If the techniques do show that the primary cause of toxicity is TDS ions, the TNRCC will evaluate, or require the permittee to evaluate, the use of an alternative test species or modified test protocol.

If a permittee believes that effluent toxicity in a 48-hour acute or chronic test is due to dissolved salts, then a permittee may use the same techniques to confirm TDS as the cause of toxicity. If TDS is not coming from source water, the permittee may conduct a biological study to evaluate instream impacts. The evaluation should follow USEPA's Rapid Bioassessment Protocols (Plafkin *et al.*, 1989).

The *in situ* evaluation of aquatic communities via impairment studies can be important because laboratory WET caused by TDS ions does not necessarily reflect adverse impacts in receiving waters. Because of the rapid dilution that can occur in receiving water bodies, ion imbalances may be eliminated quickly, although the ratio of effluent to receiving stream (Instream Waste Concentration, IWC) must be considered. Laboratory manipulation of effluent also can affect ion concentrations and thus result in artifactual toxicity, which can complicate efforts to identify real effluent toxicity (Douglas *et al.*, 1996).

In summary, toxicity in WET tests due to TDS ions has proven to be a concern for some effluents from certain industries. Ion toxicity also is something that can appear occasionally in many effluents, including municipal discharges. Although adverse effects to laboratory test organisms due to TDS are a concern and must be addressed, there are considerations that should be taken into account both in the potential for ecological impacts as well as in the identification of toxicants. Many of these TDS ions are essential for long- and short-term survival and general health. Rapid dilution with receiving waters can often correct ion imbalances quickly and, although organisms certainly accumulate many of these ions (e.g., Ca^{2+} in skeletal structures), most species have also evolved elaborate mechanisms for transporting and storing common TDS ions. Therefore, they generally do not bioaccumulate in the same, potentially deleterious, manner as other chemicals. The following sections detail many of these issues.

Section 2
IONIC COMPOSITION OF WATER

SALINITY

Salinity originally was intended to be a measure of the mass of dissolved salts in a given volume of solution. An accurate measure of the salinity of a natural water would, therefore, require a complete analysis of all ions in solution, which would be time-consuming, expensive, and ultimately impractical. It became apparent to early researchers that the ratios of ions in seawater were very constant. Therefore, measurement of a single major parameter would allow calculation of the remaining ion concentrations. Chloride was chosen early on as this single parameter. However, the measurement of chloride, though not difficult, included the addition of $AgNO_3$, which also precipitates bromide (Br^-) and iodide (I^-) (Pytkowicz, 1983). Therefore, chlorinity was introduced as a chloride equivalent and is currently defined as the weight of silver needed to precipitate all of the Cl^-, Br^-, and I^- in 0.32867 kg of seawater (Pytkowicz, 1983).

Although chlorinity is still used, salinity is usually considered a unitless measure in which a physical property of a solution (conductivity, density, refractive index, or sound speed) is used to represent salinity (APHA, 1989). Typically, conductivity (presented as micromhos per centimeter [μmhos/cm] or millisiemens per meter [mS/m]) is used as a measure of salinity, with a conductivity ratio (*R*), representing the ratio between the conductivity of the seawater sample being tested and the conductivity of a standard KCl solution (Brown *et al.*, 1995). The resulting number is unitless, although sometimes referred to as *practical salinity units* (p.s.u.).

While there are difficulties in accurately quantifying the salinity of natural waters, it is relatively easy to determine salinity as a mass measurement in solutions prepared in the laboratory. To prepare the solutions, reagent-grade compounds are added to deionized water in known amounts. With careful measurements, nominal concentrations can be quite accurate, and samples can be analyzed to verify concentrations. In these artificial solutions, therefore, salinity can be accurately described as mg/L of solutes, although more traditionally salinity is referred to as g/L (or parts per thousand [ppt]). In this document salinity will generally be considered a true measure of the concentration of dissolved material in solution, with the units being g/L or ppt (often designated by the symbol ‰). A salinity value not accompanied by units should be considered a unitless value determined indirectly through the measurement of some physical property of the water (such as refractive index), although somewhat representative of the concentration of dissolved materials.

Salinity is often considered roughly equivalent to the TDS of a solution, TDS being the material remaining in a sample of water after it is passed through a membrane filter (e.g., 1.2 μm glass fiber) and dried at 180°C (APHA, 1989). TDS, however, is generally reported in mg/L rather than g/L. Although TDS is not always equal to salinity, because most studies use laboratory-prepared solutions, TDS will be considered roughly equivalent to salinity, with the exception of the units in which each term is presented (i.e., 100,000 mg/L TDS = 100‰ salinity). Other terms that are typically used in toxicological discussions are defined in the Glossary.

IONS IN FRESHWATER

There is a great deal of variation in the ionic composition of the freshwater systems of the world. The salinity of a water body depends upon the geological and biological composition of the watershed, atmospheric sources of ions, precipitation, evaporation, and exchange with sediments within the water body. The average salinity of the world's river water is 120 mg/L (Wetzel, 1983), however, the actual ion concentrations in individual lotic systems vary with location and time of year. Table 2-1 lists the concentrations of some common ions from several rivers from around the world. Sodium concentration, for example, ranges from 2.5 mg/L in the Wisconsin to over 800 mg/L in the Powder (after addition of oil-field produced water). Chemistry will fluctuate with the seasons as patterns of precipitation, sunshine, vegetation, etc. change. Spring snowmelt, for example, often significantly alters water chemistry via dilution and transport of materials accumulated over the winter. Manmade structures, such as dams, also affect downstream water chemistry through deposition of certain minerals (e.g., silica). This effect, in turn, can have dramatic impacts on biological communities (e.g., diatoms). Lake chemistry is often more stable than that of rivers, although seasonal changes and watershed activities can impact lake chemistry as well.

Freshwater bodies are greatly influenced by precipitation and the materials provided from the watershed by the resulting runoff. Precipitation contains dissolved constituents due to 1) droplet formation and 2) particles flushed from the atmosphere by the physical and chemical action of precipitation. Rain and snow droplets form around nuclei, which are primarily soil and dust particles, combustion products, and sea salts (Berner and Berner, 1987). The nuclei may contain any number of ions, depending upon their source. Ions such as Ca^{2+}, Mg^{2+}, and K^{+} originate commonly from soil dust, but also are generated anthropogenically and via sea salt aerosols. The ocean can contribute significantly to precipitation-forming nuclei, although the relative importance of the ocean as a source of ions in inland precipitation varies with distance from the coast (Berner and Berner, 1987).

Table 2-1. Major Dissolved Ion Concentrations in Selected Rivers of the World.

River	Concentration (mg/L)									Reference
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	SO_4^{2-}	HCO_3^{-}	SiO_2	TDS	
Colorado	83	24	95	5.0	82	270	135	9.3	703	Meybeck, 1979
Yukon	31	5.5	2.7	1.4	0.7	22	104	6.4	174	Meybeck, 1979
Mississippi[a]	39	10.7	17	2.8	19.3	50.3	118	7.6	265	Meybeck, 1979
Rio Grande	109	24	117	6.7	171	238	183	30	881	Livingstone, 1963
Clark Fork (MT)[b]	65.7	14.1	15.4	4.0	6	79.5	185	NM	298	ENSR, 1996
Powder (WY)[c]	146	60	176	5	78	275	224	NM	NM	Boelter *et al.*, 1992
Powder (WY)[c,d]	49	36	801	9	689	228	568	NM	NM	Boelter *et al.*, 1992
San Joaquin (CA)	88	40	236	NM	253	316	215	NM	1160	Saiki *et al.*, 1992
Pecos (NM)	394	93	333[e]	333[e]	538	1150	157	17	2610	Hem, 1985
Wisconsin (WI)	18	8.5	2.5	2.4	1.5	9.5	90	42	106	Hem, 1985
Nile	25	7.0	17	4.0	7.7	9	134	21	225	Meybeck, 1979
Ganges	24.5	5.0	4.9	3.1	3.4	8.5	105	12.8	167	Meybeck, 1979

[a] Data from 1965 to 1967.
[b] Mean of data from four seasons, 1994-1995.
[c] Mean of high-, medium-, and low-flow concentrations in 1988.
[d] Downstream of confluence with tributary containing oil-field produced water.
[e] Combined sodium and potassium measurement.

TDS in rainwater averages approximately 5 mg/L, while TDS in river water is approximately 100 mg/L. While evaporation tends to concentrate rainwater, much of this increase in TDS is due to contributions from watershed soils and rocks during weathering. Ca^{2+} and HCO_3^- are the dominant ions in the world's rivers, and are primarily the product of limestone weathering (Berner and Berner, 1987). The relative contribution of other ions varies with location. In Australia, for example, Ca^{2+} concentrations tend to be lower than Na^+ and Mg^{2+}, while in other parts of the world, Ca^{2+} tends to be higher (Williams and Wan, 1972).

IONS IN SALTWATER

Unlike freshwater, seawater tends to be consistent in its salinity and ionic composition, regardless of location on the planet. The surface water salinity of the open oceans is within the range of 33 to 37‰, although the average salinity is approximately 35‰ (Brown *et al.*, 1995). Na^+ and Cl^- are the most abundant ions in seawater, making up approximately 11 and 19 g/L, respectively (Table 2-2). However, in estuarine areas that experience significant tidal influence as well as freshwater input, salinity can decrease substantially. Brackish water found in

estuaries generally has a salinity of less than 25, although tides and river discharge rate play an important role in the actual salinity at any particular time.

Table 2-2. Average Concentrations of the Major Ions in Seawater.

Ion	Conc. (mg/L)	Molarity (moles/L)
Cl^-	18,980	0.535
SO_4^{2-}	2,649	0.028
HCO_3^-	140	2.3×10^{-3}
Br^-	65	8.1×10^{-4}
$H_2BO_3^-$	26	4.3×10^{-4}
F^-	1	5.3×10^{-5}
Na^+	10,556	0.459
Mg^{2+}	1,272	0.052
Ca^{2+}	400	0.010
K^+	380	9.7×10^{-3}
Sr^{2+}	13	1.5×10^{-4}
Total Salinity	34.482‰	

Source: Brown *et al.* (1995)
Note: HCO_3^- includes carbonate, CO_3^{2-}

At the extreme end of the salinity spectrum are inland saline lakes containing hypersaline water with a salinity in excess of approximately 40‰. Typically, these saline lakes exist in relatively arid regions and are closed basins, having no outflow. Ions, accumulated from the drainage basin, are therefore trapped in the water and sediments (due to the precipitation of minerals). Because of the accumulation of salts in saline lakes, a characteristic feature is the presence of relatively high concentrations of some unusual minerals, such as glauberite [Na_2Ca-$(SO_4)_2$], trona [$Na_2(CO_3)$•$Na(HCO_3)$•$2H_2O$], pirssonite [Na_2Ca-$(CO_3)_2$•$2H_2O$], and sepiolite [$Mg_4(Si_2O_5)_3$-$(OH)_2$•$6H_2O$] (Berner and Berner, 1987). The only loss of ions occurs through wind deflation during periods of drought and exposure of the sediments (Wetzel, 1983). The Great Salt Lake in the United States and the Dead Sea in Israel have salinities near 200‰.

Section 3
ION IMBALANCE IN EFFLUENTS

There are several industries that produce effluent that is ionically imbalanced, such that the ratios or concentrations of ions deviate significantly from what is typical in either freshwater or seawater, depending upon where the effluent will be discharged. This imbalance may be due to an excess or deficiency of ions, or to an overall imbalance of the ionic composition. Ionically-imbalanced effluents may originate as a result of 1) high-salinity source water, 2) addition of salts during a treatment process in a production stream, or 3) manipulation of the water via a chemical or physical process (e.g., evaporation). Effluents from different sources may all exhibit toxicity associated with ion imbalance, yet the processes that formed the effluents, and the ion composition of the effluents, can be vastly different. Subsequently, the methods required to ameliorate the toxicity also may be markedly dissimilar.

The purpose of this section is to describe some of the more common sources of effluents that exhibit ion-related toxicity and to identify procedures and techniques developed by regulatory agencies to address ion toxicity. Also included is a discussion of the application of Toxicity Identification Evaluation (TIE) methods that can be employed to help isolate ion toxicity.

SOURCES AND CHARACTERISTICS OF HIGH TDS WATERS

Produced Water

The production of oil and gas typically results in the concurrent production of water that shares the pore space in reservoir rocks. More often than not, the water that is produced along with the hydrocarbons tends to be saline, although the salinity varies regionally. The oil and gas industry produces approximately 14 billion barrels of saline water annually, the majority of which is associated with the oil industry (Daly *et al.*, 1995). The TDS of the produced water ranges from relatively fresh to several times that of seawater. TDS of 100,000 mg/L are not uncommon and some effluents may reach 200,000 mg/L or more (Tibbetts *et al.*, 1992). Typically, the predominant cation in produced water is Na^+ and the predominant anion is Cl^-. However, produced waters can vary in composition depending upon the type of production operation, geologic source of the water, and the treatment of the water once it is brought to the surface. For example, HCO_3^- (>9,000 mg/L), rather than Cl^-, was reported as the dominant anion in coalbed methane-produced water from Colorado (Simmons, 1992). In the United States some of the more saline gas production-related waters are obtained at operations in East Texas and the Arkansas/Louisiana area, with TDS concentrations in the range of 150,000 mg/L (Daly *et al.*, 1995). Produced water from the Rocky Mountain and Appalachian regions, on the other hand, tend to have low TDS concentrations, in some cases less than 5,000 mg/L.

Reverse Osmosis Membrane (Desalination Water)

Ever-increasing human population densities have strained freshwater drinking supplies in many areas. In coastal areas, infiltration of saline water into formerly freshwater supply wells has further reduced available drinking water. In response to the need for drinking water, desalination plants have been developed in coastal areas which use reverse-osmosis (RO) and membrane technology to reduce TDS in water to a level that is sufficient for drinking water. In the United States these plants are especially predominant in Florida. The concentrate (or "reject" water) from desalination plants typically contains elevated levels of common ions that may be in different proportions than are found in natural seawater (FDEP, 1995). Generally, the salinity of the membrane concentrates is lower than that of seawater.

Hydrostatic Water

State and federal laws require natural gas companies to maintain the integrity of transport pipelines. The primary purpose of this maintenance is to ensure public safety by preventing ruptures or failure of the pipelines. The integrity of pipelines is usually verified with a hydrostatic test of the pipeline using water. The test is performed by sealing the pipe and providing a fill location for water as well as air venting locations. Water is pumped into the pipe and the pressure is slowly increased until the desired pressure is achieved and then held for a predetermined time, typically eight hours (GRI, 1989). When the test is complete, the water is released and frequently is discharged to surface waters. State or federal regulations may require an NPDES discharge permit before the hydrostatic test water can be released.

The constituents of hydrostatic test water will vary with several factors, including the age of the pipe, composition of material transported, and test source water. Hydrostatic test waters may contain elevated levels of oil and grease as well as some metals that are flushed out of the pipe. Test waters can also contain elevated levels of TDS. Concentrations of TDS are highly variable and can range from under 1,000 to over 13,000 mg/L (GRI, 1989). The concentration of TDS in hydrostatic discharge water is controlled primarily by the salinity of the source water; for example, freshwater would produce a low-TDS effluent.

Agricultural Irrigation Drainwater

Agriculture in arid regions of the western United States is highly dependent upon intensive irrigation to sustain production. Evaporation and sparse rainfall often result in the concentration of ions from mineralized groundwater. Saiki *et al.* (1992) reported TDS concentrations in excess of 20,000 mg/L in tile drainwater collected from the Westlands Water District in Fresno County, California. In the same sample the SO_4^{2-} concentration was 13,300 mg/L and the Cl^- concentration was 1,240 mg/L (geometric means). Ingersoll *et al.* (1992) measured salinity of

23 g/L (23,000 mg TDS/L), and SO_4^{2-} and Cl^- concentrations of 2,660 and 11,200 mg/L, respectively, from the Stillwater Wildlife Management Area in Nevada, which receives irrigation drain water. Such high concentrations of salts can have adverse effects on organisms living in the aquatic systems that receive and accumulate irrigation waters.

Mining/Metals Industry

Obtaining and processing metals can result in high-TDS effluent at various points in the process stream. Groundwater used in mining operations may be naturally high in some ions and may also acquire ions during leaching or other processes. Toler (1980) found that, in a survey of streams in surface-mined areas of Illinois, SO_4^{2-} was the major mineral constituent from all sites, with concentrations ranging from 25 to 4,100 mg/L. Smelting operations can also result in effluents with elevated TDS ion levels, along with trace metals (e.g., cadmium, chromium, copper, lead, zinc). Hemens and Warwick (1972), for example, reported F^- concentrations of 40 to 60 mg/L in effluent from an aluminum smelting plant.

Other Water

Effluents from several other sources may potentially be high in TDS. Municipal as well as industrial effluents have been found to have TDS concentrations high enough to cause toxicity to test organisms. The causative agent of ion toxicity varies with the effluent source and may be associated with other toxicants. Mining effluents, as indicated, have been found to have toxicity due to common ions (e.g., SO_4^{2-}). However, these effluents also may have toxicity associated with metals such as copper, cadmium, or zinc. Fortunately, metals can generally be removed during TIE procedures through the use of sodium thiosulfate or ethylenediaminetetraacetic acid (EDTA).

TDS toxicity can be seasonal or associated with precipitation which, by flushing materials into a stream or runoff collection system, can cause concentrations of TDS and TSS (Total Suspended Solids) to increase. Toxicants other than TDS can also be flushed from the watershed. Municipal effluents, for example, which normally do not display WET, may demonstrate toxicity in the spring (April through June) due to increased levels of pesticides. Source water can also impact the toxicity of an effluent. Water used for cleaning, flushing, cooling, or other activities may start out with elevated levels of TDS if, for example, it is taken from a saline surface water lake or impoundment. Therefore, if ion-associated toxicity is discovered in an effluent, it may be important to test the source water to determine if toxicity exists prior to its use for a specific activity.

METHODS FOR IDENTIFYING ION TOXICITY

The presence of toxicity in an effluent regulated under the NPDES often triggers the need for a TIE. Although guidance for conducting TIEs has been established (USEPA 1989, 1991a, 1991b, and 1993), methods do not specifically target toxicity due to salinity or ion imbalance. Nevertheless, traditional TIE methods can be coupled with other tools to identify TDS toxicity.

The typical Phase I TIE characterization pattern for samples containing TDS toxicity is the failure of any of the manipulations to markedly reduce or remove effluent toxicity (Table 3-1). This characterization pattern is the first indication that TDS may be responsible for the observed toxicity. However, this pattern is also indicative of other toxicants (e.g., anionic metals such as hexavalent chromium). If Phase I manipulations do not reduce toxicity, and the conductivity and/or salinity measurements of the sample are consistent with toxicity due to TDS (e.g., conductivity measurements of 2,000 μmhos/cm or higher in fresh waters), additional studies must be conducted to determine if TDS is, in fact, the cause of toxicity.

If an effluent sample demonstrates toxicity in a baseline study and Phase I manipulations are ineffective in characterizing the toxicant, then a series of steps may be employed if TDS or ion imbalance toxicity is suspected (Figure 3-1). Toxicity may be due to excessive quantities of one or more ions, a deficiency of ions, or an overall imbalance of ions. Mathematical models can be used to analyze ion concentrations to determine if they approach or exceed those found to be toxic to test organisms (Pillard *et al.*, 1996; Douglas and Horne, 1997, Mount *et al.*, 1997; Pillard *et al.*, 1998a). Although toxicity typically is considered as reflecting an excess of a chemical, some saltwater species are adversely affected by both an excess and a deficiency of some ions, particularly Ca^{2+} and K^{+} (Douglas and Horne, 1997; Pillard *et al.*, 1998a). Even if ion concentrations fall within an acceptable range for organism survival, overall ion imbalance may still trigger significant reductions in survival or sublethal effects (Tucker *et al.*, 1997).

As described in Section 1, protocols developed to supplement and enhance traditional TIE techniques depend heavily on ion balancing and mock effluent studies. In both cases reagent-grade salts are added to either the effluent (for ion balancing) or deionized water (for mock effluent studies); both often are very effective in identifying ion toxicity.

Tietge *et al.* (1997) used mock effluents and computer models to analyze the toxicity of six produced waters collected at various sites in the United States. They found that the toxicity of mock effluents, in which the concentrations of major ions (Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}, Cl^{-}, HCO_3^{-}, and SO_4^{2-}) matched that of the effluents, was very similar to the toxicity of the actual effluent for four of the six effluents for *D. magna*, *C. dubia*, and *P. promelas*. In addition, for the latter two

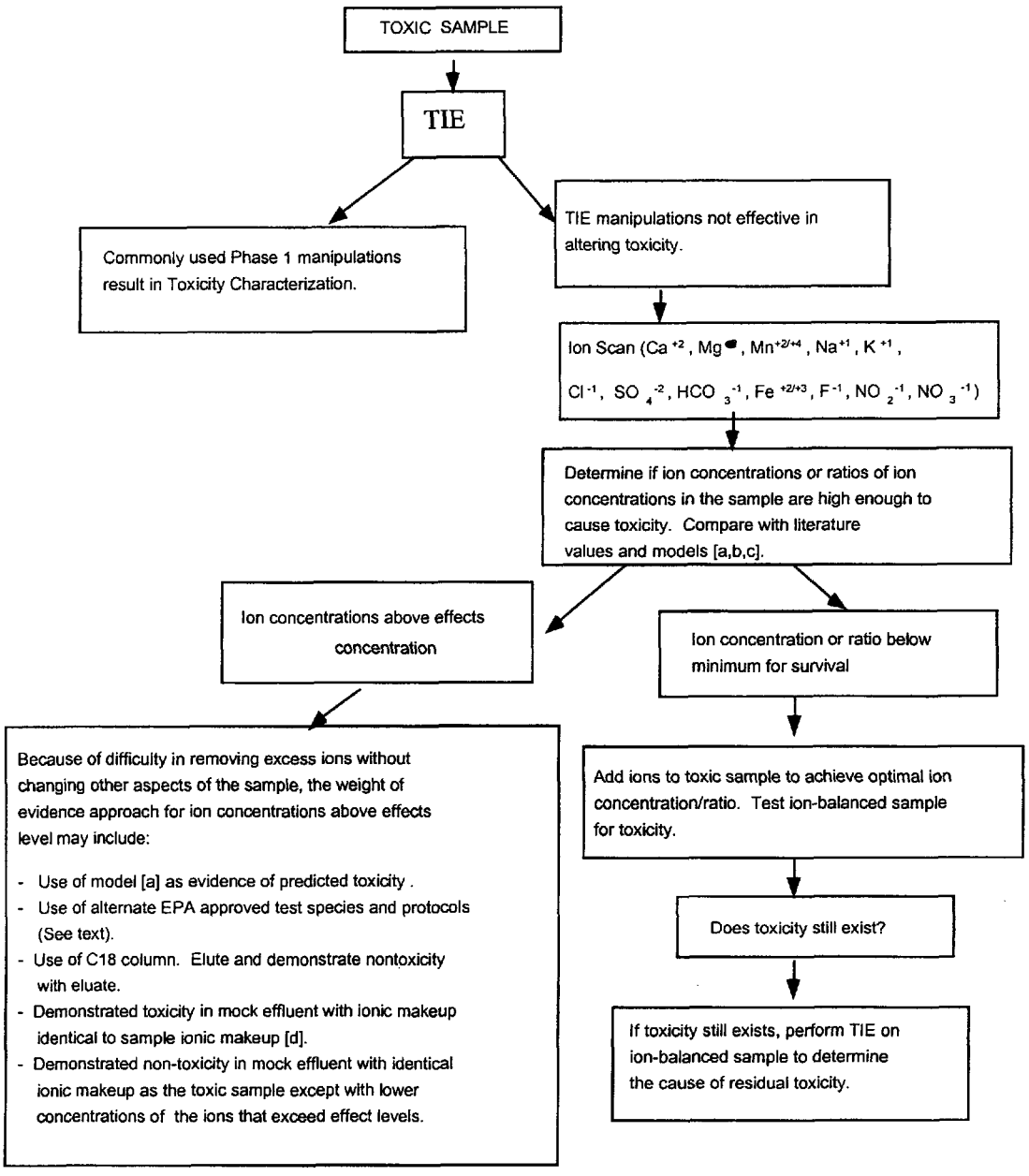
Table 3-1. Results of Acute Phase I Toxicity Characterization of an Industrial Effluent Using *D. magna* Where TDS Toxicity is Suspected.

Phase I Toxicity Characterization Test	48-Hour LC_{50}(%)
Baseline Toxicity (Unaltered Effluent)	40
pH 3 Adjustment	40
pH 3 Filtration	59
pH 3 Aeration	55
pH 3 SPE	62
Ambient pH Filtration	42
Ambient pH Aeration	35
Ambient pH SPE	55
pH 11 Adjustment	35
pH 11 Aeration	35
pH 11 Filtration	40
pH 9 SPE	42
Oxidant Reduction	40
EDTA Addition	35
Graduated pH	20

Source: Hockett and Mount (1993)

species, the actual and mock effluent study results correlated well with predictions made using a computer model (Tietge *et al.*, 1994). Toxicity in two of the produced water effluents was attributed to constituents other than ions. McCulloch and Smith (1994) used synthetic effluents to evaluate ion requirements of *Mysidopsis bahia* and found that combinations of ions could be added to effluents to offset the effects of ion imbalance.

For chronic TIE studies, mock effluent studies may be used to identify TDS toxicity. Results of a chronic Phase I Toxicity Characterization using *C. dubia* are provided in Table 3-2 (Hockett and Mount, 1993). None of the Phase I manipulations markedly reduced sample toxicity and TDS toxicity was suspected due to relatively high conductivity (2,420 μS/cm). The effluent sample was analyzed for TDS ions and a mock sample was then prepared and a chronic



References: [a] Mount *et al.*, 1997
[b] Tietge *et al.*, 1997
[c] Douglas and Horne, 1997
[d] McCulloch *et al.*, 1993

Figure 3-1. Framework for Isolating Ion Toxicity in a Toxic Sample.

Source: Ho and Caudle (1997)
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toxicity test with *C. dubia* was conducted (Figure 3-2). The toxicity of each of these solutions was very similar (IC_{25} [concentration at which there is a 25% reduction in organism performance] values were within 1% of one another). Based on the results of the Phase I TIE and Phase II TIE mock solution studies, common ions were judged responsible for the toxicity observed in this sample.

Identifying Ion-Specific TDS Toxicity

As illustrated in the examples presented above, mock effluents can be used to confirm TDS ion toxicity in effluents. Other manipulations can be used to identify and eliminate ion-specific toxicity. These methods take advantage of the fact that ions differ substantially in their toxicity to test organisms. The sensitivities of three common freshwater organisms are (Mount *et al.*, 1997):

C. dubia	(More Toxic) $K^+ > HCO_3^- > Mg^{2+} > Cl^- > SO_4^{2-}$ (Less Toxic)
D. magna	(More Toxic) $K^+ > Mg^{2+} > HCO_3^- > Cl^- > SO_4^{2-}$ (Less Toxic)
P. promelas	(More Toxic) $K^+ > Mg^{2+} > HCO_3^- > Cl^- > SO_4^{2-}$ (Less Toxic)

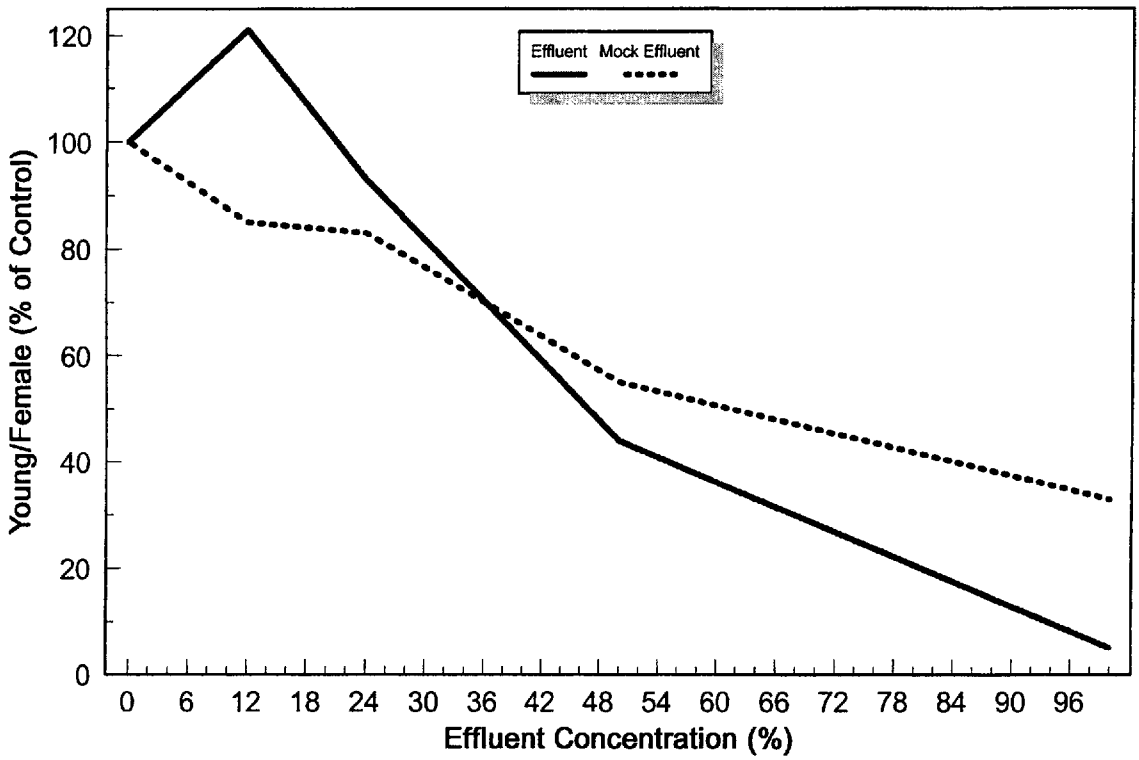
All three organisms are most sensitive to K^+ and least sensitive to SO_4^{2-}. Na^+ and Ca^{2+} do not appear to be directly toxic to freshwater species but are important to measure to ensure proper charge balance (i.e., they provide a quality assurance check during ion analysis). Ca^{2+} is, however, a significant toxicant in saltwater effluents. Pillard *et al.* (1998a; 1998b) studied the effects of several ions on marine species using 48-hour acute tests. They found that marine organisms are highly sensitive not only to excess quantities of certain ions but also to deficiencies. In addition, the interactions that occur in high ionic strength solutions seriously complicates efforts to identify a single cause of toxicity. Nevertheless, some generalizations can be made about the toxicity of ions (in excess) to marine species. The toxicity of ions (determined as unequilibrated mass concentration) to three common marine organisms are (Pillard *et al.*, 1998a):

M. bahia	(More Toxic) $B_4O_7^{2-} > K^+ > HCO_3^- > Ca^{2+} > Mg^{2+} > Br^- > SO_4^{2-}$ (Less Toxic)
Cyprinodon variegatus	(More Toxic) $B_4O_7^{2-} > K^+ > HCO_3^- > Ca^{2+} > Mg^{2+}$ (Less Toxic)
Menidia beryllina	(More Toxic) $Sr^{2+} > B_4O_7^{2-} > HCO_3^- > K^+ > Mg^{2+} > Ca^{2+} > Br^- > SO_4^{2-}$ (LT)

Table 3-2. Results of Chronic Phase I Toxicity Characterization of an Industrial Effluent Using *C. dubia* Where TDS Toxicity is Suspected.

Phase I Toxicity Characterization Test	IC_{25} (%)
Baseline Toxicity (Unaltered Effluent)	30.4
Filtration	19.8
Aeration	18.1
SPE	37.0
EDTA Addition	<50
Oxidant Reduction	<50

Source: Hockett and Mount (1993)



Note: The IC_{25} for the effluent was 30.4%; the IC_{25} for the mock effluent was 30.8%.

Figure 3-2. Observed Chronic Toxicity to *C. dubia* in Effluent and Mock Effluent (data from Hockett and Mount, 1993).

Sr^{2+} was not found to cause acute toxicity to *M. bahia* or *C. variegatus* within the concentrations tested, although significant effects to *M. beryllina* were reported. Br^- caused no significant effects to any of the three species tested, although an LC_{50} could nevertheless be extrapolated (using a mathematical model) for *M. bahia* and *M. beryllina*. SO_4^{2-} caused no acute toxicity to *C. variegatus* although adverse effects to *M. bahia* and *M. beryllina* were noted at very high SO_4^{2-} concentrations. Douglas and Horne (1997) reported a similar pattern of Ca^{2+}, K^+, Mg^{2+}, and Br^- toxicity to *M. bahia*. They also found that *M. bahia* mortality would occur if these four ions were deficient in the test water; Pillard *et al.* (1998a) reported similar results. The most dramatic effect occurs when Ca^{2+} or K^+ are absent from test solutions. Without these ions, even in the presence of other ions such as Mg^{2+}, Na^+, Br^-, and Cl^-, death occurs rapidly. Both Mg^{2+} and Br^- are also essential ions, although only partial mortality will occur, even over 96 hours, if these ions are absent from test solutions (Douglas and Horne, 1997).

As mentioned previously, the interactions that occur in high-ionic strength solutions, such as seawater and seawater-salinity effluents, may make the use of simple toxicity interpretations difficult. Oddo and Tomson (1994) developed saturation indices for ions such as Ca^{2+} to predict how scale forms during petroleum extraction activities. Using this information, Pillard *et al.* (1998a) developed a Chemical Equilibration Model (CEM) that estimates the concentration of an ion in solution by predicting complexation and precipitation. The relative ion toxicity to the three marine species presented above are based on nominal total ion concentrations. Equilibrated ion concentrations in a solution may be lower, especially HCO_3^- and Ca^{2+}, which are the most dramatically affected by interactions with other ions and specific water conditions (e.g., pH, temperature, pressure). Ca^{2+}, for example, will coprecipitate with HCO_3^- and SO_4^{2-}, thereby substantially reducing the amount of these ions that are in solution, and thus bioavailable. What appears to be toxicity due to HCO_3^- or SO_4^{2-}, may be due to a Ca^{2+} deficiency, if a sufficient amount is precipitated. Increasing pH tends to result in an increase in soluble HCO_3^- concentration but a decrease in Ca^{2+} concentration (above a pH of approximately 8.2). The interactions and accompanying precipitates are, however, solution specific and cannot be generalized; they must be calculated for each individual solution.

Although the chemical interactions described above can confound and frustrate TIE efforts, they also can be used to facilitate toxicant identification. MacGregor *et al.* (1996) studied ion imbalance in a TIE of a chemical manufacturing plant and found that the addition of Na_2SO_4

caused the formation of an insoluble $CaSO_4$ salt. Precipitation of this material improved the ion balance of the effluent and reduced or eliminated toxicity. Mickley *et al.* (1996) used ion balancing to reduce toxicity in membrane concentrates where the primary toxicants were identified as Ca^{2+}, K^+, and F^-. Ca^{2+} was also found to be at least partially responsible for toxicity in four out of five effluents studied by Douglas and Horne (1997). Ion balance in the effluents was restored using several methods. Some effluents only required the addition of reagent-grade salts to correct ion deficiency. In some cases excess ions first were reduced with cation-exchange resin, followed by spiking with reagent-grade salts. In other cases, salinity first was increased to 34‰ before ion addition occurred.

Elevated HCO_3^- concentrations (1,805 mg/L) were suspected as being responsible for toxicity to *C. dubia* (Hockett and Mount, 1993). To determine if HCO_3^- was, in fact, responsible for this toxicity, specific volumes of sulfuric acid were added to aliquots of the sample and then the aliquots were tested for toxicity to *C. dubia*. By adding sulfuric acid to the solution, the sample HCO_3^- concentration was decreased (exchanged with the SO_4^{2-} anion). Because SO_4^{2-} is substantially less toxic to *C. dubia* than is HCO_3^-, it was predicted that sample toxicity would decrease as SO_4^{2-} increased and HCO_3^- decreased; this prediction was confirmed experimentally (Table 3-3). These results provided additional evidence that the sample toxicity was attributable to elevated TDS ions, and particularly to HCO_3^-.

Table 3-3. Results of a Confirmation Study for Bicarbonate Toxicity in a Produced Water Effluent.

Solution	Bicarbonate Concentration (mg/L)	*C. dubia* Survival (%)
Unaltered Produced Water Sample (US)	1,805	0
100 ml US + 2.5 ml H_2SO_4	302	80
100 ml US + 3 ml H_2SO_4	48	100

Source: Hockett and Mount (1993)

Ion deficiencies in saltwater (e.g., Ca^+ deficient solutions, when compared to the ionic composition of seawater) can also cause toxicity. Because of this effect, it is helpful to determine the ionic composition of effluents (e.g., produced waters) prior to initiating formal TIE

studies. The ionic composition of the sample should then be compared to that of seawater at the salinity of the sample. Using this method, it may be possible to identify potentially deficient ions, and add them back into the effluent with reagent-grade salts (Douglas and Horne, 1997). A series of mock effluent tests that can be conducted to determine if the deficiency is responsible for sample toxicity include:

- unaltered sample
- unaltered sample with deficient ions added in to more closely match the proportions found in seawater
- mock sample
- mock sample with deficient ions added in to more closely match the proportions found in seawater

Samples Containing both TDS and Non-TDS Sources of Toxicity

Effluent samples may be toxic due to TDS and other non-TDS toxicants. In these instances, computer models (where applicable) and mock effluent studies can be used to characterize and identify non-TDS sources of toxicity. Hockett and Mount (1993) measured total salinity (5,704 mg/L) and ion concentrations in a sample that was found to be acutely toxicity to *C. dubia* and fathead minnows, with LC_{50} (concentration lethal to 50% of the test organisms) values of 2% and 35% sample, respectively. Based on the sample ionic composition, a computer model predicted LC_{50} values of 32% sample for *C. dubia* and 36% sample for fathead minnows. Mock effluent studies resulted in LC_{50} values of 38% for *C. dubia* and 58% for fathead minnows. Based on the results of the sample toxicity tests, mock solution tests, and the computer model predictions, the observed toxicity to fathead minnows in this sample was consistent with expected toxicity due to TDS ions although it was apparent that there was a non-TDS toxicant affecting *C. dubia*. An acute Phase I TIE was conducted with this sample using *C. dubia* (Table 3-4). Extraction with C_{18} at all three pHs completely removed the sample non-TDS toxicity, suggesting toxicity attributable to one or more non-polar organic compounds. This non-polar organic toxicity was then quantitatively recovered from the SPE column.

In summary, the identification of TDS ion-specific toxicity in effluents often requires corroborative evidence from several sources. Analytical data provide first-hand, direct information that can be interpreted in light of what is known about the ion tolerance of the organism(s) in question. However, because of the possible existence of 1) multiple TDS ion

toxicants 2) non-TDS toxicants, and 3) ion interactions, it may be necessary to utilize several lines of evidence (mock effluents, salt additions, and computer models) in the identification process.

Table 3-4. Results of Phase I Toxicity Characterization of a Produced Water Sample Using *C. dubia*. Manipulations that Reduced Toxicity are Shown in Italics and Bold.

Test Type	LC_{50} (%)
Baseline (Unaltered Sample)	<6.25
Mock Effluent	38
Computer Model Prediction	32
pH 3 Adjustment	<6.25
pH 3 Filtration	<6.25
pH 3 Aeration	<6.25
pH 3 SPE	***35***
Filtration	<6.25
Aeration	<6.25
SPE	***35***
pH 11 Adjustment	<6.25
pH 11 Filtration	<6.25
pH 11 Aeration	<6.25
pH 9 SPE	***35***
EDTA Addition	<25
Oxidant Reduction	<25
Graduated pH	<6.25

Source: Hockett and Mount (1993)

Section 4
TOXICITY OF MAJOR IONS TO AQUATIC ORGANISMS

A variety of organisms, from algae to chordates, have been used to study the effects of ions. This review concentrates on the effects of ions to animals, particularly from a physiological point of view. Plant studies also are included because a goal was to provide a review of all pertinent toxicological information. Many different life stages have been studied and test durations vary. Toxicity studies can be grouped broadly as either acute or chronic. Generally, any short-term (e.g., 48- to 96-hour) study that refers to the survival of an organism as an endpoint can be considered acute, and any longer term study that typically measures sublethal effects such as reproduction or growth can be considered as representative of chronic effects. Although many researchers have conducted long-term studies that encompass a substantial portion of an organism's life span and thus may be considered truly chronic (Petrocelli, 1985), short-duration (i.e., short-term chronic) studies are more common (e.g., 7-day fathead minnow tests). The majority of available data are for acute toxicity tests where the endpoint measured is typically the median lethal concentration (LC_{50}), although several other acute and chronic endpoints have been reported. It is sometimes difficult to discriminate between acute and chronic studies; LC_{50}s, for example, may be based on responses observed over long exposure periods, and sublethal endpoints (typically monitored in chronic studies) may be measured during acute (i.e., short-term) studies.

A review of the existing literature was conducted to identify concentrations of ions that have been shown to cause lethality and/or sublethal effects. Some of the studies present results as the concentration of a single ion from a given salt (e.g., an LC_{50} of 6,000 mg/L Cl^- as NaCl), while other studies expressed the endpoint as the concentration of the salt (e.g., an LC_{50} of 9,891 mg/L NaCl). In a few studies it was not possible to determine how the endpoint was expressed. Discussion is limited to those studies where it is clear what the endpoint concentrations represented. An attempt was made to gather data on the more commonly used test organisms (e.g., *D. magna*, *C. dubia*, and *P. promelas* in freshwater, and *M. bahia*, *C. variegatus*, and *M. beryllina* in seawater), although a variety of species that have been used to investigate ion toxicity are included in this review. The databases were searched using various salts as key search terms. For example, information on Na^+ salts was obtained by using sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), sodium bromide (NaBr), sodium bisulfate (Na_2SO_4), and sodium bromate ($NaBrO_3$) as key words. Because of the nature of the

AQUIRE literature search, it was possible to summarize the data in tabular form. These data are available from API under separate cover.

SALINITY TOLERANCE

Studies have demonstrated that salinity or TDS is indirect and imprecise measures of the potential toxicity of a solution. The individual ions that comprise TDS often play an important role in determining toxicity. However, assuming no single ion is present at toxic levels, organisms have salinity tolerance ranges that can be predicted. The tolerance ranges of stenohaline species are relatively narrow while euryhaline species, such as those that might be found in estuaries, can tolerate wide fluctuations in salinity. For most species, salinity tolerance is not fixed but can vary with other factors, such as temperature, pH, and dissolved oxygen concentrations. Acclimation to a given salinity, either natural or artificial, will also extend the tolerance range of a species.

Acclimation appears to be a critical factor in salinity tolerance, and has been demonstrated in laboratory studies as well as field observations. Populations of *Ophiothrix angulata*, an echinoderm, were found to differ in their tolerance of salinity, depending upon their geographic location (Stancyk and Shaffer, 1977). Specimens collected from a Florida estuary, which has lower salinity, were more tolerant of low salinity test water than those collected from seawater along the South Carolina coast. Kangas and Skoog (1978) found that specimens of the gastropod, *Theodoxus fluviatilis*, collected from higher salinity habitats were more tolerant of high salinity waters, while those found in near freshwater conditions were more tolerant of reduced salinity waters. *Tigriopus brevicornis* is an estuarine harpacticoid copepod that can be found in rock pools with salinities ranging from 5 to 200‰. It was found that, when *T. brevicornis* was slowly acclimated to solutions of higher salinity, its tolerance of higher saline solutions improved in laboratory experiments (Damgaard and Davenport, 1994). In addition, its tolerance of low salinities was unimpaired. When given a choice, however, the copepod preferred water closer to the salinity of seawater. Finally, in studies conducted by the Florida Department of Environmental Protection (FDEP, 1997), mysid shrimp were cultured in 20‰ artificial or natural seawater and exposed to natural and artificial seawater with salinities ranging from 4 to 20‰ either with or without prior acclimation to lower salinities. Unacclimated mysids taken directly from 20‰ culture water showed significant mortality when placed in 4, 5, 6, 7, and 8‰ test media; mortality decreased as salinity increased. However, if mysids were acclimated to the lower salinities prior to testing, survival significantly improved.

Three common marine test organisms, mysid shrimp (*M. bahia*), sheepshead minnow (*C. variegatus*), and inland silverside (*M. beryllina*), were acclimated to seawater at 25‰ and were then exposed to test solutions with salinities ranging from near zero to as high as 80‰ (Pillard *et al.*, 1998a). There were substantial differences among the three species in their ability to tolerate the stress of salinity changes. *Mysidopsis bahia* exhibited significant mortality at salinities greater than approximately 40‰ (LC_{50}=42.24‰). However, it also showed significant mortality when salinity dropped below 7‰ (LC_{50}=3.86‰) . *Cyprinodon variegatus* was the most tolerant of the three species tested, with 100 percent survival in some studies up to at least 80‰. The LC_{50} for *C. variegatus* was estimated at 69.47‰. *Menidia beryllina* was more sensitive than *C. variegatus*, and exhibited significant mortality at salinities greater than approximately 40‰ (LC_{50}=47.74‰).

For some related species, variations in salinity tolerance may be due to slight physiological differences. Piller *et al.* (1995) found that two euryhaline crab species, *Callinectes sapidus* and *C. similis*, inhabited 15 to 30‰ estuarine waters. While both species are hyperosmotic regulators, *C. sapidus* is able to tolerate waters to near 0‰. The better physiological tolerance of *C. sapidus* at low salinities is due to higher hemolymph concentrations of Na^+ and Cl^- (Engel, 1977), and may be a consequence of reduced permeability of gill surfaces. *C. similis* must expend more energy (i.e., ATP) to maintain an ion balance, and at lower salinities is simply unable to compensate for diffusive ion loss.

Kültz and Onken (1993) acclimated tilapia (*Oreochromis mossambicus*) to several test solutions adjusted to different salinities and measured various physiological changes in the fish, including density and diameter of chloride cells. As salinity increased from freshwater to seawater (≈35‰), the density and diameter of chloride cells increased, thus increasing the capacity for active ion secretion. Above seawater salinity, however, there was an indication that cellular tight junctions were becoming less permeable. Therefore, up to a certain level, salinity stress is reduced by increasing the capacity for active ion transport. At higher salinities, however, a preventive mechanism is employed by a reduction in overall permeability. This may be an adaptive mechanism to control the energetic costs of ion regulation.

ION INTERACTIONS

An important consideration in the interpretation of ion toxicity is the compound used. Ions are not presented as individual constituents but as combinations with other ions (e.g., NaCl, $CaCl_2$,

KCl). Therefore, the toxicity of an individual cation or anion in a compound may be masked by its respective anion or cation. For example, Mount *et al.* (1997) found that, while the 48-hour LC_{50} for *C. dubia* was 1,190 mg Cl^-/L when exposed to NaCl, it was 300 mg Cl^-/L when exposed to KCl, indicating the K^+ ion is much more toxic than the Na^+ ion. To address this concern in this report, an attempt was made in this document to limit descriptions to those studies where the ion in question is likely to be the principle cause of toxicity. Although single ion compounds, or salts, are typically tested, actual effluents (e.g., produced waters) often contain several salts, and thus multiple combinations of ions. The presence of more than one salt in a solution does affect the toxicity, and consequently, the ability to predict toxicity based on ion concentration. Mount *et al.* (1997) reported that when two cations (e.g., Na^+ and K^+) were added to freshwater test solutions as Cl^- salts, the solutions were less toxic, particularly to *C. dubia*, than solutions with a single cation (e.g, Na^+ or K^+) . This "protective" effect of multiple cations was also observed by de March (1988) who exposed the freshwater amphipod, *Gammarus lacustris*, to mixtures of two (out of five) cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+). Although K^+ and Mg^{2+} showed additive effects in combination with Cu^{2+}, Cd^{2+}, or Zn^{2+}, they showed less-than-additive effects when tested together.

Evaluation of the effects of ionic composition in saltwater is more difficult than in freshwater, because ion deficiencies, as well as excesses, can result in adverse effects. While freshwater organisms also will die if completely deprived of environmental ions (e.g., in deionized water), the required concentrations of essential ions are low enough that effluents discharged to freshwater receiving streams are seldom lacking; this is not always the case with marine effluents. Apparent toxicity of SO_4^{2-} to *M. bahia*, for example, may be due to a deficiency of Ca^{2+} through the formation and precipitation of calcium sulfate ($CaSO_4$). The same is also true for HCO_3^-, which can precipitate as $Ca(HCO_3)_2$. The formation of these precipitates has been a historic concern for the petroleum industry because the presence of high concentrations of ions in produced water can result in scale formation and accelerated corrosion of pipes and equipment (Oddo and Tomson, 1994). Using equations originally developed to predict scale formation, Pillard *et al.* (1998a) developed chemical equilibration models to predict actual ion concentrations in solution, based on measured ion concentrations. Use of the equilibrated ion concentrations greatly improved the predictive ability of some models to estimate ion toxicity to marine organisms.

Given the interactions that can occur in an effluent, including complexation, precipitation, antagonistic and possibly additive effects, toxicity measurements (e.g., LC_{50}s, IC_{25}s, etc.) must be viewed in light of specific conditions of a toxicity test. Tables 4-1 through 4-10 list much, although not all, of the toxicity data found through searches conducted for this report. The data in the tables are divided into "Invertebrates", "Vertebrates", and "Plants and Algae." Where available, more common freshwater test organisms are listed first, followed by more obscure species. If available, toxicity data for marine species are also presented.

TOXICITY AND BIOACCUMULATION OF IONS

The data presented in Tables 4-1 through 4-10 demonstrate that ions vary substantially in their influence on aquatic organisms. Despite this variability, there is some consistency in the relative toxicity among different species, as illustrated in Figures 4-1 to 4-6. From these data it is apparent, for example, that ions such as SO_4^{2-} and Br^- are two of the least acutely toxic ions. While chronic toxicity due to SO_4^{2-} is also likely to occur only at high concentrations, chronic effects due to Br^- may occur at much lower concentrations. As might be expected, sublethal effects have been shown to occur at lower concentrations than acute effects; however, the differences may be slight. Pillard *et al.* (1998a) found that 7-day IC_{25}s were often very similar to 48-hour LC_{50}s for *M. bahia*, *C. variegatus*, and *M. beryllina*.

For freshwater organisms, F^-, Mg^{2+}, K^+, and Sr^{2+} are four of the more toxic ions studied, although fewer Sr^{2+} toxicity data are available. LC_{50}s and acute EC_{50}s for these ions were all less than 1,000 mg/L for *D. magna* and *P. promelas* and less than 500 mg/L for *C. dubia*. To marine species, particularly *M. bahia*, F^- was one of the more toxic ions (Figure 4-4); $B_4O_7^{2-}$ also appears to have adverse effects at lower concentrations relative to other ions (Figs. 4-4, 4-5, and 4-6). In the evaluation of the potential for causing adverse effects, the absolute toxicity as defined by the mass of an ion per volume (mg/L) is less important than the environmental concentrations that are likely to be encountered in effluents. For example, although $B_4O_7^{2-}$ is more toxic than Ca^{2+} and K^+ to marine organisms, the latter two ions are likely to be present at higher concentrations in many marine-discharged effluents, and thus are more frequently identified as potential toxicants.

As indicated in Tables 4-1 to 4-10 and illustrated in Figures 4-1 to 4-6, there can be substantial variations in the results of toxicity studies from different laboratories, even using the same test species and test chemical (salt). Some differences are to be expected based on differences in

Table 4-1. Results of Selected Toxicity Studies with Bicarbonate.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as HCO_3^- (mg/L)
Invertebrates					
Daphnia magna	Hoke *et al.*, 1992	1,133-2,211	48H LC_{50}	$NaHCO_3$	823-1,606
Daphnia magna	Mount *et al.*, 1997	2,380	24H LC_{50}	$NaHCO_3$	1,730
Daphnia magna	Mount *et al.*, 1997	1,640	48H LC_{50}	$NaHCO_3$	1,190
Ceriodaphnia dubia	Hoke *et al.*, 1992	983-1,159	48H LC_{50}	$NaHCO_3$	714-842
Ceriodaphnia dubia	Mount *et al.*, 1997	1,420	24H LC_{50}	$NaHCO_3$	1,030
Ceriodaphnia dubia	Mount *et al.*, 1997	1,020	48H LC_{50}	$NaHCO_3$	740
Culex sp.	Dowden & Bennett, 1965	2,000	1 & 2D LC_{50}s	$NaHCO_3$	1,450
Mysidopsis bahia	Pillard *et al.*, 1998a	1,086[a]	48H LC_{50}	HCO_3^- as $NaHCO_3$	1,086
Mysidopsis bahia	Pillard *et al.*, 1998a	1,068[a]	7D IC_{25}	HCO_3^- as $NaHCO_3$	1,068
Vertebrates					
Pimephales promelas	Mount *et al.*, 1997	4,850	24H LC_{50}	$NaHCO_3$	3,520
Pimephales promelas	Mount *et al.*, 1997	2,500	48H LC_{50}	$NaHCO_3$	1,820
Pimephales promelas	Mount *et al.*, 1997	<850	96H LC_{50}	$NaHCO_3$	<620
Lepomis macrochirus	Patrick *et al.*, 1968	8,600	4D LC_{50}	$NaHCO_3$	6,250
Gambusia affinis	Wallen *et al.*, 1957	7,550	2D LC_{50}	$NaHCO_3$	5,480
Gambusia affinis	Wallen *et al.*, 1957	7,700	1D LC_{50}	$NaHCO_3$	5,590
Cyprinodon variegatus	Pillard *et al.*, 1998a	1,672[a]	48H LC_{50}	HCO_3^- as $NaHCO_3$	1,672
Cyprinodon variegatus	Pillard *et al.*, 1998a	2,105[a]	7D IC_{25}	HCO_3^- as $NaHCO_3$	2,105
Menidia beryllina	Pillard *et al.*, 1998a	671[a]	48H LC_{50}	HCO_3^- as $NaHCO_3$	671
Menidia beryllina	Pillard *et al.*, 1998a	976[a]	7D IC_{25}	HCO_3^- as $NaHCO_3$	976
Plants and Algae					
Algae	Dickman, 1973	45	Abundance	$NaHCO_3$	34
Nitzschia linearis	Patrick *et al.*, 1968	650	5D LC_{50}	$NaHCO_3$	470

[a] Unequilibrated concentrations; equilibrated concentrations are likely to be lower.

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-2. Results of Selected Toxicity Studies with Borate.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as $B_4O_7^{2-}$ (mg/L)
Invertebrates					
Limnora ligorum	Robinson & Perkins, 1977	250	1D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	102
Gammarus tigrinus	Mann, 1973	10,000	1D LC_{100}	$Na_2B_4O_7 \cdot 10H_2O$	4,070
Tubifex tubifex	Mann, 1973	2,000	1D LD_{100}	$Na_2B_4O_7 \cdot 10H_2O$	814
Orichidoris fusca	Robinson & Perkins, 1977	>250	1D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	>102
Mysidopsis bahia	Pillard *et al.*, 1998a	379	48H LC_{50}	$B_4O_7^{2-}$ as $Na_2B_4O_7 \cdot 10H_2O$	379
Vertebrates					
Oncorhynchus mykiss	Alabaster, 1969	2,800	24H LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	1,140
Oncorhynchus mykiss	Alabaster, 1969	1,800	48H LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	730
Poecilia reticulata	Mann, 1973	5,000	1D LC_{100}	$Na_2B_4O_7 \cdot 10H_2O$	2,030
Carassius auratus	Birge & Black, 1977	65	7D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	26
Carassius auratus	Birge & Black, 1977	71	3D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	29
Ictalurus punctatus	Birge & Black, 1977	155	9D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	63
Ictalurus punctatus	Birge & Black, 1977	71	9D LC_{50}	$Na_2B_4O_7 \cdot 10H_2O$	29
Cyprinodon variegatus	Pillard *et al.*, 1998a	455	48H LC_{50}	$B_4O_7^{2-}$ as $Na_2B_4O_7 \cdot 10H_2O$	455
Menidia beryllina	Pillard *et al.*, 1998a	298	48H LC_{50}	$B_4O_7^{2-}$ as $Na_2B_4O_7 \cdot 10H_2O$	298
Plants and Algae					
Elodea canadensis	Nobel, 1981	2	Photosynthesis	$Na_2B_4O_7 \cdot 10H_2O$	0.81
Myriophyllum spicatum	Stanley, 1974	171	EC_{50} Shoot Growth	$B_4O_7^{2-}$	171
Myriophyllum spicatum	Stanley, 1974	152	EC_{50} Root Growth	$B_4O_7^{2-}$	152

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-3. Results of Selected Toxicity Studies with Bromide.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Br^- (mg/L)
Invertebrates					
Daphnia magna	Slooff & Canton, 1983	3,200	21D NOEC Mort.	NaBr	2,490
Daphnia magna	Slooff & Canton, 1983	10	21D NOEC Reprod.	NaBr	7.8
Daphnia magna	Canton *et al.*, 1983	7.8	NOEC Reprod.	Br^- as NaBr	7.8
Daphnia magna	Hermens *et al.*, 1984	13,500	2D LC_{50}	NaBr	10,500
Daphnia magna	Hermens *et al.*, 1984	29	16D EC_{50} Reprod.	NaBr	23
Daphnia magna	Kühn *et al.*, 1989	7,219	24H EC_{50}	Br^- as NaBr	7,219
Daphnia magna	Kühn *et al.*, 1989	91	21D NOEC	Br^- as NaBr	91
Daphnia magna	Cohen & Stubblefield, 1991	7,980	2D LC_{50}	Br^- as NaBr	7,980
Ceriodaphnia dubia	Cohen & Stubblefield, 1991	3,470	2D LC_{50}	Br^- as NaBr	3,470
Ceriodaphnia dubia	Cohen & Stubblefield, 1991	1.6	ChV	Br^- as NaBr	1.6
Chironomus tentans	Cohen & Stubblefield, 1991	3,125	4D LC_{50}	Br^- as NaBr	3,125
Culex pipiens	Slooff & Canton, 1983	100	24D NOEC Mort.	NaBr	78
Physa gyrina lymnaea	Cohen & Stubblefield, 1991	2,030	4D LC_{50}	Br^- as NaBr	2,030
Mysidopsis bahia	Pillard *et al.*, 1998a	7,991	48H LC_{50}	Br^- as NaBr	7,991
Vertebrates					
Pimephales promelas	Alexander *et al.*, 1981	18,441	24H LC_{50}	NaBr	14,320
Pimephales promelas	Alexander *et al.*, 1981	17,757	48H LC_{50}	NaBr	13,790
Pimephales promelas	Alexander *et al.*, 1981	16,479	96H LC_{50}	NaBr	12,797
Pimephales promelas	Cohen & Stubblefield, 1991	16,981	4D LC_{50}	Br^- as NaBr	16,981
Pimephales promelas	Cohen & Stubblefield, 1991	1,767	ChV	Br^- as NaBr	1,767
Oncorhynchus mykiss	Cohen & Stubblefield, 1991	13,350	4D LC_{50}	Br^- as NaBr	13,350
Poecilia reticulata	Canton *et al.*, 1983	12,000	28D LC_{50}	Br^- as NaBr	12,000
Poecilia reticulata	Canton *et al.*, 1983	16,000	1D LC_{50}	Br^- as NaBr	16,000
Oryzias latipes	Canton *et al.*, 1983	1,500	34D LC_{50}	Br^- as NaBr	1,500
Oryzias latipes	Canton *et al.*, 1983	370	34D EC_{50} Behavior	Br^- as NaBr	370
Cyprinodon variegatus	Pillard *et al.*, 1998a	>2,950	48H LC_{50}	Br^- as NaBr	>2,950
Menidia beryllina	Pillard *et al.*, 1998a	18,299	48H LC_{50}	Br^- as NaBr	18,299
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	430	LC_{50}	Br^- as NaBr	430
Scenedesmus pannonicus	Canton *et al.*, 1983	5,800	1D EC_{50} Growth	Br^- as NaBr	5,800
Scenedesmus pannonicus	Canton *et al.*, 1983	10,000	4D EC_{50} Growth	Br^- as NaBr	10,000
Scenedesmus subspicatus	Kühn & Pattard, 1990	6,000	4D EC_{50}	NaBr	4,660
Lemna minor	Slooff & Canton, 1983	3,200	7D NOEC Growth	NaBr	2,490

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-4. Results of Selected Toxicity Studies with Calcium.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Ca^{2+} (mg/L)
Invertebrates					
Daphnia magna	Khangarot & Ray, 1989	383.6	48H EC_{50} Immobiliz.	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	383.6
Daphnia magna	Mount *et al.*, 1997	3,250	24H LC_{50}	$CaCl_2$	1,170
Daphnia magna	Mount *et al.*, 1997	2,770	48H LC_{50}	$CaCl_2$	1,000
Daphnia magna	Mount *et al.*, 1997	>1,970	24&48H LC_{50}s	$CaSO_4$	>580
Daphnia hyalina	Baudouin & Scoppa, 1974	3,000	48H LC_{50}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	3,000
Ceriodaphnia dubia	Mount *et al.*, 1997	2,260	24H LC_{50}	$CaCl_2$	820
Ceriodaphnia dubia	Mount *et al.*, 1997	1,830	48H LC_{50}	$CaCl_2$	660
Ceriodaphnia dubia	Mount *et al.*, 1997	>1,940	24H LC_{50}	$CaSO_4$	>570
Ceriodaphnia dubia	Mount *et al.*, 1997	>1,910	48H LC_{50}	$CaSO_4$	>560
Nitocra spinipes	Bengtsson, 1978	580	96H LC_{50}	Ca^{2+} as $CaCl_2$	580
Polycelis nigra	Jones, 1940	2,600	48H Tox. Threshold	Ca^{2+} as $CaCl_2$	2,600
Tubifex tubifex	Khangarot, 1991	814	1D EC_{50} Immobilization	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	814
Tubifex tubifex	Khangarot, 1991	281	4D EC_{50} Immobilization	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	281
Mysidopsis bahia	Dorn & Rodgers, 1989	1,140 & 1,178	96H LC_{50}s	Ca^{2+} as $CaCl_2$	1,140 & 1,178
Mysidopsis bahia	Pillard *et al.*, 1998a	1,098[a]	48H LC_{50}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	1,098
Mysidopsis bahia	Pillard *et al.*, 1998a	1,022[a]	7D IC_{25}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	1,022
Vertebrates					
Pimephales promelas	Mount *et al.*, 1997	>6,660	24H LC_{50}	$CaCl_2$	>2,410
Pimephales promelas	Mount *et al.*, 1997	>6,560	48H LC_{50}	$CaCl_2$	>2,370
Pimephales promelas	Mount *et al.*, 1997	4,630	96H LC_{50}	$CaCl_2$	1,670
Pimephales promelas	Mount *et al.*, 1997	>1,970	24, 48, & 96H LC_{50}s	$CaSO_4$	>580
Lepomis macrochirus	Trama, 1954	876	4D Mort.	Ca^{2+} as $CaSO_4$	876
Lepomis macrochirus	Brown *et al.*, 1973	200	180D Mort.	Ca	200
Gambusia affinis	Wallen *et al.*, 1957	>56,000	24H LC_{50}	Ca^{2+} as $CaSO_4$	>56,000
Gambusia affinis	Wallen *et al.*, 1957	13,400	24H LC_{50}	$CaCl_2$	8,561
Oryzias latipes	Tsuji *et al.*, 1986	>1,000	24 & 48H LC_{50}s	Ca^{2+} as $CaCl_2$	>1,000
Cyprinodon variegatus	Dorn & Rodgers, 1989	2,766	96H LC_{50}	Ca^{2+} as $CaCl_2$	2,766
Cyprinodon variegatus	Pillard *et al.*, 1998a	4,409[a]	48H LC_{50}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	4,409
Cyprinodon variegatus	Pillard *et al.*, 1998a	3,996[a]	7D IC_{25}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	3,996

Table 4-4. Continued.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Ca^{2+} (mg/L)
Menidia beryllina	Pillard *et al.*, 1998a	4,609[a]	48H LC_{50}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	4,609
Menidia beryllina	Pillard *et al.*, 1998a	1,591[a]	7D IC_{25}	Ca^{2+}as $CaCl_2 \cdot 2H_2O$	1,591
Plants and Algae					
Chlorella vulgaris	Becker & Keller, 1973	1,872	Growth	Ca^{2+} as $CaSO_4$	1,872
Chlorella vulgaris	DeJong, 1965	>280	Growth	Ca^{2+}as $CaCl_2 \cdot 6H_2O$	>280
Nitzschia linearis	Patrick *et al.*, 1968	3,200	5D LC_{50}	Ca^{2+} as $CaSO_4$	3,200
Nitzschia linearis	Patrick *et al.*, 1968	3,130	LC_{50}	Ca^{2+} as $CaCl_2$	3,130

[a] Unequilibrated concentrations; equilibrated concentrations are likely to be lower.

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-5. Results of Selected Toxicity Studies with Chloride.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Cl^- (mg/L)
Invertebrates					
Daphnia magna	Dowden, 1961	3,384	48H LC_{50}	NaCl	2,050
Daphnia magna	Dowden & Bennett, 1965	6,447	24H LC_{50}	NaCl	3,910
Daphnia magna	Dowden & Bennett, 1965	5,874	48H LC_{50}	NaCl	3,560
Daphnia magna	Hoke *et al.*, 1992	5,008	48H LC_{50}	NaCl	3,040
Daphnia magna	Mount *et al.*, 1997	6,380	24H LC_{50}	NaCl	3,870
Daphnia magna	Mount *et al.*, 1997	4,770	48H LC_{50}	NaCl	2,890
Ceriodaphnia dubia	Hoke *et al.*, 1992	738-837	48H LC_{50}s	NaCl	450-510
Ceriodaphnia dubia	Mount *et al.*, 1997	3,380	24H LC_{50}	NaCl	2,050
Ceriodaphnia dubia	Mount *et al.*, 1997	1,960	48H LC_{50}	NaCl	1,190
Dugesia gonocephala	Palladini *et al.*, 1980	1,230	Mortality	Cl^- as NaCl	1,230
Culex sp.	Dowden & Bennett, 1965	10,500	1D LC_{50}	NaCl	6,370
Culex sp.	Dowden & Bennett, 1965	10,200	2D LC_{50}	NaCl	6,190
Vertebrates					
Pimephales promelas	Adelman & Smith, 1976	7,910	24H LC_{50}	NaCl	4,800
Pimephales promelas	Mount *et al.*, 1997	8,280	24H LC_{50}	NaCl	5,020
Pimephales promelas	Mount *et al.*, 1997	6,510	48H LC_{50}	NaCl	3,950
Pimephales promelas	Mount *et al.*, 1997	6,390	96H LC_{50}	NaCl	3,880
Lepomis macrochirus	Dowden & Bennett, 1965	14,125	24H LC_{50}	NaCl	8,570
Lepomis macrochirus	Patrick *et al.*, 1968	12,946	4D LC_{50}	NaCl	7,850
Gambusia affinis	Wallen *et al.*, 1957	18,100	24H LC_{50}	NaCl	10,980
Gambusia affinis	Wallen *et al.*, 1957	17,550	96H LC_{50}	NaCl	10,650
Anguilla rostrata	Hinton & Eversole, 1979	21,450	96H LC_{50}	NaCl	13,010
Rana breviceps	Mahajan *et al.*, 1979	470-6,000	Mortality	Cl^- as NaCl	470-6,000
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	590-680	Growth	NaCl	360-410
Nitzschia linearis	Patrick *et al.*, 1968	2,430	5D LC_{50}	NaCl	1,470
Myriophyllum spicatum	Stanley, 1974	8,183	EC_{50} Root Biomass	NaCl	4,960
Myriophyllum spicatum	Stanley, 1974	8,008	EC_{50} Shoot Growth	NaCl	4,860

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-6. Results of Selected Toxicity Studies with Fluoride.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as F^- (mg/L)
Invertebrates					
Daphnia magna	Anderson, 1946	504	Threshold Conc. Immobility	NaF	230
Daphnia magna	LeBlanc, 1980	340	48H LC_{50}	NaF	154
Daphnia magna	Dave, 1984	453	24H EC_{50}	NaF	210
Daphnia magna	Dave, 1984	216	48H EC_{50}	NaF	100
Daphnia magna	Fieser *et al.*, 1986	180-350	48H LC_{50}s	F^- as NaF	180-350
Daphnia magna	Kühn *et al.*, 1989	352	24H EC_{50}	F^- as NaF	352
Daphnia magna	Kühn *et al.*, 1989	14	21D NOEC	F^- as NaF	14
Mytilus edulis	Wright and Davison, 1975	10	21D LD_{100}	F^-	10
Penaus indicus	McClurg, 1984	1,118	96H LC_{50}	F^- as NaF	1,118
Hydropsyche bronta	Camargo, 1996	15.8	96H LC_{50}	F^- as NaF	15.8
Hydropsyche occidentalis	Camargo, 1996	34.0	96H LC_{50}	F^- as NaF	34.0
Cheumatopsyche pettiti	Camargo, 1996	42.5	96H LC_{50}	F^- as NaF	42.5
Grandideirella lutosa & *G. lignorum*	Connell and Airey, 1982	5.0-6.2	MATC Popul. Growth	F^- as NaF	5.0-6.2
Barytelphusa guerini	Reddy *et al.*, 1989	89.13	96H LC_{50}	NaF	40
Mysidopsis bahia	Lixey *et al.*, 1997	11	Mean 24H LC_{50}	F^- as NaF	11
Vertebrates					
Pimephales promelas	Smith *et al.*, 1985	180-315	96H LC_{50}s	F^- as NaF	180-315
Oncorhynchus mykiss	Neuhold and Sigler, 1960	2.7-4.7	20D LC_{50}s	F as NaF	2.7-4.7
Oncorhynchus mykiss	Angelovic *et al.*, 1961	2.6-6.0	10D LC_{50}s	F^- as NaF	2.6-6.0
Oncorhynchus mykiss	Smith *et al.*, 1985	200	96H LC_{50}	F^-	200
Cyprinus carpio	Neuhold and Sigler, 1960	75-91	LC_{50}s	F^- as NaF	75-91
Channa punctatus	Chitra & Rao, 1981	10	96H LC_{50}	NaF	4.5
Boleophtalmus dussuieri	Shaikh and Hiradhar, 1988	120	96H LC_{50}	F^- as NaF	120
Gasterosteus aculeatus	Smith *et al.*, 1985	340-460	96H LC_{50}s	F^- as NAF	340-460
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	48-72	Growth	NaF	20-30
Porphyra tenera	Ishio and Nakagawa, 1971	376	20 & 30D NOEC	F^- as CaF_2	376
Nitzschia palea	Joy and Balakrishnan, 1990	10-110	Growth Stimul.	F^- as NaF	10-110

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-7. Results of Selected Toxicity Studies with Magnesium.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Mg^{2+} (mg/L)
Invertebrates					
Daphnia magna	Dowden & Bennett, 1965	3,803	96H LC_{50}	$MgSO_4$	790
Daphnia magna	Biesinger & Christensen, 1972	125	Reprod.	Mg^{2+} as $MgCl_2$	125
Daphnia magna	Khangarot & Ray, 1989	406	24H LC_{50}	Mg^{2+} as $MgSO_4 \cdot 7H_2O$	406
Daphnia magna	Khangarot & Ray, 1989	344	48H LC_{50}	Mg^{2+} as $MgSO_4 \cdot 7H_2O$	344
Daphnia magna	Mount *et al.*, 1997	1,560	24H LC_{50}	$MgCl_2$	400
Daphnia magna	Mount *et al.*, 1997	1,330	48H LC_{50}	$MgCl_2$	340
Daphnia magna	Mount *et al.*, 1997	2,360	24H LC_{50}	$MgSO_4$	480
Daphnia magna	Mount *et al.*, 1997	1,820	48H LC_{50}	$MgSO_4$	370
Daphnia hyalina	Baudouin & Scoppa, 1974	32	48H LC_{50}	Mg^{2+} as $MgCl_2 \cdot 6H_2O$	32
Ceriodaphnia dubia	Mount *et al.*, 1997	1,270	24H LC_{50}	$MgCl_2$	320
Ceriodaphnia dubia	Mount *et al.*, 1997	880	48H LC_{50}	$MgCl_2$	220
Ceriodaphnia dubia	Mount *et al.*, 1997	1,770	24 & 48H LC_{50}	$MgSO_4$	450
Gammarus lacustris	de March, 1988	64.7	LC_{50}	Mg^{2+}	64.7
Nitocra spinipes	Bengtsson, 1978	720	96H LC_{50}	Mg^{2+} as $MgCl_2$	720
Orconectes limosus	Boutet & Chaisemartin, 1973	760	4D LC_{50}	Mg^{2+} as $MgCl_2$	760
Polycelis nigra	Jones, 1940	970	48H Tox. Thresh.	Mg^{2+} as $MgCl_2$	970
Tubifex tubifex	Khangarot, 1991	165	48H LC_{50}	Mg^{2+} as $MgSO_4 \cdot 7H_2O$	165
Mysidopsis bahia	Pillard *et al.*, 1998a	2,650[a]	48H LC_{50}	Mg^{2+} as $MgCl_2 \cdot 6H_2O$	2,650
Mysidopsis bahia	Pillard *et al.*, 1998a	2,560[a]	7D IC_{25}	Mg^{2+} as $MgCl_2 \cdot 6H_2O$	2,560
Vertebrates					
Pimephales promelas	Mount *et al.*, 1997	3,520	24H LC_{50}	$MgCl_2$	900
Pimephales promelas	Mount *et al.*, 1997	2,840	48H LC_{50}	$MgCl_2$	730
Pimephales promelas	Mount *et al.*, 1997	2,120	96H LC_{50}	$MgCl_2$	540
Pimephales promelas	Mount *et al.*, 1997	4,630	24H LC_{50}	$MgSO_4$	940
Pimephales promelas	Mount *et al.*, 1997	3,510	48H LC_{50}	$MgSO_4$	710
Pimephales promelas	Mount *et al.*, 1997	2,820	96H LC_{50}	$MgSO_4$	570
Lepomis macrochirus	Dowden & Bennett, 1965	19,000	24H LC_{50}	$MgSO_4$	3,800
Lepomis macrochirus	Brown *et al.*, 1973	200	180D Mortality	Mg^{2+}	200
Gambusia affinis	Wallen *et al.*, 1957	4,210-4,780	LC_{50}	Mg^{2+} as $MgCl_2$	4,210-4,780

Table 4-7. Continued.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Mg^{2+} (mg/L)
Gambusia affinis	Wallen *et al.*, 1957	3,100	LC_{50}	Mg^{2+} as $MgSO_4$	3,100
Cyprinodon variegatus	Pillard *et al.*, 1998a	4,741[a]	48H LC_{50}	Mg^{2+} as $MgCl_2$	4,741
Cyprinodon variegatus	Pillard *et al.*, 1998a	3,479[a]	7D IC_{25}	Mg^{2+} as $MgCl_2$	3,479
Menidia beryllina	Pillard *et al.*, 1998a	2,796[a]	48H LC_{50}	Mg^{2+} as $MgCl_2$	2,796
Menidia beryllina	Pillard *et al.*, 1998a	3,054[a]	7D IC_{25}	Mg^{2+} as $MgCl_2$	3,054
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	980-1,230	Growth	$MgSO_4 \cdot 7H_2O$	97-120

[a] Unequilibrated concentrations; equilibrated concentrations are likely to be slightly lower.

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-8. Results of Selected Toxicity Studies with Potassium.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as K^+ (mg/L)
Invertebrates					
Daphnia magna	Dowden & Bennett, 1965	679	96H LC_{50}	KCl	360
Daphnia magna	Biesinger & Christensen, 1972	68	EC_{50} Reprod.	K^+ as KCl	68
Daphnia magna	Khangarot & Ray, 1989	328	24H EC_{50} Immobilization	K^+ as KCl	328
Daphnia magna	Khangarot & Ray, 1989	141	48H EC_{50} Immobilization	K^+ as KCl	141
Daphnia magna	Mount *et al.*, 1997	740	24H LC_{50}	KCl	390
Daphnia magna	Mount *et al.*, 1997	660	48H LC_{50}	KCl	350
Ceriodaphnia dubia	Mount *et al.*, 1997	630	24 & 48H LC_{50}s	KCl	330
Cricotopus trifascia	Hamilton *et al.*, 1975	2,987	48H LC_{50}	KCl	1,570
Dreissena polymorphia	Fisher *et al.*, 1991	138	24H LC_{50}	KCl	72
Dreissena polymorphia	Fisher *et al.*, 1991	112	24H LC_{50}	K_2SO_4	65
Hydroptila augusta	Hamilton *et al.*, 1975	4,415	48H LC_{50}	KCl	2,320
Musculium transversum	Sparks and Anderson, 1977	2,700	2D LC_{50}	K^+	2,700
Musculium transversum	Sparks and Anderson, 1977	168	5D LC_{50}	K^+	168
Nais variabilis	Hamilton *et al.*, 1975	143	48H LC_{50}	KCl	75
Nitocra spinipes	Bengtsson, 1978	450	96H LC_{50}	K^+ as KCl	450
Polycelis nigra	Jones, 1940	350	48H LC_{50}	K^+ as KCl or KNO_3	350
Tubifex tubifex	Khangarot, 1991	1,320	48H LC_{50}	K^+ as Kcl	1,320
Mysidopsis bahia	Pillard *et al.*, 1998a	786	48H LC_{50}	K^+ as KCl	786
Mysidopsis bahia	Pillard *et al.*, 1998a	489	7D IC_{25}	K^+ as KCl	489
Vertebrates					
Pimephales promelas	Mount *et al.*, 1997	950	24H LC_{50}	KCl	500
Pimephales promelas	Mount *et al.*, 1997	910	48H LC_{50}	KCl	480
Pimephales promelas	Mount *et al.*, 1997	880	96H LC_{50}	KCl	460
Lepomis macrochirus	Trama, 1954	1,060	4D LC_{50}	K^+ as KCl	1,060
Lepomis macrochirus	Dowden & Bennett, 1965	5,500	1D LC_{50}	KCl	2,880
Gambusia affinis	Wallen *et al.*, 1957	5,300	24H LC_{50}	K^+ as KCl	5,300
Gambusia affinis	Wallen *et al.*, 1957	2,200	48H LC_{50}	K^+ as KCl	2,200
Cyprinodon variegatus	Pillard *et al.*, 1998a	1,212	48H LC_{50}	K^+ as KCl	1,212
Cyprinodon variegatus	Pillard *et al.*, 1998a	766	7D IC_{25}	K^+ as KCl	766
Menidia beryllina	Pillard *et al.*, 1998a	1,103	48H LC_{50}	K^+ as KCl	1,103
Menidia beryllina	Pillard *et al.*, 1998a	618	7D IC_{25}	K^+ as KCl	618

Table 4-8. Continued.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as K^+ (mg/L)
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	600-670	Growth	KCl	320-350
Nitzschia linearis	Patrick *et al.*, 1968	705	5D LC_{50}	K^+ as KCl	705

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-9. Results of Selected Toxicity Studies with Strontium.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as Sr^{2+} (mg/L)
Invertebrates					
Daphnia magna	Biesinger & Christensen, 1972	60	EC_{50} Reproduction	Sr^{2+} as $SrCl_2$	60
Daphnia magna	Khangarot & Ray, 1989	163	24H EC_{50} Immobilization	Sr^{2+}as $SrCl_2 \cdot 6H_2O$	163
Daphnia magna	Khangarot & Ray, 1989	94	48H EC_{50} Immobilization	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	94
Daphnia hyalina	Baudouin & Scoppa, 1974	75	48H LC50	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	75
Culex pipiens	Suzuki, 1959	5,500	ET_{50} Immobilization	Sr^{2+} as $SrCl_2$	5,500
Culex pipiens	Suzuki, 1959	5.5	ET_{50}	Sr^{2+} as $SrCl_2$	5.5
Polycelis nigra	Jones, 1940	6,600	48H Tox. Threshold	Sr^{2+} as $SrCl_2$	6,600
Tubifex tubifex	Khangarot, 1991	540	24H EC_{50} Immobilization	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	540
Tubifex tubifex	Khangarot, 1991	320	48H EC_{50} Immobilization	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	320
Mysidopsis bahia	Pillard *et al.*, 1998a	>332	48H LC_{50}	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	>332
Vertebrates					
Carassius auratus	Birge, 1978	8.58	7D LC_{50}	Sr^{2+} as $SrCl_2$	8.58
Oncorhynchus mykiss	Birge, 1978	0.2	28D LC_{50}	Sr^{2+} as $SrCl_2$	0.2
Cyprinodon variegatus	Pillard *et al.*, 1998a	>332	48H LC_{50}	Sr^{2+} as $SrCl_2 \cdot 6H_2O$	>332
Menidia beryllina	Pillard *et al.*, 1998a	215	48H LC_{50}	Sr^{2+}as $SrCl_2 \cdot 6H_2O$	215
Plants and Algae					
Chlorella vulgaris	DeJong, 1965	>150	Growth	$SrCl_2 \cdot 6H_2O$	>50

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

Table 4-10. Results of Selected Toxicity Studies with Sulfate.

Taxon	Source	Conc. (mg/L)	Endpoint	Ion/Compound	Conc. as SO_4^{2-} (mg/L)
Invertebrates					
Daphnia magna	Anderson, 1946	5,960	Thesh. Conc. Immobilization	Na_2SO_4	4,030
Daphnia magna	Dowden & Bennett, 1965	6,800	24H LC_{50}	Na_2SO_4	4,600
Daphnia magna	Dowden & Bennett, 1965	6,100	48H LC_{50}	Na_2SO_4	4,130
Daphnia magna	Mount *et al.*, 1997	6,290	24H LC_{50}	Na_2SO_4	4,255
Daphnia magna	Mount *et al.*, 1997	4,580	48H LC_{50}	Na_2SO_4	3,100
Ceriodaphnia dubia	Mount *et al.*, 1997	3,590	24H LC_{50}	Na_2SO_4	2,430
Ceriodaphnia dubia	Mount *et al.*, 1997	3,080	48H LC_{50}	Na_2SO_4	2,080
Culex sp.	Dowden & Bennett, 1965	11,430	24H LC_{50}	Na_2SO_4	7,730
Culex sp.	Dowden & Bennett, 1965	13,350	48H LC_{50}	Na_2SO_4	9,030
Artemia salina	Saliba & Ahsanullah, 1973	5,400	Mortality	SO_4-S as Na_2SO_4	16,178
Mysidopsis bahia	Pillard *et al.*, 1998a	16,715[a]	48H LC_{50}	SO_4^{2-} as Na_2SO_4	16,715
Mysidopsis bahia	Pillard *et al.*, 1998a	4,986[a]	7D IC_{25}	SO_4^{2-} as Na_2SO_4	4,986
Vertebrates					
Pimephales promelas	Mount *et al.*, 1997	>8,080	24H LC_{50}	Na_2SO_4	>5,460
Pimephales promelas	Mount *et al.*, 1997	7,960	96H LC_{50}	Na_2SO_4	5,380
Lepomis macrochirus	Dowden & Bennett, 1965	17,500	24H LC_{50}	Na_2SO_4	11,840
Cyprinidae	Turoboyski, 1960	4,500	Mortality	SO_4-S as Na_2SO_4	13,480
Gambusia affinis	Wallen *et al.*, 1957	5,400	24H LC_{50}	SO_4-S as Na_2SO_4	16,180
Gambusia affinis	Wallen *et al.*, 1957	3,940	48H LC_{50}	SO_4-S as Na_2SO_4	11,800
Morone saxatilis	Hughes, 1973	450	24H LC_{50}	SO_4-S as Na_2SO_4	1,350
Morone saxatilis	Hughes, 1973	220	48H LC_{50}	SO_4-S as Na_2SO_4	660
Cyprinodon variegatus	Pillard *et al.*, 1998a	>17,000[a]	48H LC_{50}	SO_4^{2-} as Na_2SO_4	>17,000
Cyprinodon variegatus	Pillard *et al.*, 1998a	11,720[a]	7D IC_{25}	SO_4^{2-} as Na_2SO_4	11,720
Menidia beryllina	Pillard *et al.*, 1998a	26,705[a]	48H LC_{50}	SO_4^{2-} as Na_2SO_4	26,705
Menidia beryllina	Pillard *et al.*, 1998a	14,006[a]	7D IC_{25}	SO_4^{2-} as Na_2SO_4	14,006
Plants and Algae					
Chlorella vulgaris	Becker & Keller, 1973	1,497	Growth	SO_4-S as Na_2SO_4	4,480
Nitzschia linearis	Patrick *et al.*, 1968	430	LC_{50}	SO_4-S as Na_2SO_4	1,288
Myriophyllum spicatum	Stanley , 1974	10,228	EC_{50} Root Biom.	Na_2SO_4	6,920
Myriophyllum spicatum	Stanley , 1974	10,370	EC_{50} Root Grth.	Na_2SO_4	7,010

[a] Unequilibrated concentrations; equilibrated concentrations are likely to be lower.

Note: Where the information is available, salts used in toxicity tests are shown, including waters of hydration.

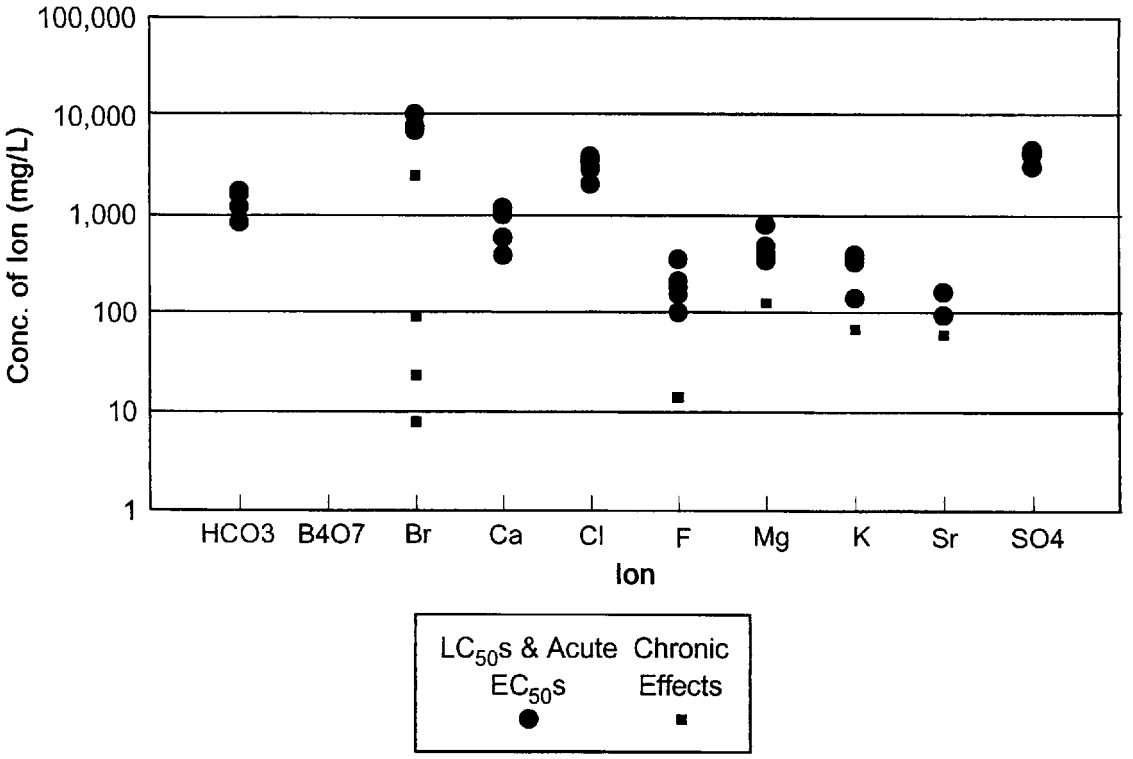


Figure 4-1. Acute and Chronic Test Results for *D. magna* (see Tables 4-1 to 4-10 for actual data).

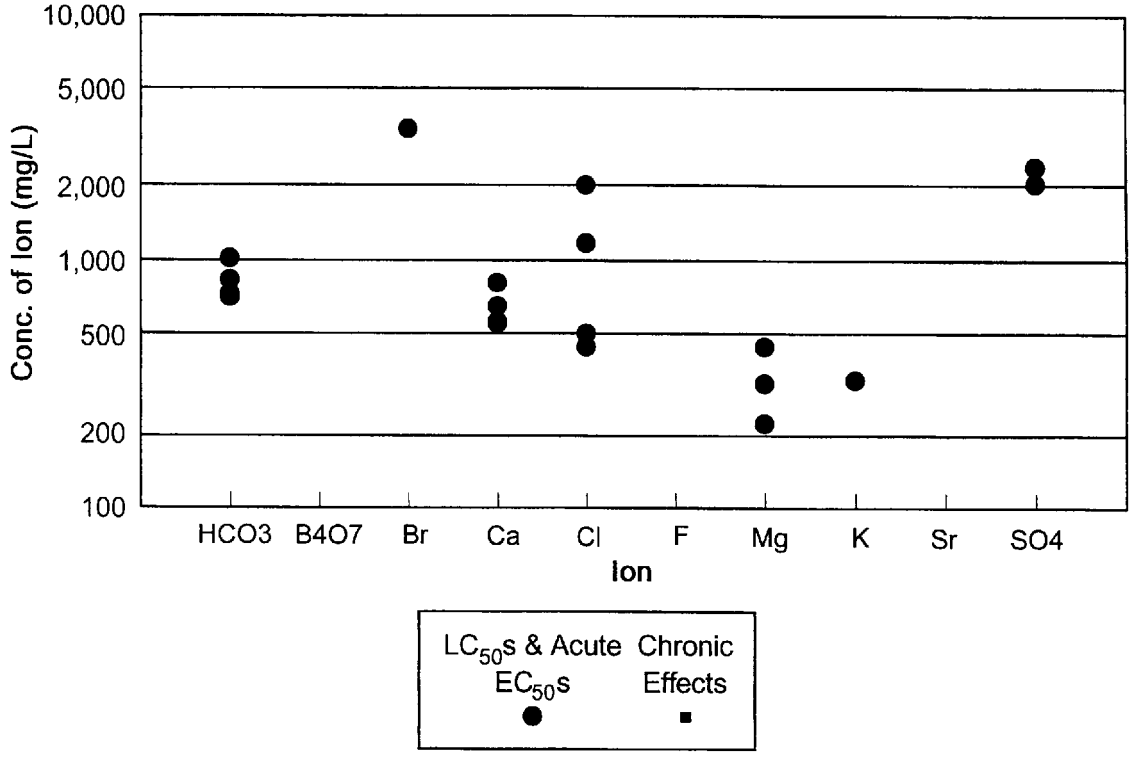


Figure 4-2. Acute and Chronic Test Results for *C. dubia* (see Tables 4-1 to 4-10 for actual data).

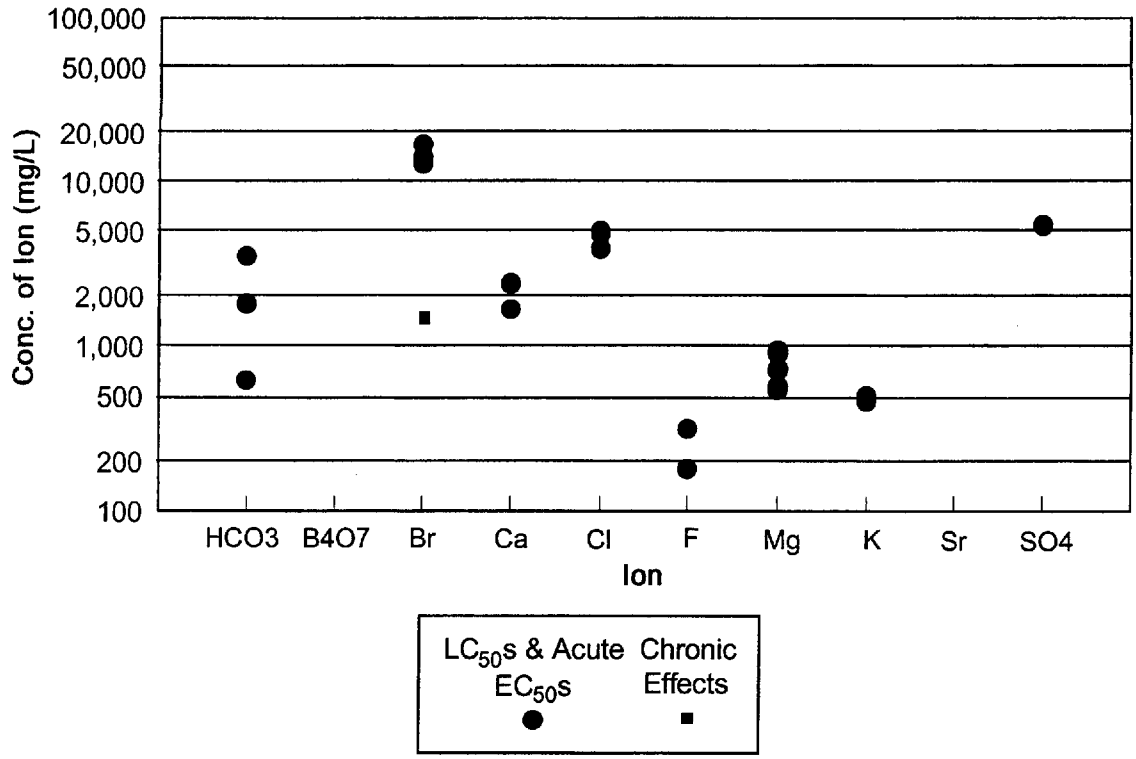


Figure 4-3. Acute and Chronic Test Results for *P. promelas* (see Tables 4-1 to 4-10 for actual data).

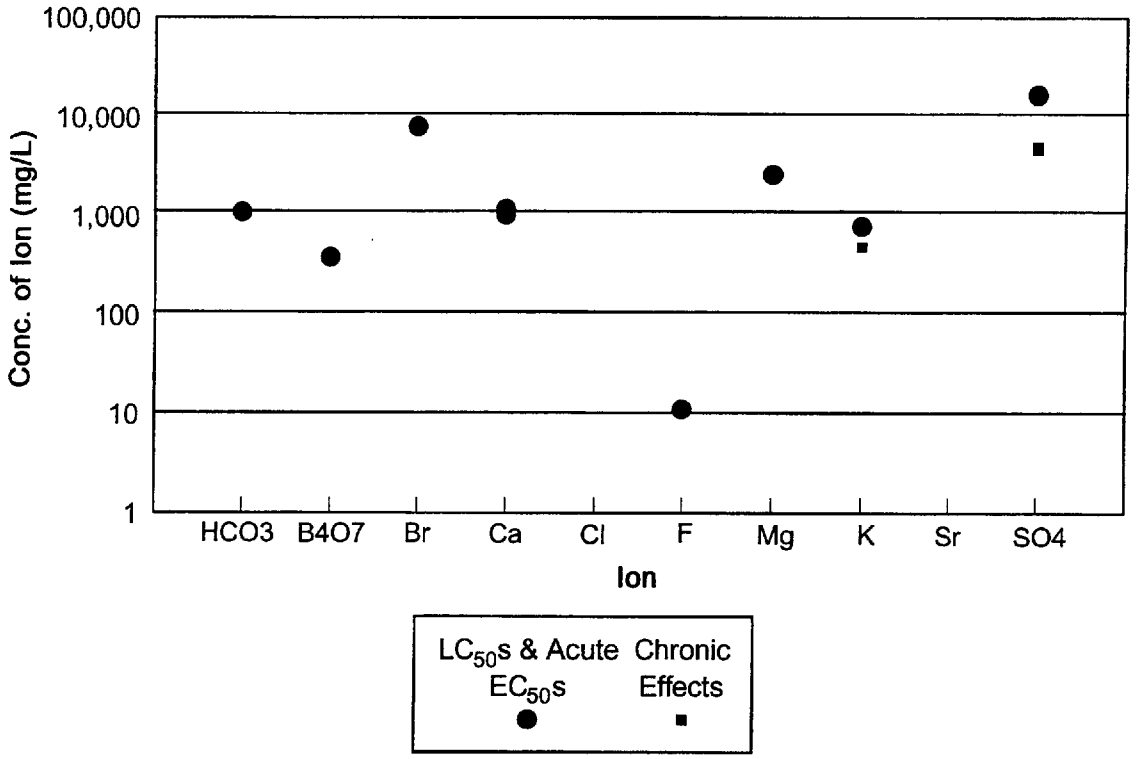


Figure 4-4. Acute and Chronic Test Results for *M. bahia* (see Tables 4-1 to 4-10 for actual data).

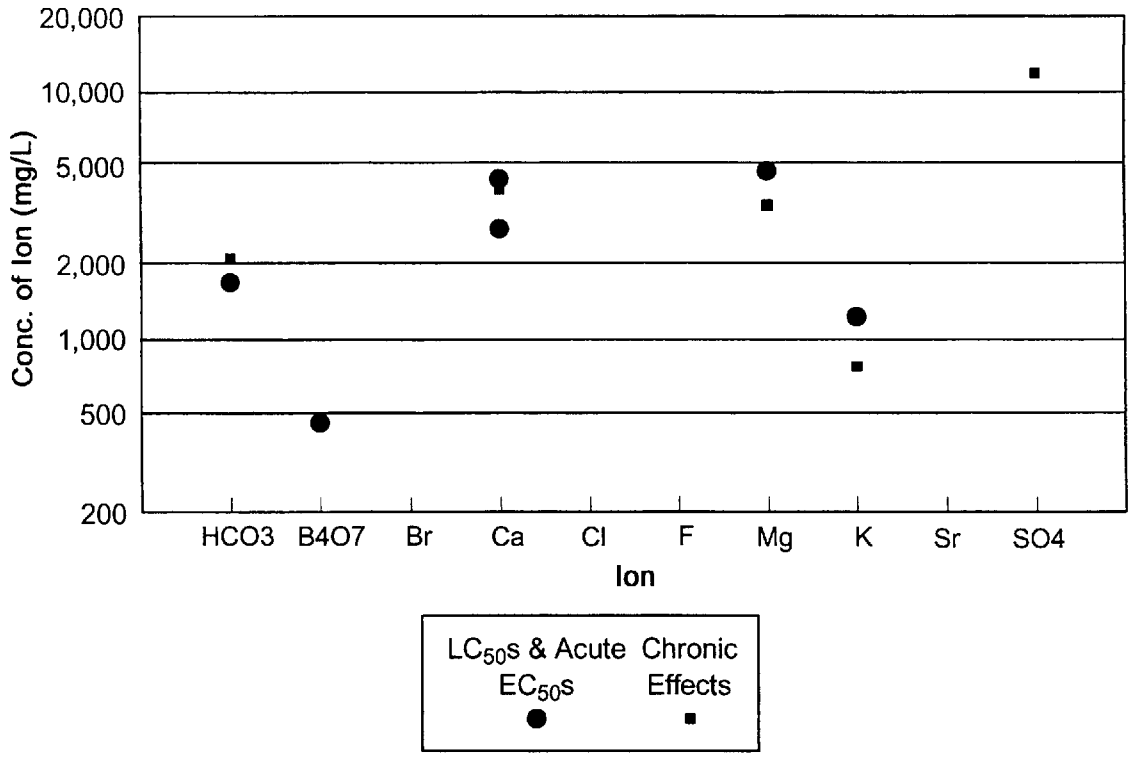


Figure 4-5. Acute and Chronic Test Results for *C. variegatus* (see Tables 4-1 to 4-10 for actual data).

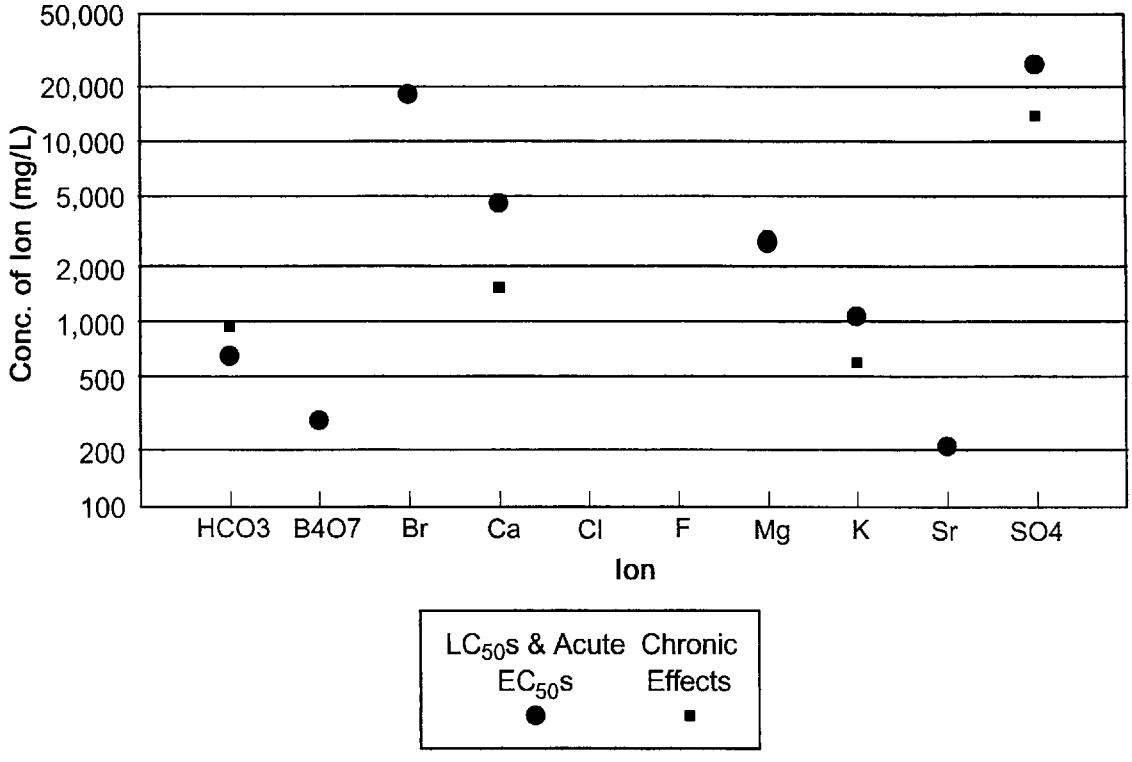


Figure 4-6. Acute and Chronic Test Results for *M. beryllina* (see Tables 4-1 to 4-10 for actual data).

test waters, culture conditions, food quality, and genetic strains of test organisms. The most reliable interspecies comparisons can be done by using data collected under the same or similar conditions. The studies conducted by Mount *et al.* (1997) meet these requirements. All studies were conducted in the same laboratory using identical equipment and organisms from the same culture. For all salts tested, *C. dubia* is more sensitive than either *D. magna* or *P. promelas* (Figure 4-7). For some salts the toxicity differences among species are substantial (e.g., NaCl), while toxicity due to $CaSO_4$ is similar among all three species.

The absence of certain essential ions in the exposure medium can cause toxicity, particularly to marine species. Pillard *et al.* (1998a) found that a lack of Ca^{2+} or K^+ in artificial seawater resulted in rapid mortality of *M. bahia*, *C. variegatus*, and *M. beryllina.* The deficiency 48-hour LC_{50}s for these three species for Ca^{2+} were 99.4, 218.4, and 5.8 mg/L, respectively. The deficiency 48-hour LC_{50} s for *M. bahia* and *C. variegatus* for K^+ were 115.3 and 24.0 mg/L, respectively. Douglas and Horne (1997) found that, in addition to Ca^{2+} and K^+, Mg^{2+} and Br^- are needed for long-term survival of *M. bahia.*

Because many of the ions discussed are essential to aquatic animals, they often are readily accumulated in plasma and tissues. Ca^{2+}, for example, is stored in hard structures such as bone and shells. Organisms are generally adapted for absorbing, storing, and excreting essential ions through physiological mechanisms as described in Section 5. Although there are definite limits to the concentrations of ions that organisms can tolerate, most can withstand and regulate within a species-specific range. Accumulation of nonessential ions may pose greater risks to aquatic organisms, particularly when ambient concentrations undergo a rapid, dramatic change. F^-, for example, has been shown to accumulate primarily in skeletal structures of both vertebrates (Neuhold and Sigler, 1960; Wright and Davison, 1975; McClurg, 1984; Camargo, 1996) and invertebrates (Barbaro *et al.*, 1981; McClurg, 1984). Because F^- forms tight bonds with Ca^{2+} in skeletal structures, Ca^{2+} deficiency can result if the body needs to sequester Ca^{2+} from bone to bolster low levels in intracellular and extracellular fluids; tetany and death may result.

Plants have been shown to accumulate and tolerate some ions to a much higher degree than animals. This difference is often related to the fact that while some elements are not needed by animals, they are essential to plants. Boron (B^-) is one such element. Saiki *et al.* (1993) found that B^- (along with molybdenum) concentrations were higher in filamentous algae than in

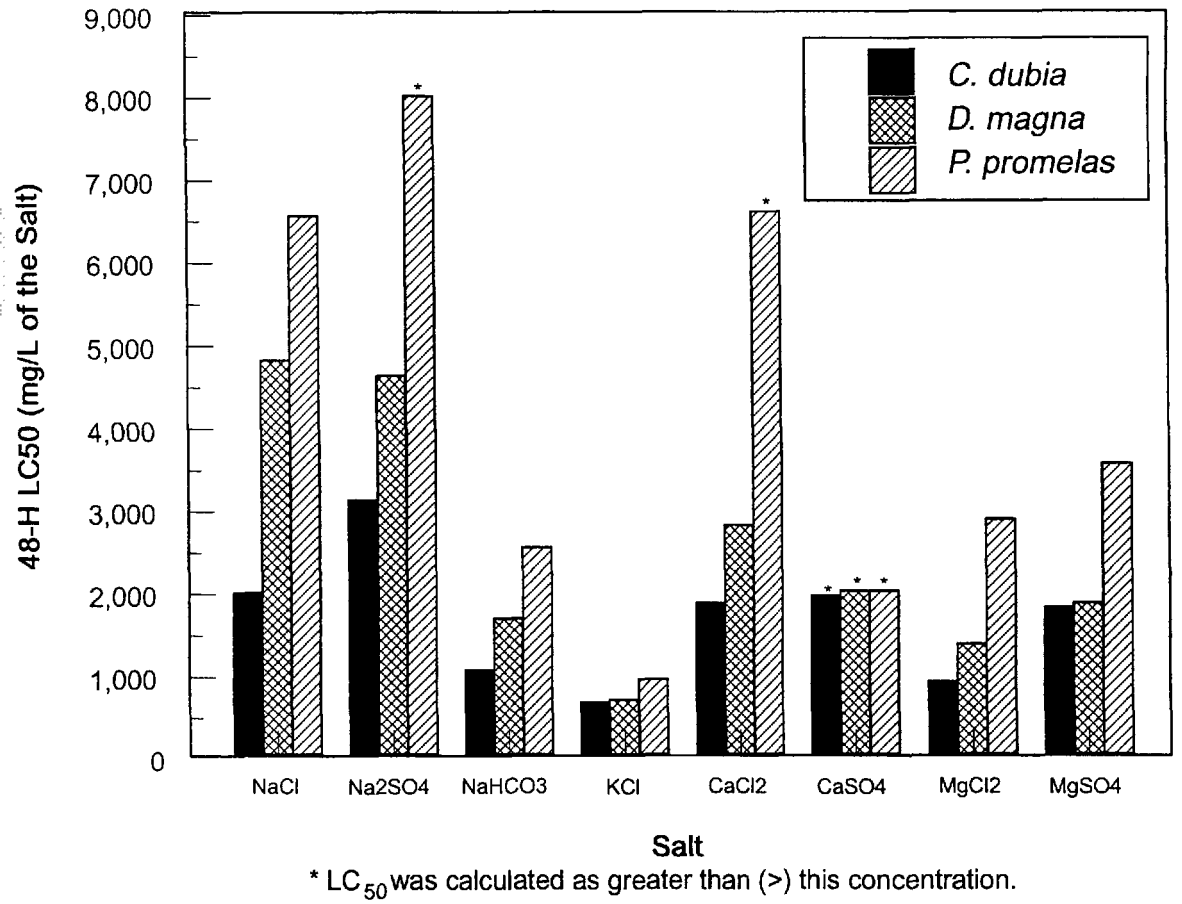


Figure 4-7. 48-Hour LC_{50} s of Three Species Exposed to Different Salts (data from Mount *et al.*, 1997).

invertebrates or fish in the saline waters of the San Joaquin, CA river system. Dickerson and Ramirez (1997) found that concentrations of B^- in the pondweed, *Potamogeton vaginatus*, regularly exceeded 300 μg/g and occasionally exceeded 900 μg/g. However, B^- concentration in invertebrates, fish, or bird eggs was not elevated. These studies indicate that while elements such as B^- may accumulate in food plants, they are not biomagnified by consumers.

Section 5
PHYSIOLOGY OF MAJOR IONS IN AQUATIC SYSTEMS

The seven ions most prevalent in the aquatic environment are Ca^{2+}, Cl^-, HCO_3^-, K^+, Mg^{2+}, Na^+, and SO_4^{2-}. They are involved in nearly all physiologic processes and share essential, fundamental roles in biology. Toxicity might be expected only when ambient concentrations fall outside the range of an organism's capacity to regulate uptake and release. All of these ions are included in USEPA recipes for artificial freshwater and saltwater, which can be used for culturing and testing (Weber, 1993). Other minerals (e.g., selenium, copper, iron) are also essential nutrients for aquatic organisms (National Research Council, 1993). The importance of some minerals is yet to be defined. It has been suggested, for example, that F^- may be beneficial to fish by reducing bacterial kidney disease (Bowser *et al.*, 1988). However, while this action may be possibly advantageous, such benefits do not denote essentiality. Approximate dietary requirements of essential ions to rainbow trout are presented in Table 5-1.

This review provides a summary of the relevant physiologic considerations and attempts to focus on the ionic dependence of processes common to all organisms. While it is well beyond the scope of this monograph to comprehensively describe the comparative physiology of salt and water balance among the aquatic organisms, it does provide an initial framework with which the physiological effects of inappropriate environmental levels of common ions can be viewed. The first few pages of this section describe some of the general mechanisms used by freshwater and marine organisms to control the loss and/or gain of ions and water in different environments. The importance of different organs and tissues is then discussed, followed by a more detailed description of the roles individual ions play in biochemical reactions and homeostasis.

A discussion of the physiological roles of ions and the implications of inappropriate environmental levels sometimes requires detailed descriptions of organ, tissue, and even cellular structures, mechanisms, and processes. As a result, the reader is likely to find this section more technical than the rest of the report.

Table 5-1. Inorganic Ion Requirements for Rainbow Trout as a Percent of Diet.

Ion	Percent of Diet
Sodium	0.6[a]
Calcium	1.0[a]
Magnesium	0.05
Potassium	0.7
Chlorine	0.9[a]

Source: NRC (1993).
[a] Estimate.

GENERAL OSMO- AND IONOREGULATION IN AQUATIC ANIMALS

Invertebrates

For the most part, the internal fluids of invertebrate organisms in freshwater systems are hypertonic to the external media. There is, therefore, a tendency for continuous movement of water into body tissues through all permeable surfaces, including cuticle, chitin, gills, and epithelia. The permeability of surfaces varies among taxonomic groups (Pennak, 1978). Age is also likely to play a role because older organisms may have thicker, and thus less permeable, surfaces. Freshwater invertebrates rid themselves of excess water through specialized structures that function to produce a highly dilute urine. Those structures include contractile vacuoles (in protozoans), flame bulb systems, nephridia, and different types of glandular structures (Pennak, 1978). Many freshwater invertebrates, including Crustacea and Insecta, have a relatively hard exoskeleton which must be periodically shed, or molted, to allow the body to grow. When the hard exoskeleton is shed the soft body immediately is subject to an influx of water. This influx expands the body before the new exoskeleton can harden and thus is an important factor in invertebrate growth (Krogh, 1965).

Passive infiltration of excess water into body tissues is not generally a problem for marine invertebrates due to the higher ion concentrations of seawater. The water transport mechanisms that developed in freshwater species are reduced or absent in their marine counterparts. Marine protozoans, for example, lack the contractile vacuoles found in freshwater protists (Pennak, 1978). The osmoregulatory glands of marine isopods, amphipods, and decapods are smaller than those in their freshwater relatives. This characteristic indicates a reduced capacity for water transport (Pennak, 1978).

Marine invertebrates tend to be osmoconformers, relying on osmotic pressures to maintain their internal ionic balance. Invertebrates in euryhaline environments face a wider range of osmotic conditions and thus have an increased need to respond to environmental stress. Various adaptive strategies exist for survival under these conditions. The estuarine turbellarian, *Gunda olvae*, for example, allows its body water volume to increase or decrease, depending upon the salinity of the external media (Krogh, 1965). *Callinectes sapidus*, a euryhaline crab, increases its salinity tolerance range by reducing the permeability of its gill surfaces, thus conserving energy that would otherwise be used in the active transport of ions out of the body (Piller *et al.*, 1995). Larvae of several species of the genus *Aedes* have adapted to osmotic conditions ranging from very dilute water to several times the concentration of seawater. Species in a saline media constantly lose water to the external environment. Compensation is achieved through a high rate of water ingestion and the formation of hyperosmotic urine (Bradley *et al.* 1984).

Fish

The tissues of freshwater fish are hypertonic relative to the external environment, so there is a constant passive flow of ions out of the body, and a passive movement of water into the body (Figure 5-1). Because freshwater fish tend to gain water, primarily through the gill, they actually drink little water and do not absorb much water through the intestine. In addition, large volumes of water are excreted by the kidney (Takei, 1993). Dietary uptake of Na^+ and Cl^-, as well as active uptake of these ions at the gills, compensate for the loss through diffusion as well as through the urine (Lin and Randall, 1995). The amount of salt lost by freshwater fish varies with species. A goldfish (*Carassius auratus*) loses approximately 5% of its body chlorides in a day, while the Atlantic salmon (*Salmo salar*) in freshwater may lose up to 17% (Lagler *et al.*, 1977).

It is also evident that energy must be expended to accumulate K^+, Mg^{2+}, Ca^{2+}, and SO_4^{2-}. The fundamental task of ion regulation in freshwater fish (and invertebrates as well) is the acquisition and retention of scarce macro- and micronutrients. The exchange mechanisms used for NaCl uptake in very dilute environments depend on concomitant acid-base regulation. Therefore, compensation for inappropriate NaCl levels can compromise acid-base balance.

The gills account for as much as 50% of a fish's surface area and invariably dominate the task of salt acquisition. In many fishes, however, the integument is salt-absorbing (and often respiratory as well) so that smaller organisms with a large surface area-to-volume ratio can

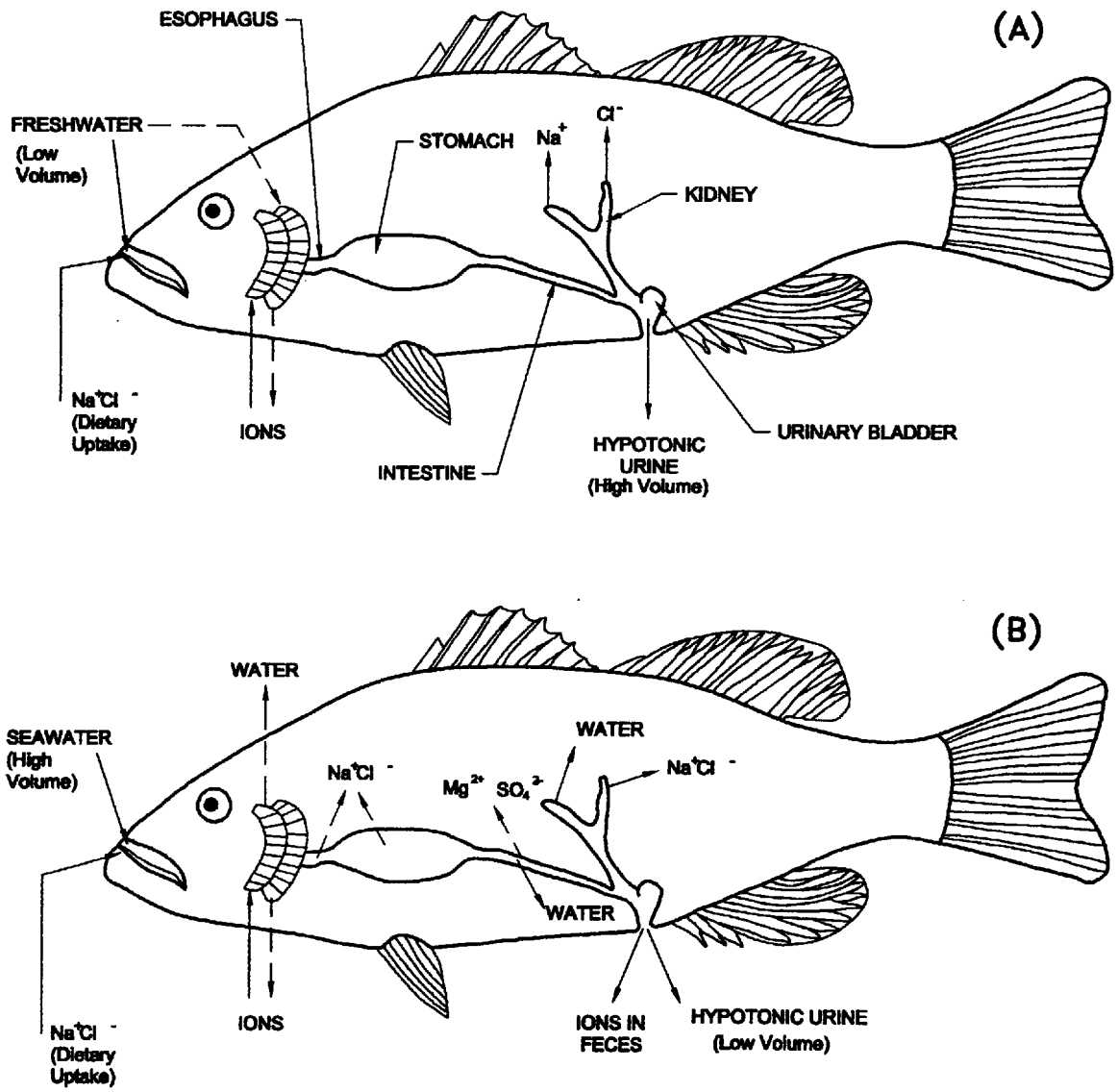


Figure 5-1. Inflow and Outflow of Water and Salts in Freshwater (A) and Marine (B) Teleosts. Broken Lines Represent Passive Movement While Solid Lines Represent Active Transport.

accommodate the large salt losses that such geometry promotes. Many of the mineral nutrient needs of freshwater animals are met only through the food chain. Viability, therefore, depends to a large extent on intestinal absorptive mechanisms and the continued viability of critical food chain carriers of essential nutrients. Because of this dependence, freshwater animals are somewhat less independent than their marine counterparts, which obtain some minerals via direct uptake from the media.

Marine fish have evolved a variety of strategies for dealing with the hypertonic environment. The myxinoids (hagfish) are unique in that the extracellular fluids generally resemble seawater in ionic composition, which is similar to marine invertebrates (Rankin *et al.*, 1983; Takei, 1993). Hagfish (e.g., *Myxine glutinosa*) have only a limited ability to osmoregulate and generally do not need to do so. However, hagfish do have serum Mg^{2+} levels approximately 1/10 that of seawater; serum Na^{+} levels are correspondingly increased (Rankin *et al.*, 1983).

Marine elasmobranchs (e.g., sharks and rays) accumulate urea and trimethylamine oxide (TMAO) in their plasma to maintain a blood osmolality slightly higher than that of seawater (Takei, 1993). Because the concentration of NaCl is still higher in seawater, there tends to be a net passive flow of these ions into the bodies of elasmobranchs. Osmoregulation therefore primarily occurs through ionoregulation, i.e., the elimination of excess NaCl. This elimination occurs through the shark rectal gland, which secretes a fluid containing high levels of NaCl (Valentich *et al.*, 1995). The coelocanth (*Latimeria*) has evolved similar specialized devices for the excretion of excess salt. These animals may also employ their kidneys where urea and TMAO are reabsorbed to offset losses across the integument and the gills.

Marine teleosts have evolved regulatory mechanisms that allow them to live in seawater while maintaining the body fluid osmolalities characteristic of freshwater fishes. In general, the blood of teleost fishes has an osmolality of 300 mOsm/kg, while the seawater around them has an osmolality of up to 1,000 mOsm/kg (Takei, 1993). Saltwater fishes therefore are faced with the diffusional entry of salts through membranes as well as the osmotic loss of water, principally across the gill epithelium. To replace the water, marine teleosts drink copious amounts of saltwater, which cannot be absorbed immediately (because of the osmotic gradient), but first must be stripped of much of its NaCl (Figure 5-1). This process is accomplished by the diffusion of NaCl across the wall of the esophagus and stomach (Rankin *et al.*, 1983). With the change in osmotic gradient, water can be absorbed across the wall of the intestine (Loretz,

1995). The Na^+ and Cl^- gained through ion uptake from ingested seawater and passive diffusion at the gills are eliminated by active extrusion by the chloride cells of the operculum and gill (Takei, 1993; Loretz, 1995). Saltwater fish generally produce very small volumes of urine that contain divalent ions in high concentrations (Rankin and Davenport, 1981). Divalent ions are also lost in the feces.

In all fishes, ion-transporting epithelia are of crucial importance in maintaining homeostasis by regulating such things as plasma ion levels, pH, and water balances (Valentich *et al.*, 1995). In addition to the chloride cells present in gill lamellar epithelium, which play an important role in ionoregulation, the mucous (or goblet) cells and the epithelial (or pavement) cells are also present. Mucous cells, as the name implies, secrete a thin layer of mucous onto the gill surface. Gas exchange is accomplished primarily through the epithelial cells which are permeable to O_2, CO_2, and NH_3 (Lin and Randall, 1995). Because of the importance of the epithelia in maintaining ion and water balances, they are sensitive to several control mechanisms. It is likely that chloride cells, for example, are subject to multihormonal control (Marshall, 1995).

Although osmo- and ionoregulation required by freshwater and marine stenohaline organisms may seem daunting, euryhaline organisms, which move between fresh and saltwater during their lifetime, face an even greater challenge. Euryhaline organisms include those inhabiting estuaries, where dramatic changes in salinity associated with tides can occur four times a day, and those that travel between fresh and saltwater to breed and lay eggs. Euryhaline organisms must possess the ability to secrete salt in marine conditions and absorb it from freshwater. Teleosts with this character include the intertidal blenny (*Xiphister*) and the killifish or mummichog (*Fundulus*). Anadromous fish, that spend the majority of their life in the sea but return to freshwater to breed, include the lamprey and several salmonid species. Catadromous fish, on the other hand, breed in the sea after migrating from freshwater (e.g., eels).

For fish moving from salt to freshwater, the containment of ion loss is critical. When eels (*Anguilla anguilla*) are transferred from saltwater to freshwater, there is an immediate reduction in Na^+ outflux, which is high in saltwater. An additional reduction occurs approximately 30 minutes later (Rankin and Davenport, 1981). Marine fish that lack an ability to reduce ion loss will die if transferred to freshwater. Transfer of an eel back to saltwater causes an increase in Na^+ outflux and a concurrent increase in chloride cells and Na^++K^+-activated ATPase. The

hormone cortisol, secreted by the interrenal gland, appears to play a major role in the physiological changes that occur in eels transferred between salt and freshwater (Forrest *et al.*, 1973). The permeability of the esophagus also is reduced in freshwater eels, an effect that is influenced by both cortisol and prolactin (Hirano, 1980).

Freshwater teleosts that migrate to saltwater face the task of reducing the amount of dilute urine produced. Rainbow trout show a substantial decrease in both the glomerular filtration rate (GFR) and urine flow when they are transferred from fresh to saltwater (Rankin and Davenport, 1981). Certain neurohypophysial hormones, including arginine vasotocin (AVT) and isotocin, may play a critical role in reducing urine flow (Rankin and Davenport, 1981). AVT appears to act as an antidiuretic hormone by afferent arteriolar constriction (Takei, 1993).

FLUIDS

To understand the manner in which altered levels of common ions in the environment might impact organisms, it is necessary to consider some of the characteristics that influence toxicity. The observed strategy is to protect cellular metabolism by generation of an idealized ionic environment inside cells and to progressively refine the nature of the environment outside the cell membrane. These fluid environments are in constant contact through channels that permeate the cell walls. Control mechanisms therefore are critical in preventing disruptive changes within the cell due to inappropriate ion levels in surrounding fluids.

Intracellular Fluid

The intracellular fluid (ICF) is an extensively regulated solution because it is the bathing medium in which all critical metabolic processes ultimately occur. The ICF environment shows little variance across most taxa. Osmolality of ICF generally is adjusted with altered levels of organic compounds. In most organisms, the following ICF characteristics are common:

- K^+ is the dominant cation, followed by Mg^{2+};
- Na^+ and Cl^- are maintained at comparatively low levels considering their usual dominance of the external environment and the extracellular fluid;
- Free Ca^{2+} concentration is very low.

ICF is very dependent on high capacity regulatory mechanisms because the requirements of metabolic processes are often very narrow. Dependence of enzymatic processes on an electrolyte environment, as in the ICF, was demonstrated by Hastings (1941). He showed experimentally that rat liver slices could not synthesize glycogen from glucose in simulated,

high Na^+, extracellular fluid (ECF), but that *in vitro* synthesis did take place in a zero-Na^+, high K^+, high Mg^{2+} medium (simulating ICF).

The ICF is maintained by both passive and active transport processes. High K^+ and low Na^+ result from the continuous operation of a membrane-bound, Mg^{2+}-dependent Na^+-K^+-ATPase, referred to as the "sodium pump" (Figure 5-2). With energy derived from the conversion of ATP to ADP and inorganic phosphate, the pump moves 3 Na^+ ions out of the cell in exchange for 2 K^+ ions. Backleak of these ions is through a variety of channels that can act as exchange mechanisms (antiporters), or coupled carriers (symporters) and thus affect the distribution of other solutes as well. The asymmetric pumping of univalent cations (3 for 2) results in an electronegative interior that further contributes to the forces that dictate ion distribution. Internal Ca^{2+} is at very low concentrations (0.1 to 1.0 µM) despite extracellular concentrations in the millimolar (mM) range. These low internal Ca^{2+} levels are achieved by a Mg^{2+}-requiring, Ca^{2+}-ATPase in both the plasma membrane and the endoplasmic reticulum that pumps Ca^{2+} ions out of the cytosol, and by an antiport channel that shuttles 3 Na^+ ions into the cell while expelling one Ca^{2+} ion. Mg^{2+} ions are routinely present at between 25 and 45 mEq/L in the cytosol, but free ion concentrations are from 2 to 6 mEq/L because most of the ion is bound (probably to negative sites on cytoplasmic proteins).

The maintenance of Mg^{2+} out of electrochemical equilibrium is generally observed but its basis is not understood. Cl^- ions are distributed at electrochemical equilibrium with high permeability coefficients across the plasma membrane; the electronegative interior (-60 to -90 mV) results in cytosolic Cl^- values 10 to 15 times lower than those of the ECF. Intracellular macromolecules (proteins, nucleic acids) are polyanionic, but because of the need for electroneutrality, the cell contains an excess of mobile cations, over anions, to shield these negative charges. This arrangement results in a Donnan distribution of ions with a resulting osmotic pressure profile that would swell the cell were it not for the action of the sodium pump that maintains Na^+ outside the cell to balance osmotic pressures across the cell membrane (Figure 5-3) (Rankin and Davenport, 1981).

Extracellular Fluid

The ECF directly surrounds the cells and represents the medium which, in a controlled way, interacts with the intracellular fluid. For protozoans, this is their external environment and these cells are, therefore, exposed directly to environmental variations. The large size of protists

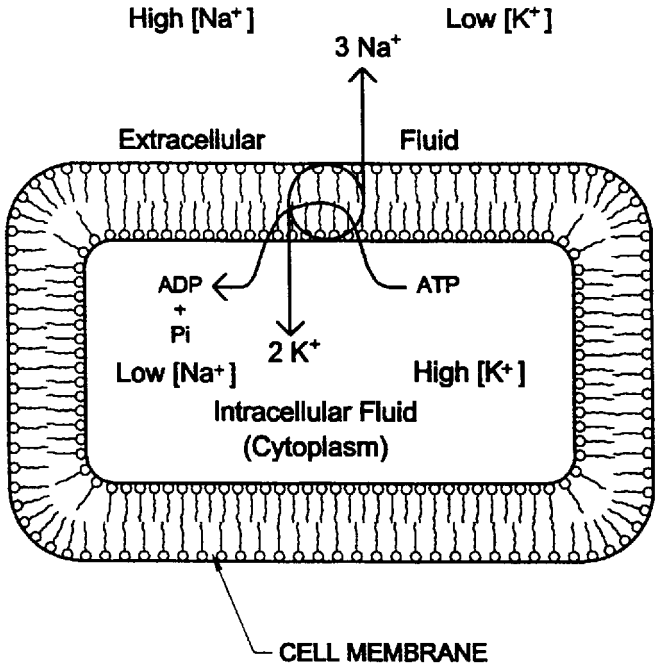


Figure 5-2. The Mg^{2+} - Dependent Sodium Pump.

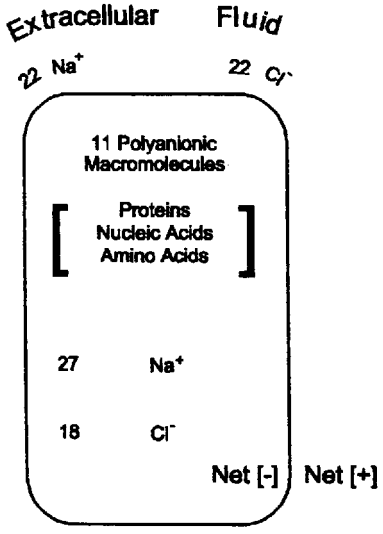


Figure 5-3. Hypothetical Donnan Distribution of Ions Maintained by the Sodium Pump in Animal Cells.

(relative to single cells in multicellular organisms) results in a low membrane surface area-to-cell volume ratio, which minimizes unfavorable ionic exchange. In multicellular animals (metazoans), there is a blastocoel, originating as the central cavity formed by cleavage of the fertilized egg, and, in more advanced metazoans, a coelom, that is established as a separate compartment by folding of the mesoderm (Hickman *et al.*, 1974). The primitive phyla (e.g., Porifera, Cnidaria) have an extracellular fluid space between epidermis and endodermis; in many marine species this space is in near-equilibrium with the external environment. The flatworms and the nemerteans have a blastocoel filled with loose tissue (parenchyma) but no formed coelomic cavity (Figure 5-4). Pseudocoelomate species (e.g., nematodes, rotifers) do have a body cavity, although it is not formed within embryonic mesoderm, but represents a persistent blastocoel. The more advanced marine and freshwater species regulate the composition of the blastocoel fluid. More complex organisms (annelids to mammals) are coelomate (Figure 5-4). In some invertebrates, the coelom is not extensive and the blastocoel is the dominant extracellular compartment referred to as the hemocoel and contains the blood of an open circulatory system. Closed vascular systems form via restriction of the blastocoel space and contain blood as a solution apart from the interstitial fluid (lymph) that bathes the cells (the ECF).

There is a range of strategies for maintenance of extracellular fluids. Almost all organisms are ionoregulators, maintaining ECF ions at different levels than in the external environment. While ionoconformers are scarce (jellyfish and the hagfish approximate this condition) many organisms possess body fluids at total osmolyte concentrations matching the ambient environment and are therefore osmoconformers. This characteristic is not necessarily restrictive; the shrimp, *Callianassa*, adjusts body fluid osmolality in parallel with environmental changes from 300 to 1200 mOsm/kg. Osmoregulators maintain an ECF different from their environment in osmotic strength. For example, *Upogebia*, also a shrimp, preserves an ECF about 200 mOsm/kg above ambient levels of 200 - 700 mOsm/kg but becomes an osmoconformer as external osmolality increases further. To osmoconform and ionoregulate, animals complement a reduced ionic total with organic molecules. *Carcinus* (a crab) uses amino acids while *Squalus* (the dogfish) enhances osmolality with a balance of counteracting solutes including urea, trimethylamine oxide (TMAO) and betaine. Numerous marine vertebrates, notably the teleosts, iono- and osmoregulate by maintaining Na^+, Cl^-, and total osmolytes at levels less than half those found in the sea.

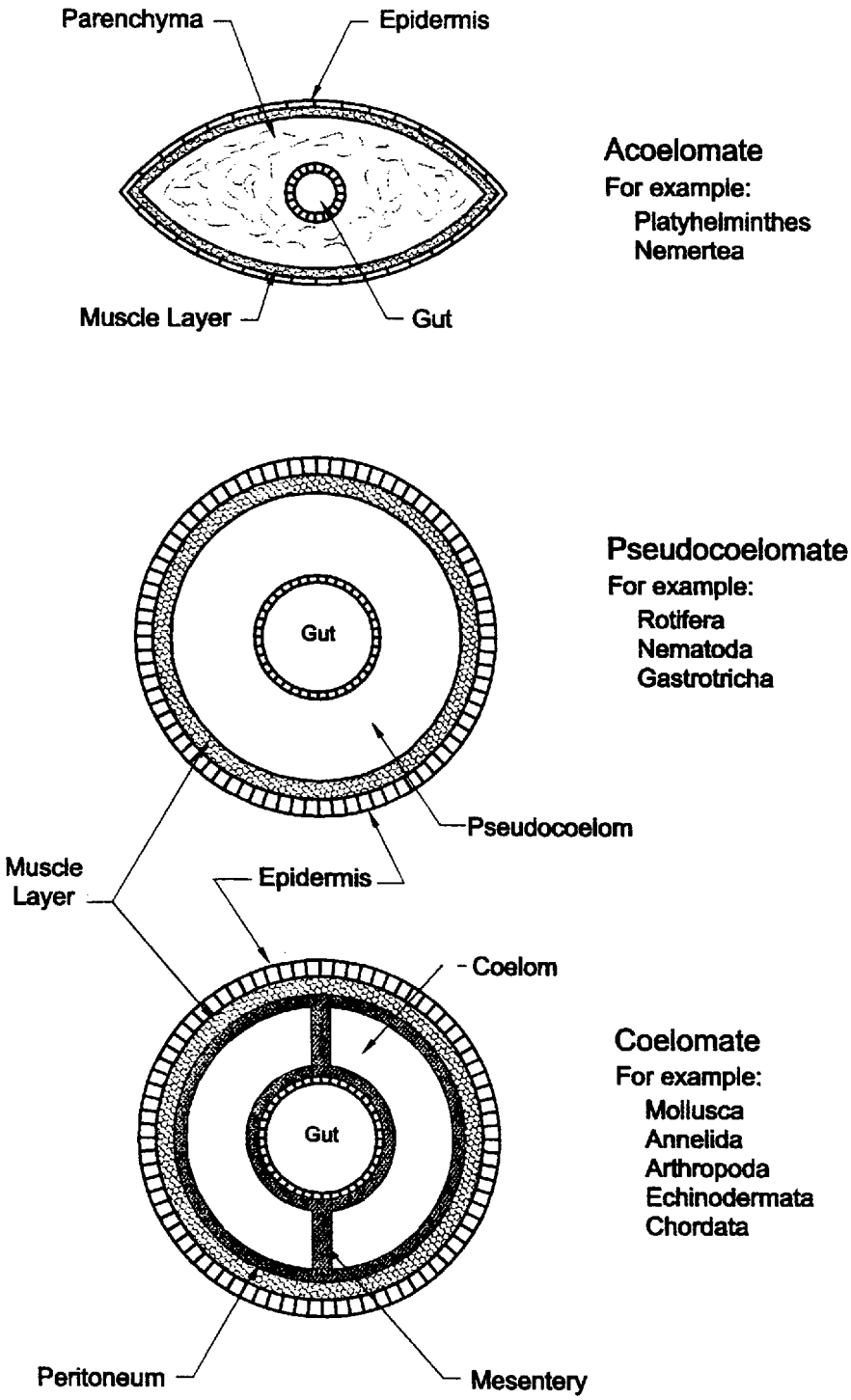


Figure 5-4. Generalized Body Plans of Metazoans.

In all cases, from near conformity with the environment to strict preservation of a markedly different solution, the maintenance of the ECF in a steady-state (i.e., the preservation of homeostasis) is a first priority (Cannon, 1929). There are situations where, at least for short term crisis-management, organisms turn to a strategy labeled enantiostasis (Mangum and Towle, 1977). This term describes situations in which function is maintained by counteracting a change in one physiologic parameter with a compensatory change in another. Over time, with continuous exposure, organisms acclimate to hostile environments, thus securing homeostasis of the ECF.

ABSORPTION AND EXCRETION IN AQUATIC ORGANISMS

For all aquatic organisms, the essential need for gas exchange (primarily oxygen and carbon dioxide) is often in direct conflict with the need to maintain a balance of ions and water. The permeability of surfaces that allows movement of gases also promotes passive exchange of other materials. Some exchange of ions and water occurs through the integument; this is especially true for some invertebrates. For vertebrates and many higher invertebrates, absorptive and excretory activities are dominated by the gills and excretory organs.

Gills

The gills constitute the major ion-exchange organs for a great many aquatic organisms. The various highly folded surfaces presumably have evolved to optimize gas exchange for respiration. Gills are also well-suited for absorption or secretion of salt and/or water with a large surface area and a rich vasculature to maintain gradients for solute and solvent transport. Gills are similar in salt absorption characteristics among vertebrates and invertebrates as illustrated by comparing the studies of Degnan *et al.* (1977) on *Fundulus heteroclitus* (killifish) with that of Burnett and Tole (1990) on *C. sapidus* (blue crab). Each of these organisms hyper-ionoregulates in low salinities, displaying vigorous Na^+-K^+-ATPase activity on the inner, serosal surface and Na^+/H^+ exchangers on the external surface in keeping with the design of salt-absorbing epithelia. Similar results were found in a comparative study by Kirschner *et al.* (1973), which used the diuretic, amiloride, as a probe (Kleyman and Cragoe, 1988) to establish the presence of externally disposed Na^+/H^+ antiporters in gills of both trout and crayfish (*Procambarus*). There is also evidence for Na^+/ammonia (NH_4^+) exchange on gill external surfaces.

Cl^- absorption from very low environmental concentrations is also associated with an exchange process where Cl^- is exchanged for HCO_3^- or OH^-. It is possible that salt acquisition may be more dependent on the simultaneous activities tied to acid-base balance than to the classical model where the sodium pump is the site of primary active transport (Kerstetter and Keeler, 1976). Mechanisms of the saltwater gill generally are defined more clearly than those of the freshwater gill. For the saltwater gill the fundamental requirement for salt balance is NaCl secretion; a detailed mechanistic model exists which is generally accepted (Karnaky, 1986).

Excretory Organs

Kidneys or analogous organs provide primary excretory mechanisms in multicellular organisms. The metanephridia of higher invertebrates, like vertebrate kidneys, are positioned between a coelom and the exterior of the animal. They have an organized distribution of "podocytes" overlying the basal lamina of small blood vessels to provide a filter for muscle-mediated expression of vascular fluid into the coelom and then to an open duct for both reabsorption of water and selected solutes as well as transport of metabolic waste products to the outside. With minor differences (e.g., cilia-driven fluid transport and distribution over multiple sites) the protonephridia of lower invertebrates perform comparable functions.

The nephrons of a typical fish kidney are made up of a renal corpuscle and a kidney tubule (Figure 5-5). The tubules collect urine to be transported to the external environment via the mesonephric duct. Afferent and efferent arterioles lead into, and away from, the renal corpuscle. Inside the capsule of the corpuscle, the tightly coiled arterioles are referred to as the gomerulus. From this structure water is filtered, as appropriate, from the blood, and salts are reabsorbed or excreted. In freshwater fish, the glomeruli are generally well-developed and are present in greater numbers than in marine fish. In saltwater species that must retain water against an unfavorable gradient, the number and size of glomeruli are reduced. Although ion reabsorption occurs in the tubules of freshwater fish kidneys, some loss, particularly of Cl^- ions, also occurs. This loss is countered by the chloride cells at the gill epithelia.

Kidney structure varies substantially in marine fish. Hagfish retain a pronephros as an adult; the mesonephros is primitive in this group. Other species of bony fish may also have functional pronephroi (Lagler *et al.*, 1977). Surplus salts in marine bony fishes are generally eliminated through the gut and the gills (chloride cells); only trace amounts leave in the urine. Hagfish may also rid their bodies of excess Mg^{2+}, Ca^{2+}, and K^+ in the copious slime produced by the

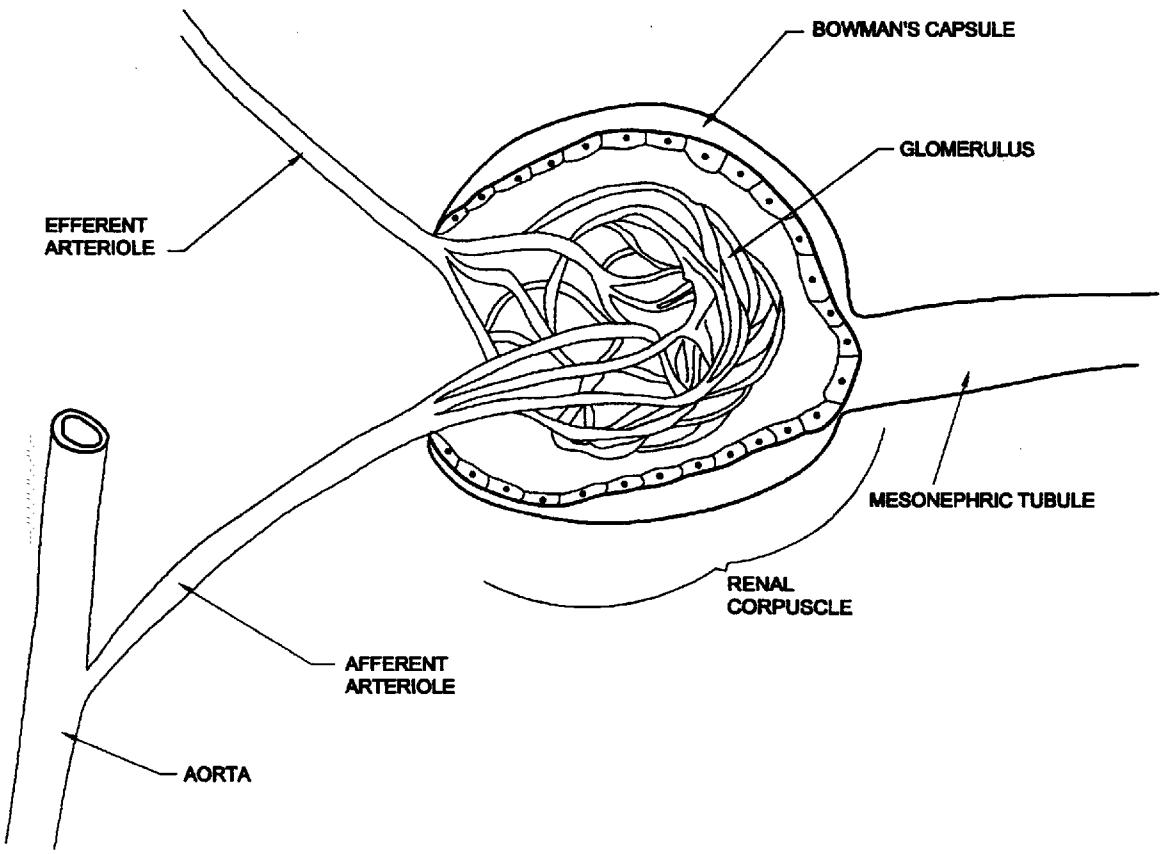


Figure 5-5. Teleost Mesonephron.

skin. Marine elasmobranchs have a specific organ, the rectal gland, which aids in the secretion of salts, primarily NaCl.

ROLES OF THE COMMON IONS IN THE PHYSIOLOGY OF AQUATIC ANIMALS

The roles of specific ions are, to a large extent, based on their essential nature as enzyme cofactors or in structural situations where size, charge, and charge density are sufficiently proscribed so that only one ion can meet the need. The essential nature of an individual ion may depend on the simultaneous presence of a specific group of others ions. This is well-illustrated by the role that any of the cations (e.g., Ca^{2+}, K^+) play in the maintenance of cell membrane potential, which is a function of numerous gradients. Consequently, excess or deficient quantities of a particular common ion may be dangerous only to the extent that critical ionic ratios are disrupted. This potential danger is especially evident in the case of protons (H^+) and HCO_3^- in that the role these ions play in transmembrane exchanges will affect distributions of Na^+, Ca^{2+}, and Cl^-.

Virtually all cells exist close to an osmotic equilibrium with the medium surrounding them because "actively" transporting water is thermodynamically inefficient. Freshwater protozoans are obviously not at osmotic equilibrium and utilize contractile vacuoles to prevent osmotic swelling. Multicellular organisms maintain an ECF that bathes the cells in a isosmotic environment. Organisms vary widely in their capacity to generate an ECF out of osmotic equilibrium with the environment so that ICF, constrained to specific ion distributions, is adjusted osmotically with nitrogenous compounds (amino acids, urea, TMAO). The polyanionic nature of intracellular macromolecules results in the retention of cations and rejection of inorganic anions to preserve electroneutrality. Cl^- is thus maintained out of the cells in the ECF; K^+ and Mg^{2+} (and to a lesser extent, Na^+) play major roles in neutralizing the negative charges on macromolecules. A major consequence of the external environment's ionic content is the degree to which it stresses the maintenance of osmotic balance.

The specific utility of individual ions is described in the following sections. The impact of a decrease or increase in the concentration of an ion, outside of expected limits, varies dramatically from ion to ion. For those ions involved in critical physiological functions, atypical concentrations result in rapid, acute responses. Other functions may be disrupted over a longer period although immediate survival is not compromised. Ca^{2+} and K^+, for example, are instrumental in maintaining cell membrane potential, and while aquatic fauna can generally

tolerate short-term media deficiencies of other ions, lack of these two causes immediate physiological dysfunction and rapid mortality. Long-term deficiency of Ca^{2+} below immediate lethal levels can result in Ca^{2+} loss in vertebrate bones, and eventual death. There are several fundamental physiologic processes that require and/or exploit the cooperative action of groups of ions. This cooperation applies to those processes that involve solution chemistry, such as membrane-bound transport and cytoplasmic metabolism. The absence or excess of one ion can often, therefore, indirectly alter fundamental processes that directly depend on other ions.

The following sections discuss only the physiological aspects of major cations and anions. There are certainly other ions that are required for long-term organism health. However, many of these ions are needed in almost undetectable levels and are poorly understood. Douglas and Horne (1997) found, for example, that in addition to Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}, long-term survival of *M. bahia* is dependent upon Br^-. The exact function of Br^- is not known, although uptake of Br^- and an increase in plasma concentrations is associated with a stoichiometrical decrease in Cl^- (Stormer *et al.*, 1996). Some of the major functions of the significant ions are presented in Table 5-2.

Bicarbonate (HCO_3^-)

The significance of HCO_3^- is almost entirely a function of its role in acid-base balance. Aquatic organisms vary widely in their capacity to regulate changes in pH. The buffer capacity (the amount of strong acid or base [in mM/L] required to change the pH by 1 unit), is a function of the fluid's buffer base (the total concentration of HCO_3^-, phosphates and protein anions). Buffer capacity ranges from near zero for annelid and echinoderm plasma, to 2.5 - 10 for crustaceans and elasmobranchs, to 7 - 20 for teleosts and amphibians and to more than 20 for reptiles and mammals.

Inappropriate HCO_3^- levels in ECF disrupt the acid-base balance. Alkalosis, produced by high ECF HCO_3^-, elicits the exit of protons from cells and an inward movement of K^+. This effect promotes K^+-secretion in the distal tubule of the kidney and, with prolonged alkalosis, a general reduction in body stores of K^+ with an elevation of ICF Na^+. Acidosis (e.g., reduced HCO_3^-) has the opposite effect, with protons entering cells and K^+ moving into the plasma. Initially lowered distal tubule K^+ results in reduced K^+-excretion but, with time, increased plasma levels result in higher K^+-loss. For those organisms with renal mechanisms of K^+-excretion, both high and low HCO_3^- levels can cause a loss of K^+. Cl^- losses are also expected because of elevated ECF levels with activation of the Cl^-/HCO_3^- antiporter via HCO_3^- movement to the ICF.

Table 5-2. Physiological Functions of Some Ions and Consequences of Abnormal Environmental Levels.

Ion	Some Important Functions	Possible Consequences of Deficiency	Possible Consequences of Excess
HCO_3^-	Maintaining acid-base balance	Acidosis; reduced K^+ excretion; respiratory effects; blood pH effects	Alkalosis; general reduction in body stores of K^+; respiratory effects; blood pH effects; paralysis
Ca^{2+}	Gating of Na^+ fluxes in nerve membranes; regulation of cell membrane permeability; cofactor for metabolic processes	Muscle cramps; tetany; seizures; reduction in bone Ca^{2+}	Paralysis
Cl^-	Regulation of intracellular pH; regulation of cellular volume	Changes in K^+ balance; cell swelling	Changes in K^+ balance; altered membrane potential
Mg^{2+}	Neutralization of polyanionic proteins; cofactor for numerous enzymes; protein, nucleic acid synthesis	Hyperexcitability; muscular weakness; seizures; tetany; can lead to prolonged Ca^{2+} deficiency	Retardation of neuromuscular transmission; central nervous system depression; disturbance of cardiac condition
K^+	Major role in establishing cell membrane potential	Hyperpolarization of cell membrane potential; paralysis	Depolarization of cell membrane potential; paralysis
Na^+	Intestinal uptake of amino acids and sugars; renal PAH transport; charge carrier at chemical synapses	Disruption of cell membrane electrochemical gradient; reduction in ECF volume and GFR	Disruption of cell membrane electrochemical gradient; expansion of extracellular and vascular volume

Extracellular fluid pH is determined by the ratio of free buffer anion to undissociated acid. For most organisms, the dominant buffer system is H_2CO_3 - $NaHCO_3$. Consequently, ECF pH is determined by the following equation:

$$ECF\ pH = 6.1 + \log [HCO_3^-]/[H_2CO_3] \qquad \text{(Equation 5-1)}$$

The generation of excess HCO_3^- can readily be caused by situations that compromise respiration. The cytoplasmic enzyme, carbonic anhydrase, catalyzes the following reaction:

$$CO_2 + [OH^-] = [HCO_3^-] \qquad \text{(Equation 5-2)}$$

The balance in this equation is driven to the right if there is excess CO_2 in the body fluids. Air-breathing aquatic organisms (e.g., marine mammals and amphibians) can make adjustments in respiration that will eliminate excess CO_2. With a constant concentration of H_2CO_3, changes in

the quantity of HCO_3^- result in inverse adjustments in proton concentration. This may affect the intracellular environment because of resident mechanisms for H^+ exchange with Na^+ and K^+. Intracellular buffering primarily is provided by the histidine residues on proteins.

Given the relatively high solubility of CO_2 compared to O_2, the partial pressure of CO_2 is low in the blood of water-breathing animals (vertebrates and invertebrates). Therefore, blood HCO_3^- concentration also is low. Acid-base balance is achieved primarily through antiporter channels that exchange Na^+ for H^+ (or NH_4^+) and Cl^- for HCO_3^- at the gills. These mechanisms are important in NaCl absorption in freshwater organisms and NaCl excretion in marine animals. Evans (1984) demonstrated that these antiport systems function in the hagfish, an extant relative of the ancestral chordates that predate the invasion of freshwater by the vertebrates. Therefore, acid-base balance and nitrogen excretion may have been selective forces for the evolution of these antiporters, and the utility for absorption of NaCl may be a secondary benefit to freshwater fish.

Calcium (Ca^{2+})

Ca^{2+} serves in a broad range of biological functions, making it one of the most important ions in organism biochemistry. Many essential roles are vulnerable to excessive or deficient external environmental concentrations. Ca^{2+} controls the gating of Na^+ fluxes in the nerve membrane via competition with Na^+ for entry sites. Through this, Ca^{2+} controls neuromuscular excitability. Ca^{2+} elevation in the cytoplasm will move electrophoretically into the mitochondria, uncoupling respiration by reducing proton flow across the inner membrane through reduction of the -150 to 180 mV potential difference. Ca^{2+} also regulates membrane permeability (through the bilayer phase) by cross-linking adjacent phosphate groups between phospholipid molecules. As a result, the ECF concentration can have a controlling effect on the permeability of hydrophobic (lipophilic) solutes.

Many essential metabolic activities depend upon Ca^{2+} as a cofactor, including the biochemistry of dehydrogenases in oxidative phosphorylation, kinase activation, receptor responses (synapses), IP_3-linked reactions, ATPases, and oxygen release in photosynthesis. Ca^{2+} specifically triggers the action of calmodulin, ATPases, and annexin. Physiologic processes that depend on Ca^{2+} include muscle contraction, microtubule function (e.g., cell mobility, mitosis), exocytosis, intracellular communication, and the maintenance of epithelial tight junction "tightness" and selective permeability. As an extracellular component, Ca^{2+} is an essential cofactor in blood clotting and for several digestive enzymes.

Perhaps the most obvious use of Ca^{2+} in the body is the formation of hard skeletal structures for support and muscle attachment. The carbonates, phosphates, and oxalates of Ca^{2+} comprise the large majority of raw materials used in biomineralization. Many calcified structures (e.g., bone) may contain Ca^{2+} in a fashion that confers stability but also allows mobilization of the ion to meet organismal priorities when exogenous resources are scarce.

Because of the numerous functions Ca^{2+} has, an excess or deficiency of this ion can cause chronic and even lethal effects. High levels of Ca^{2+} in the ECF may cause paralysis because of the role Ca^{2+} plays in neuromuscular excitability. Conversely, low levels may produce spontaneous muscle cramps, and extremely low levels may result in tetany and seizures. Ca^{2+} deficiency will severely impair several functions (e.g., muscle contraction, microtubule function. In environments where exogenous Ca^{2+} supplies are scarce, adjustments can be made to mobilize skeletal-bound Ca^{2+} on a short-term basis and thus guarantee survival under acute conditions. Unfortunately, long-term mobilization of this Ca^{2+} source could prove lethal under chronic exposure.

Chloride (Cl^-)

NaCl is the principal ion pair to which organisms are exposed. Substantial energy is devoted to adapting to Na^+ and Cl^- environmental excesses and deficiencies. Unlike Na^+, which is critical to more physiologic processes than any other ion, Cl^- is involved in a far more selective fashion. The Cl^- ion is at or near equilibrium with the cell membrane potential (within 15 mV) and, with its distribution, constitutes a major factor in the repolarization of excitable cells through Cl^- channels. A second consequence of the uniformly low intracellular concentration of Cl^- is its importance in the determination of transmembrane Na^+ and K^+ balance through the neutral cotransport system that shuttles Na^+, K^+, and 2 Cl^- ions simultaneously.

The important role of Cl^- in the regulation of intracellular pH is tied to channel-mediated exchanges with HCO_3^-. Consequently, for those animals where renal mechanisms are operative, the balance of K^+ is necessarily affected when Cl^- levels are inappropriate. Adjustments in Cl^- channel activity are important in the regulation of cellular volume in the face of external pressures for swelling and shrinkage. Salt extrusion mechanisms depend upon the polarized distribution of Cl^- channels in secretory cells and glands so that the coupling of sodium pump activity and cotransport uptake of Cl^- (restricted to the basolateral side of epithelial cell membranes) results in the transport from the extracellular fluid to the outside. In

these systems (e.g., teleost gills, shark rectal glands, salt glands of marine birds), the Na^+ ion is a passive ion partner in transport.

Magnesium (Mg^{2+})

Mg^{2+} is the most abundant intracellular cation after K^+. Despite overall intracellular concentrations from 25 to 45 mM, the free ion content is more likely to be as low as 2 to 6 mequiv/L. The large bound fraction of intracellular Mg^{2+} is related to its function in the neutralization of polyanionic proteins. It is a cofactor for numerous enzymes and an essential requirement for all of the recognized ATPases as well as for both protein and nucleic acid synthesis. Plasma levels are regularly in the range of 2 mequiv/L, of which 25 to 35% may be bound to serum proteins (e.g., albumen) with a small fraction complexed to anions like citrate, phosphate, and SO_4^{2-}. Generally, more than half of the total body Mg^{2+} is stored in skeletal structures.

Because of the essential nature of Mg^{2+}, other ions generally cannot be effectively substituted. Most Mg^{2+}-dependent enzymes will not function with alternative cofactors. For example, manganese (Mn^{2+}) can replace Mg^{2+} on catalytic ribonucleic acids (ribozymes) or on the stability-enhancing binding site of tRNA; however, function is compromised. In the former case (ribozymes), Ca^{2+} makes an apparently isostructural substitution, but the ribozyme is inactive until Mg^{2+} is restored.

Mechanisms for the accumulation of Mg^{2+} from dilute sources are not well understood. Higher organisms conserve and accumulate Mg^{2+} in the kidney at Henle's Loop and in the proximal tubule similar to Na^+ absorption. There are also apparent absorption sites in the colon. Like K^+, inappropriate levels of circulating Mg^{2+} have deleterious effects on neuromuscular function. Based on the regulatory effect of Mg^{2+} in restricting the liberation of acetyl choline at neuromuscular junctions and sympathetic ganglia, a deficiency can lead to hyperexcitablity, muscular weakness, tetanus, and seizures. Alternatively, an excess of circulating Mg^{2+} may retard neuromuscular transmission, cause central nervous system depression, and disturb cardiac conduction. Prominent effects of hypermagnesemia (lack of Mg^{2+}) may lead to prolonged Ca^{2+} deficiencies in that it impairs the secretion of parathyroid hormone. With no feedback loop, the loss of Ca^{2+} from the kidney and reduced mobilization from bone will continue until Mg^{2+} levels are restored.

Potassium (K^+)

K^+ in the extracellular fluid is held within very strict limits. The considerations for all multicellular organisms are identical and are based on potassium's influence on the electrical potential across cell membranes.

Because it is the major factor in establishing the cell membrane potential (E_m), minor alterations of the K^+ concentration in the ECF can produce major physiological changes. A typical resting membrane potential (e.g., in vertebrate muscle) is -90 mV (inside negative). The diffusion potential for K^+ is determined as follows:

$$\text{diffusion potential} = -(RT/F)\ \ln[K_i]/[K_o] \qquad \text{(Equation 5-3)}$$

The diffusion potential equals about -98 mV (inside negative) when the concentration inside the cell $[K_i]$ is 155 and the concentration outside the cell $[K_o]$ is 4. Because Cl^- is distributed at electrochemical equilibrium and the equilibrium potentials for Na^+ and Ca^{2+} are +67 and +129 mV, respectively, it is apparent that K^+ controls E_m. Consequently, a shift in extracellular K^+ from 4 to 7 mM would depolarize E_m to -76 mV. A shift from 4 to 2 mM would hyperpolarize E_m to -107 mV. The responsiveness (or excitability of nerve and muscle) depends on the difference in electrical potential of the resting cell membrane (-90 mV) and the threshold trigger for initiation of an action potential (typically, -65mV). Therefore, minor shifts in ECF concentrations of K^+ will strongly alter excitability. Very minor shifts in K^+ concentrations may not produce noticeable short term effects, but altered neuromuscular performance will be deleterious to many functions, notably respiration. Elevated levels of K^+ will produce hyperexcitability and a difficulty in repolarization after a single response, resulting in cardiac arrhythmia or paralysis.

Pearce and Scheibling (1994) found that elevated levels of K^+ (as KCl) increased metamorphosis rates in the larval echinoid *Strongylocentrotis droebachiensis.* Initiation of metamorphosis may be due to K^+-induced depolarization of excitable cells of the surface of the larvae, which starts a chain reaction in the normal morphogenetic pathway (Baloun and Morse, 1984). With lowered available K^+, decreased excitability can also result in paralysis. K^+ deficiency is also deleterious in that, with time, the high intracellular levels will be diminished in return for Na^+. Altering this ionic balance compromises enzyme action (Lubin, 1964) and developmental studies suggest that a lowered ICF K^+ concentration makes ICF Na^+ effectively toxic (Cannon *et al.*, 1953).

Sodium (Na^+)

The importance of the Na^+ ion depends on its presence in relatively large supply in the ECF. The electrochemical gradient for the influx of Na^+ is, together with the transmitochondrial gradient for protons, the most important in animal biology. Na^+ is important in both 1) coupled uptake (symport) process, such as intestinal uptake of amino acids and sugars, renal transport of para-aminohippuric acid, and transport of gamma-aminobutyric acid and serotonin in the nervous system, and 2) exchange (antiport) processes such as the Na^+/H^+ process used for acid-base regulation and salt absorption from low Na^+ concentrations. Diffusive flow of Na^+ through Na^+ channels (uniport) acts as a charge carrier for the acetylcholine receptor channels at chemical synapses and as inward current through voltage-gated channels for nerve impulse transmission.

ION REGULATION MECHANISMS

In an ideal situation, the intracellular ions and water are optimal for metabolic functions and the ECF composition puts minimal strain on the mechanisms that regulate its composition. Steady-state values for ions are dependent on energy-consuming processes because, with few exceptions, ions are not in equilibrium with the external environment. The primary regulatory mechanisms directly affect uptake and excretory processes, often in a fashion that requires adjustment of several parameters at one time (e.g., the regulation of Na^+ balance in vertebrates with glomerular kidneys). Because a major priority in salt and water balance is strict maintenance of Na^+ concentration in the extracellular fluid, a sudden ingestion of excess Na^+ leads to an expansion of both extracellular and vascular volumes. As a result, GFR increases, as does tubular Na^+, and increased Na^+ excretion. Alternatively, a major reduction in Na^+ causes a reduction in ECF volume and GFR and diminished or abolished Na^+ excretion. At the same time, the concentrations of ions other than Na^+ are also modified and may stimulate other regulatory mechanisms.

Preservation of fixed composition in the ECF is assumed to be critical in the preservation of ICF integrity for two reasons. First, there are discrete limits to the capacity of membrane-bound transport processes which would function to compensate for imbalance. Second, the finite volume of individual cells severely limits their resources for long-term maintenance of steady states. Dealing with maintenance of ECF balance involves regulatory processes that result in a successful adaptive response. Organisms display highly specialized tissues and organs for coping with environmental extremes, such as the salt glands of marine birds, rectal glands of

elasmobranchs, elaborate gill structure of the bony fishes, and urinary bladders in amphibians and aquatic reptiles.

Adaptive processes may be characterized in terms of response time (Diamond, 1982). The fastest of these (< 1 sec) are in the nervous system, which are most readily observed in secretory glands, and present, but poorly understood, in salt-transporting epithelia. Fast-acting hormones (e.g., gastrin, oxytocin, secretin, vasopressin) may be effective in the scale of one minute because receptor-binding leads directly to alteration of active sites or insertion into membranes of already assembled transport proteins. Slower acting hormones (with a time-lag of one hour or more) include aldosterone, prolactin, and vitamin D, all of which are effective through induction of transport protein synthesis. Substrate-induced carrier induction (as with glucose in the small intestine) seems to require several hours and probably is operative in salt-transporting epithelia. Trophic changes (e.g., as in gill chloride cells or salt-gland cells when exposed to major changes in ambient NaCl) may begin within hours but require days before a fully functional adaptive response is in place. Recent evidence suggests that critical-period programming, well recognized by behavioral scientists, may be a major factor in the quantitative nature of adaptive responses. Genetic programming for adaption is, perhaps, the most long-term acclimation response. This is an instrumental evolutionary adaption in the development of biological diversity.

SUMMARY

Aquatic organisms living in freshwater or seawater systems have developed physiological and chemical mechanisms to balance water and essential ions within body tissues and fluids. Because of the differences in the ionic composition of the external media versus internal fluids, however, a number of adaptations and/or physiological mechanisms have evolved for maintaining the necessary concentration gradients. In addition, different taxa have different adaptations to meet the challenge of ion and water regulation. The ECF of some invertebrate (particularly marine) species is similar in composition to external environment in which they live. This is essentially true for at least one group of marine vertebrates (hagfish) as well. Most other vertebrates, however, must commit substantial amounts of energy to mechanisms for maintaining the water and ion balance necessary to support essential biochemical activities. Dramatic changes in the environmental concentrations of many ions, especially on a long-term basis, can over-burden existing mechanisms for maintaining homeostasis, and lead to chronic or acute effects.

The impact that low or high ionic concentrations will have is associated with the function of the particular ion and the degree to which organisms can adapt to unfavorable conditions. Some organisms, for example, can sequester Ca^{2+} from skeletal structure to compensate for lowered environmental concentrations. Severe absence of environmental Ca^{2+}, however, cannot be overcome and rapid mortality is often the result. Other ions have little impact if absent from the environment, but can cause adverse effects if present at higher levels. Finally, since many mechanisms involve multiple ions (e.g., the sodium pump) inappropriate levels of one ion can indirectly impact physiology by disrupting levels of other ions.

Section 6
SUMMARY

Whole effluent toxicity (WET) tests have become a common component of freshwater and marine NPDES permits. Although WET tests undoubtedly will continue to evolve in how they are applied and interpreted, they will most certainly remain an integral part of the effluent monitoring process. WET associated with TDS ions has been identified in effluents from several, varied sources. Although USEPA Regions 9 and 10, Colorado, Florida, and Texas have drafted or implemented guidelines for identifying TDS ion toxicity and quantifying its impact, TDS toxicity remains an area of concern for several industries in many areas of the country. Common ion toxicity can be difficult to identify in some effluents, particularly when other non-TDS toxicity (e.g., organics) may also be present. However, TIE methods can be adapted and modified to isolate toxicity related to common ions. Procedures that can be used during the TIE process include:

- Analytical verification of ion concentrations,
- Phase I TIE characterization,
- Mock effluent studies, and
- Computer models.

The concentration that causes adverse effects varies substantially with the ion. There is an abundance of ion-related toxicity information dating back several years. The concentrations that cause adverse effects often vary markedly between taxonomic groups and between studies. Such variations are not surprising considering the well-documented differences in sensitivity among species (e.g., *D. magna* versus *C. dubia*), as well as expected interlaboratory differences in test procedures, equipment, water, and organisms. There is, however, some consistency between related taxa. For example, *Culex* sp., is sensitive to HCO_3^- at approximately the same concentration as *D. magna*. Similarly, sensitivity of *Gambusia affinis* and *Lepomis macrochirus* to HCO_3^- is nearly identical. In general, the data indicate that for freshwater organisms, K^+, Mg^{2+}, and HCO_3^- are the most toxic ions, and for marine species, $B_4O_7^{2-}$, K^+, and HCO_3^- are the most toxic ions on an absolute mass per volume basis. However, caution should be used in the identification of ion toxicity in saline solutions since pH, temperature, and the presence of other ions can alter the bioavailable amounts. Also, which ions will most frequently be identified as toxicants in WET tests is dependent upon the relative

concentration of ions in an effluent. For example, Ca^{2+} has been reported as being the toxic TDS ion in several marine effluents, even though it is less toxic, on an absolute mass basis, than some of the other ions.

Discerning the relative toxicities of different anions and cations can be difficult if the complementary cation or anion in the salt is also toxic. Several studies using $CaCl_2$, for example, presented results in terms of mg/L Ca^{2+}. However, Mount *et al.* (1997) did not identify Ca^{2+} as a significant factor in determining toxicity to *C. dubia*, *D. magna*, or *P. promelas*, although it does cause significant mortality to marine species in both deficient and excess amounts (Pillard *et al.*, 1998a). Knowing the concentration of all ions in solution is, therefore, often a critical factor in knowing the cause of toxicity. In saline effluents this information can be of particular concern due to chemical interactions that reduce "available" ion concentrations. Although these interactions can add to the frustration of toxicity identification, the use of computer models, mock effluents, and ion balancing can be helpful in alleviating TIE difficulties.

Freshwater and marine organisms live in an environment that constantly threatens to disrupt physiological processes by upsetting the ion balances of intra- and extracellular fluids. Depending upon the ion concentrations in the external media, organisms must deal with the continual loss, or influx, of water and ions. Organisms have evolved unique mechanisms that maintain a balance of water and ions through the active transport of these constituents and/or adjustment of cellular permeability. Freshwater fish face a constant loss of ions to the more dilute external environment while they gain water, primarily through gill tissues. To compensate, freshwater fish gain ions through their diet and active uptake at the gills. Bony marine fish face the opposite problem as ions diffuse into tissues and there is an osmotic loss of water through gill epithelium. Marine teleosts drink large volumes of water, which is then stripped of its NaCl before being absorbed through the wall of the intestine. The excess salts then are eliminated primarily through the chloride cells of the gill and operculum.

Despite these regulatory mechanisms for controlling ion and water balance, certain ions can disrupt physiological function and result in adverse effects. What those effects are depends upon the test species and ion being studied. Too much Ca^{2+}, for example, can cause paralysis, but if too little of this ion is present, muscle cramps, tetany, and seizures may result. Deficient Mg^{2+} can also cause seizures and tetany; too much retards neuromuscular transmission and causes nervous system depression. Some species are much more tolerant of wide fluctuations

in ion concentrations, while others can survive only within a narrow range of environmental conditions. *Cyprinodon variegatus*, for example, has been shown to tolerate extreme saline conditions that would be lethal to other marine species such as *M. bahia*. Even stenohaline species, however, can acclimate over extended periods of time to salinity conditions that might otherwise cause toxicity during sudden exposures.

In summary, effluent toxicity related to an imbalance of inorganic ions has been identified in permitted discharges from many different industrial and municipal sources. The acute and chronic effects of ion imbalance can be as pronounced as with any toxicant. However, many of these ions are essential nutrients and receiving water dilution may (although not always) correct imbalances almost immediately. While there is limited agreement in the regulatory community that TDS toxicity can be viewed as distinct from other toxic situations, it is unlikely that the NPDES or state PDES permitting process will allow ion imbalance to be addressed selectively as a unique issue. In fact, such isolation of one particular toxicity situation may not be desirable in the WET process. Nevertheless, there may be situations (e.g., where source water already demonstrates ion imbalance) where special consideration of WET would be prudent. Expensive treatment options that result in only moderate adjustment of ion concentrations are probably inappropriate and unjustified where receiving water ecology is not impaired.

REFERENCES

Adelman, I.R. and L.L. Smith, Jr. 1976. Fathead Minnows (*Pimephales promelas*) and Goldfish (*Carassius auratus*) As Standard Fish in Bioasssays and Their Reaction to Potential Reference Toxicants. *Journal of the Fisheries Research Board of Canada.* 33(2):209-214.

Alabaster, J.S. 1969. Survival of Fish in 164 Herbicides, Insecticides, Fungicides, Wetting Agents and Miscellaneous Substances. *International Pest Control.* 11(2):29-35.

Alexander, H.C., J.A. Quick, Jr. and E.A. Bartlett. 1981. Static Acute Toxicity of Sodium Bromide to Fathead Minnows. *Bulletin of Environmental Contamination and Toxicology.* 27:326-331.

Anderson, B.G. 1946. The Toxicity Thresholds of Various Sodium Salts Determined by the Use of *Daphnia magna*. *Sewage Works Journal.* 18(1):82-87.

Angelovic, J.W., W.F. Sigler and J.M. Neuhold. 1961. Temperature and Fluorosis in Rainbow Trout. *Journal of the Water Pollution Control Federation.* 33(4):371-381.

APHA. 1989. *Standard Methods for the Examination of Water and Wastewater*, 17th Edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation. APHA, Washington, DC.

Barbaro, A., A. Francescon and B. Polo. 1981. Fluoride Accumulation in Aquatic Organisms in the Lagoon of Venice. *Fluoride*. 14:102-107.

Baloun, A.J. and D.E. Morse. 1984. Ionic Control of Settlement and Metamorphosis in Larval *Haliotis rufescens* (Gastropoda). *Biology Bulletin.* 167:124-138.

Baudouin, M.F. and P. Scoppa. 1974. Acute Toxicity of Various Metals to Freshwater Zooplankton. *Bulletin of Environmental Contamination and Toxicology.* 12(6):745-751.

Becker, A.J., Jr. and E.C. Keller, Jr. 1973. The Effects of Iron and Sulfate Compounds on the Growth of Chlorella. *Proceedings. West Virginia Academy of Science.* 45(2):127-135.

Bengtsson, B.E. 1978. Use of a Harpacticoid Copepod in Toxicity Tests. *Marine Pollution Bulletin.* 9:238-241.

Berner, E.K. and R.A. Berner. 1987. *The Global Water Cycle, Geochemistry and Environment.* Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Biesinger, K.E. and G.M. Christensen. 1972. Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of *Daphnia magna*. *Journal of the Fisheries Research Board of Canada.* 29(12):1691-1700.

Birge, W.J. and J.A. Black. 1977. *Sensitivity of Vertebrate Embryos to Boron Compounds.* EPA-68-01-3222, Office of Toxic Substances, U.S. EPA, Washington D.C., U.S. NTIS PB-267 085.

Birge, W.J. 1978. Aquatic Toxicology of Trace Elements of Coal and Fly Ash. In. J.H.Thorp and J.W. Gibbons, eds. *Dept. Energy Symp. Ser. Vol. 48, Energy and Environmental Stress in Aquatic Systems.* Augusta, GA. pp. 219-240.

Boelter, A.M., F.N. Lamming, A.M. Farag and H.L. Bergman. 1992. Environmental Effects of Saline Oil-Field Discharges on Surface Waters. *Environmental Toxicology and Chemistry.* 11(8):1187-1195.

Boutet, C. and C. Chaisemartin. 1973. Specific Toxic Properties of Metallic Salts in *Austropotamobilus pallipes pallipes* and *Orconectes limosus. Comples Rendus des Seances. Societe de Biologie (Paris).* 167(12):1933-1938.

Bowser, P.R., R.B. Landy, G.A. Wooster and J.G. Babish. 1988. Efficacy of Elevated Fluoride for the Control of *Remibacterium salmoninarun* Infection in Rainbow Trout (*Salmo gairdneri*). *Journal of the World Aquaculture Society.* 19:1-6.

Bradley, T.J., K. Strange and J.E. Phillips. 1984. Osmotic and Ionic Regulation in Saline-Water Mosquito Larvae. In A. Pequeux, R. Gilles and L. Bolis, eds. *Lecture Notes on Coastal and Estuarine Studies 9, Osmoregulation in Estuarine and Marine Animals.* Springer-Verlag, Berlin. pp. 35-50.

Brown, E., A. Colling, D. Park, J. Phillips, D. Rothery and J. Wright. 1995. *Seawater: Its Composition, Properties, and Behavior.* The Open University, Milton Keynes, England.

Brown, E.R., L. Keith, J.J. Hazdra and T. Arndt. 1973. Tumors in Fish Caught in Polluted Waters: Possible Explanations. *Bibliotheca Haematologica.* 40:47-57.

Burnett, L.E. and D.W. Tole. 1990. Sodium Ion Uptake by Perfused Gills of the Blue Crab, *Callinectes sapidus*: Effects of Ouabain and Amiloride. *Journal of Experimental Biology.* 149:293-305.

Camargo, J.A. 1996. Estimating Safe Concentrations of Fluoride for Three Species of Nearctic Freshwater Invertebrates:Multifactor Probit Analysis. *Bulletin of Environmental Contamination and Toxicology.* 56:643-648.

Cannon, P.R., L.E. Frazier and R.H. Hughes. 1953. Sodium as Toxic Ion in Potassium Deficiency. *Metabolism.* 2:297-312.

Cannon, W.B. 1929. Organization for Physiological Homeostasis. *Physiological Reviews.* 9:399-431.

Canton, J.H., P.W. Wester and E.A.M. Mathijssen-Speikman. 1983. Study on the Toxicity of Sodium Bromide to Different Freshwater Organisms. *Food and Chemical Toxicology.* 21(4):369-378.

Chitra, T. and J.V.R. Rao. 1981. Effect of Sodium Fluoride on Blood and Liver Enzymes in *Channa punctatus* (Bloch.). *Fluoride.* 14:115-118.

Cohen, A.S. and W.A. Stubblefield. 1991. *Toxicity of Bromide to Freshwater Aquatic Species.* Paper presented at the 12th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Seattle, WA., USA, Nov. 3-7, 1991.

Connell, A.D. and D.D. Airey. 1982. The Chronic Effects of Fluoride on the Estuarine Amphipods *Grandidierella lutosa* and *G. lignorum. Water Research.* 16:1313-1317.

Daly, D.J., R.S. Stoa, J.A. Sorensen, S.A. Bassingthwaite, G.E. Mesing, J.P. Fillo, S. Pemmaraju, K.D. Martz and J.T. Tallon. 1995. *Atlas of Gas-Related Produced Water for 1990.* GRI-95/0016. Gas Research Institute. Chicago, IL.

Damgaard, R.M. and J. Davenport. 1994. Salinity Tolerance, Salinity Preference, and Temperature Tolerance in the High-Shore Harpacticoid Copepod *Tigriopus brevicornis. Marine Biology.* 118:443-449.

Dave, G. 1984. Effects of Fluoride on Growth, Reproduction, and Survival in *Daphnia magna. Comparative Biochemistry and Physiology.* 78C(2):425-431.

Degnan, K.J., K.J. Karnakey, Jr. and J.A. Zadunaisky. 1977. Active Chloride Transport in the *in vitro* Opercular Skin of a Teleost *(Fundulus heteroclitus)*, a Gill-Like Epithelium Rich in Chloride Cells. *Journal of Physiology (Lond.)* 271:155-191.

DeJong, L.E.D.D. 1965. Tolerance of *Chlorella vulgaris* for Metallic and Non-Metallic Ions. *Antonie Leeuwenhoek* (Journal of Microbiology and Serology). 31:301-313.

de March, B.G.E. 1988. Acute Toxicity of Binary Mixtures of Five Cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^{+}) to the Freshwater Amphipod *Gammarus lavustris* (Sars): Alternative Descriptive Models. *Canadian Journal of Fisheries and Aquatic Science.* 45(4):625-633.

Diamond, J.M. 1982. Adaptive Regulation of Epithelial Transport Mechanisms. In R.A. Corradino, ed. *Functional Regulation at the Cellular and Molecular Levels.* Elsevier, North Holland.

Dickerson, K. and P. Ramirez, Jr. 1997. *A Comparison of Boron and Selenium Concentrations in Biota from Irrigation Projects Throughout Wyoming.* Paper presented at the 18th annual meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA, USA, Nov. 16-20, 1997.

Dickman, M. 1973. Changes in Periphytic Algae Following Bicarbonate Additions to a Small Stream. *Journal of the Fisheries Research Board of Canada.* 30(12 Pt. 1):1882-1884.

Dorn, P.B. and J.H. Rodgers, Jr. 1989. Variability Associated with Identification of Toxics in National Pollutant Discharge Elimination System (NPDES) Effluent Toxicity Tests. *Environmental Toxicology and Chemistry.* 8(10):893-902.

Douglas, W.S., S.S, Grasso, D.G. Hutton and K.R. Schroeder. 1996. Ionic Imbalance as a Source of Toxicity in an Estuarine Effluent. *Archives of Environmental Contamination and Toxicology.* 31:426-432.

Douglas, W.S. and M.T. Horne. 1997. The Interactive Effects of Essential Ions and Salinity on the Survival of *Mysidopsis bahia* in 96-H Acute Toxicity tests of Effluents Discharged to Marine and Estuarine Receiving Waters. *Environmental Toxicology and Chemistry.* 16(10):1996-2001.

Dowden, B.F. 1961. Cumulative Toxicities of Some Inorganic Salts to *Daphnia magna* as Determined by Median Tolerance Limits. *Proceedings of the Louisiana Academy of Science.* 23:77-85.

Dowden, B.F. and H. J Bennett. 1965. Toxicity of Selected Chemicals to Certain Animals. *Journal of the Water Pollution Control Federation.* 37(9):1308-1316.

Engel, D.W. 1977. Comparison of the Osmoregulatory Capabilities of Two Portunid Crabs, *Callinectes sapidus* and *C. similis*. *Marine Biology*. 41:275-279.

ENSR. 1996. *Development of Site-Specific Water Quality Criteria for Copper in the Upper Clark Fork River. Phase III WER Program, Testing Results and Final Report.* 0480-277.01.22. ENSR Consulting and Engineering, Ft. Collins, CO.

Evans, D.H. 1984. Gill Na^+/H^+ and $Cl^-/HCO3^-$ Exchange Systems Evolved Before the Vertebrates Entered Freshwater. *Journal of Experimental Biology*. 113:465-469.

FDEP. 1995. *Protocols for Determining Major-Seawater-Ion Toxicity in Membrane-Technology Water-Treatment Concentrate*, DRAFT. Florida Department of Environmental Protection, Bureau of Laboratories.

FDEP. 1997. *Results of Salinity Tolerance Tests Conducted on the Mysid Shrimp,* Americamysis bahia, *on 6/20/97, 7/21/97, and 9/2/97.* October 23, 1997. Florida Department of Environmental Protection, Biology Section, Division of Administrative and Technical Services.

Fieser, A.H., J.L. Sykora, M.S. Kostalos, Y.C. Wu and D.W. Weyel. 1986. Effect of Fluorides on Survival and Reproduction of *Daphnia magna*. *Journal of the Water Pollution Control Federation*. 58(1):82-86.

Fisher, S.W., P. Stromberg, K.A. Bruner and L.D. Boulet. 1991. Molluscicidal Activity of Potassium to the Zebra Mussel, *Driessenia polymorphia*: Toxicity and Mode of Action. *Aquatic Toxicology*. 20:219-234.

Forrest, J.N., W.C. Mackay, B. Gallagher and F.H. Epstein. 1973. Plasma Cortisol Response to Saltwater Adaption in the American Eel. *American Journal of Physiology*. 224:714-717.

Goodfellow, W.L., P.B. Dorn, L.W. Ausley, D.T. Burton, D.L. Denton, D.R. Grothe, M.A. Heber, T.J. Norberg-King and J.H. Rodgers, Jr. The Role of Inorganic Ion Imbalance in Aquatic Toxicity Testing. Society of Environmental Toxicology and Chemistry Foundation Work Product. In preparation for submission.

GRI. 1989. *Proposed Study Plan for the Investigation of Water Discharges from Hydrostatic Testing of Natural Gas Pipelines.* Gas Research Institute, Chicago, Illinois.

Hamilton, R.W., J.K. Buttner, and R.G. Brunetti. 1975. Lethal Levels of Sodium Chloride and Potassium Chloride for an Oligochaete, Chironomid Midge, and a Caddisfly of Lake Michigan. *Environmental Entomology.* 4(6):1003-1006.

Hastings, A.B. 1941. Electrolytes of Tissue and Body Fluids. *Harvey Lecture Series.* 36:91-125.

Hem, J.D. 1985. *Study and Interpretation of the Chemical Characteristics of Natural Water.* United States Geological Survey, Water-Supply Paper 2254. 263 pp.

Hemens, J. and R.J. Warwick. 1972. The Effects of Fluoride on Estuarine Organisms. *Water Research*. 6:1301-1308.

Hermens, J., H. Canton, N. Steyger and R. Wegman. 1984. Joint Effects of a Mixture of 14 Chemicals on Mortality and Inhibition of Reproduction in *Daphnia magna*. *Aquatic Toxicology*. 5(4):315-322.

Hickman, C.P., C.P. Hickman, Jr. and F.M. Hickman. 1974. *Integrated Principles of Zoology*, Fifth Ed. The C.V. Mosby Company, St. Louis, 1025 pp.

Hinton, M.J. and A.G. Eversole. 1979. Toxicity of Ten Chemicals Commonly Used in Aquaculture to the Black Eel Stage of the American Eel. *Proceedings of the World Maricultural Society*. 10:554-560.

Hirano, T. 1980. Effects of Cortisol and Prolactin on Ion Permeability of the Eel Oesophagus. In B. Lahlou, ed. *Epithelial Transport in Lower Vertebrates*. Cambridge University Press, Cambridge. pp. 143-149.

Ho, K. and D. Caudle. 1997. Ion Toxicity and Produced Water. *Environmental Toxicology and Chemistry*. 16(10):1993-1995.

Hockett, J.R. and D.R. Mount. 1993. *Toxicity Identification Evaluations:Problems, Solutions, and Future Research Needs.* Paper presented at the 14th annual meeting of the Society of Environmental Toxicology and Chemistry, Houston, TX, USA, Nov. 14-18, 1993.

Hoke, R.A., W.R. Gala, J.B. Drake and J.P. Giesy. 1992. Bicarbonate as a Potential Confounding Factor in Cladoceran Toxicity Assessments of Pore Water from Contaminated Sediments. *Canadian Journal of Fisheries and Aquatic Sciences*. 49:1633-1640.

Hughes, J.S. 1973. Acute Toxicity of Thirty Chemicals to Striped Bass (Morone saxatilis). In: *Louisiana Department of Wildlife and Fisheries.* 318-343-2417.

Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler and J.B. Hunn. 1992. The Use of Freshwater and Saltwater Animals to Distinguish Between the Toxic Effects of Salinity and Contaminants in Irrigation Drain Water. *Environmental Toxicology and Chemistry*. 11(4):503-511.

Ishio, S. and H. Nakagawa. 1971. Susceptibility of Marine Organisms to Calcium Fluoride. *Bulletin of the Japanese Society of Scientific Fisheries*. 372:98-104.

Jones, J.R.E. 1940. A Further Study of the Relation Between Toxicity and Solution Pressure, with *Polycelis nigra* as Test Animal. *Journal of Experimental Biology*. 17:408-415.

Joy, C.M. and K.P. Balakrishnan. 1990. Effect of Fluoride on Axenic Cultures of Diatoms. *Water, Air, and Soil Pollution*. 49:241-249.

Kangas, P. and G. Skoog. 1978. Salinity Tolerance of *Theodoxus fluviatilis* (Mollusca, Gastropoda) from Freshwater and from Different Salinity Regimes in the Baltic Sea. *Estuarine Coastal Marine Science*. 6:409-416.

Karnaky, K.J. 1986. Structure and Function of the Chloride Cell of *Fundulus heteroclitus* and Other Teleosts. *American Zoologist*. 26:209-224.

Kerstetter, T.H. and M. Keeler. 1976. On the Interaction of NH_4^+ and Na^+ Fluxes in the Isolated Trout Gill. *Journal of Experimental Biology*. 64:517-527.

Khangarot, B.S. and P.K. Ray. 1989. Investigation of Correlation Between Physiochemical Properties of Metals and their Toxicity to the Water Flea *Daphnia magna Straus*. *Ecotoxicology and Environmental Safety*. 18(2):109-120.

Khangarot, B.S. 1991. Toxicity of Metals to a Tubicifid Worm, *Tubifex tubifex* (Muller). *Bulletin of Environmental Contamination and Toxicology*. 46:906-912.

Kirschner, L.B., L. Greenwald and T.H. Kerstetter. 1973. Effect of Amiloride on Sodium Transport Across Body Surfaces of Freshwater Animals. *American Journal of Physiology*. 224:832-837.

Kleyman, T.R. and E.J. Cragoe, Jr. 1988. Amiloride and Its Analogs as Tools in the Study of Ion Transport. *Journal of Membrane Biology*. 105:1-21.

Krogh, A. 1965. *Osmotic Regulation in Aquatic Animals*. Dover Publications, Inc. New York, New York.

Kühn, R., M. Pattard, K-D. Pernak, and A. Winter. 1989. Results of the Harmful Effects of Water Pollutants to *Daphnia magna* in the 21 Day Reproduction Test. *Water Research*. 23(4):501-510.

Kühn, R. and M. Pattard. 1990. Results of the Harmful Effects of Water Pollutants to Green Algae (*Scenedesmus subspicatus*) in the Cell Multiplication Inhibition Test. *Water Research*. 24(1):31-38.

Kültz, D. and H. Onken. 1993. Long-Term Acclimation of the Teleost *Oreochromis mossambicus* to Various Salinities: Two Different Strategies in Mastering Hypertonic Stress. *Marine Biology*. 117:527-533.

Lagler, K.F., J.E. Bardach, R.R. Miller and D.R.M. Passino. 1977. *Ichthyology*, 2nd ed. John Wiley and Sons, New York, New York.

LeBlanc, G.A. 1980. Acute Toxicity of Priority Pollutants to Water Flea (*Daphnia magna*). *Bulletin of Environmental Contamination and Toxicology*. 24(5):684-691.

Lin, H. and D. Randall. 1995. Proton Pumps in Fish Cells. In C.M. Wood and T.J. Shuttleworth, eds. *Cellular and Molecular Approaches to Fish Ionic Regulation*. Academic Press, San Diego, California. pp. 229-255.

Livingstone, D.A. 1963. *Chemical Composition of Rivers and Lakes*. United States Geological Survey. Professional Paper 440G.

Lixey, S.M., N.H. Neubotter and J.D. Horne. 1997. *Salinity-Mediated Response of Mysidopsis bahia to Fluoride-Ion Toxicity*. Paper presented at the 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA, USA, Nov. 16-20, 1997.

Loretz, C.A. 1995. Electrophysiology of Ion Transport in Telost Intestinal Cells. In C.M. Wood and T.J. Shuttleworth, eds. *Cellular and Molecular Approches to Fish Ionic Regulation*. Academic Press, San Diego, California. pp. 25-56.

Lubin, M. 1964. Intracellular Potassium and Control of Protein Synthesis. *Proceedings. Federation of American Societies for Environmental Biology*. 23:994-1001.

MacGregor III, R., M. Tucker and J.S. Qi. 1996. *Application of Ion-Balancing Methods for Marine Whole Effluent Toxicity Tests.* Paper presented at the 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington DC, USA, Nov. 17-21, 1996.

Mahajan, C.L., S.D. Sharma and S.P. Sharma. 1979. Tolerance of Aquatic Organisms to Chloride Salts. *Indian Journal of Experimental Biology.* 17(11):1244-1245.

Mangum, C.P. and D.W. Towle. 1977. Physiological Adaptation to Unstable Environments. *American Scientist.* 65:67-75.

Mann, H. 1973. Untersuchungen Uber Die Wirkung Von Borverbindungen Auf Fisce Und Einege Andere Wasserorganismen. (Effect of Borates on Fishes and Some Other Aquatic Organisms.) *Archive Für Fischereiwissenschaft.* 24(1-3):171-175.

Marshall, W.S. 1995. Transport Processes in Isolated Teleost Epithelia: Opercular Epithelium and Urinary Bladder. In C.M Wood and T.J. Shuttleworth, eds. *Cellular and Molecular Approaches to Fish Ionic Regulation.* Academic Press, San Diego. pp. 1-23.

McCulloch, W.L., W.L. Good fellow and J.A. Black. 1993. Characterization, Identification and Confirmation of Total Dissolved Solids as Effluents Toxicants. In J.W. Gorsuch, F.J. Dwyer, C.J. Ingersoll, and T.W. LaPoint, eds. *Environmental Toxicology and Risk Assessment: 2nd Volume.* STP 1216. American Society for Testing and Materials, Philadelphia, PA, USA. pp. 213-227.

McCulloch, W.L. and L.A. Smith. 1994. *Synthetic Effluent Studies Evaluating the Major Trace Ions Requirements of the Opossum Shrimp, Mysidopsis bahia.* Paper presented at the 15th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Denver CO, USA, Oct. 30 - Nov. 3, 1994.

McClurg, T.P. 1984. Effects of Fluoride, Cadmium, and Mercury on the Estuarine Prawn *Penaeus indicus. Water SA.* 10(1):40-45.

Meybeck, M. 1979. Concentrations des eux Fluviales en Éléments Majeurs et Approts en Solution aux Océans. Rev. Géol. Dyn. Géogr. Phys. 21:215-246.

Mickley, H., J. Briceño, B. Borkman, K. Fucik, J. Case and T. Nickel. 1996. *Common Ion Toxicity in R.O. Membrane Concentrate.* Paper presented at the 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington DC, USA, Nov 17-21, 1996.

Mount, D.R., D.D. Gulley, J.R. Hockett, T.D. Garrison and J.M. Evans. 1997. Statistical Models to Predict the Toxicity of Major Ions to *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas* (Fathead Minnows). *Environmental Toxicology and Chemistry.* 16(10):2009-2019.

National Research Council. 1993. *Nutrient Requirements of Fish.* National Academy Press, Washington, DC, USA, 114 pp.

Neuhold, J.M. and W.F. Sigler. 1960. Effects of Sodium Fluoride on Carp and Rainbow Trout. *Transactions of the American Fisheries Society.* 89(4):358-370.

Nobel, W. 1981. To the Effect of Boron Pollution on Submerged Softwater-Macrophytes. (Zum Einfluss Von Bor Auf Submerse Weichwasser-Makrophyten). *Angewande Botanik.* 55:501-514.

Oddo, J.E. and M.B. Tomson. 1994. Why Scale Forms and How to Predict It. *SPE Production and Facilities.* SPE 21710:47-54.

Palladini, G., V. Margotta, A. Carolei and M.C. Hernandez. 1980. Dopamine Agonist Performance in Planaria After Manganese Treatment. *Experientia.* 36(4):449-450.

Patrick, R., J. Cairns, Jr. and A. Scheier. 1968. The Relative Sensitivity of Diatoms, Snails, and Fish to Twenty Common Constituents of Industrial Wastes. *Progressive Fish-Culture.* 30(3):137-140.

Pearce, C.M. and R.E. Scheibling. 1994. Induction of Metamorphosis of Larval Echinoids (*Strongylocentrotis droebachiensis* and *Echinarachnius parma*) by Potassium Chloride (KCl). *Invertebrate Reproduction and Development.* 26:213-220.

Pennak, R.W. 1978. *Fresh-Water Invertebrates of the United States.* John Wiley & Sons, New York.

Petrocelli, S.R. 1985. Chronic Toxicity Tests. In G.M. Rand and S.R. Petrocelli, eds. *Fundamentals of Aquatic Toxicology.* Hemisphere Publishing Corporation, Washington. pp. 96-109.

Pillard, D.A., D.L. DuFresne and J. Evans. 1996. *Toxicity of Common Ions to Marine Organisms.* Paper presented at the 17th annual meeting of the Society of Environmental Toxicology and Chemistry, Washington, DC, USA, Nov. 17-21, 1996.

Pillard, D.A., D.L. DuFresne, J.E. Tietge and D. Caudle. 1998a. *Development of Salinity Toxicity Relationships for Produced Water Discharges to the Marine Environment.* GRI-97/0168. Gas Research Institute, Chicago, IL.

Pillard, D.A., J.T. Tallon and J.M. Evans. 1998b. *A Computer Program to Facilitate the Identification of Toxicity to Marine Organisms in Produced, and Other High-Salinity, Water.* Paper presented at the 5th International Petroleum Environmental Conference, Albuquerque, NM, USA, Oct. 20-23, 1998.

Piller, S.C., R.P Henry, J.E. Doeller and D.W. Kravs. 1995. A Comparison of the Gill Physiology of Two Euryhaline Crab Species, *Callinectes sapidus* and *Callinectes similis*: Energy Production, Transport-Related Enzymes and Osmoregulation as a Function of Acclimation Salinity. *Journal of Experimental Biology.* 198:349-358.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish.* EPA/444/4-89-001. United States Environmental Protection Agency, Office of Water.

Pytkowicz, R.M. 1983. *Equilibria, Nonequilibria, and Natural Waters.* John Wiley & Sons, New York.

Rand, G.M. and S.R. Petrocelli, eds. 1985. *Fundamentals of Aquatic Toxicology.* Hemisphere Publishing Corporation, Washington, D.C.

Rankin, J.C. and J.A. Davenport. 1981. *Animal Osmoregulation.* Blackie, Glasgow, Scotland.

Rankin, J.C., I.W. Henderson and J.A. Brown. 1983. Osmoregulation and the Control of Kidney Function. In J.C. Rankin, T.J. Pitcher, and R.T. Duggan, eds. *Control Processes in Fish Physiology.* John Wiley and Sons, New York, New York. pp. 66-88.

Reddy, S.L.N., N.B.R.K. Venu Gopal, A. Nagender Reddy and J.V. Ramana Rao. 1989. Fluoride-Induced Changes in Carbohydrate Metabolism in the Tissues of Freshwater Crab *Baryelphusa guerini. Ecotoxicology and Environmental Safety.* 18:59-67.

Robinson, D.J.S. and E.J. Perkins. 1977. The Toxicity of Some Wood Pulp Effluent Constituents. *Cumbra Sea Fish, Comm. Sci. Rep. No. 74/1*, The Courts, Carlisle, England.

Saiki, M.K., M.R. Jennings and R.H. Weidmeyer. 1992. Toxicity of Agricultural Subsurface Drainwater from the San Joaquin Valley, California, to Juvenile Chinook Salmon and Striped Bass. *Transactions of the American Fisheries Society.* 121:78-93.

Saiki, M.K., M.R. Jennings and W.G. Brumbaugh. 1993. Boron, Molybdenum, and Selenium in Aquatic Food Chains from the Lower San Joaquin River and its Tributaries, California. *Archives of Environmental Contamination and Toxicology.* 24:307-319.

Saliba, L.J. and M. Ahsanullah. 1973. Accimation and Tolerance of *Artemia salina* and *Ophryotrocha labronica* to Copper Sulphate. *Marine Biology.* 23(4):297-302.

Shaikh, Y.A. and P.K. Hiradhar. 1988. Alterations in the Acid and Alkaline Phosphatase Quantities in Fluoride-Exposed Estuarine Goby, *Boleophthalmus dussumieri. Fluoride.* 21(3):131-136.

Simmons, P.F. 1992. Treatment and Disposal of Wastewaters Produced with Coalbed Methane by Reverse Osmosis. In J.P. Ray and F.R. Engelhardt, eds. *Produced Water Technological/Environmental Issues and Solutions.* Plenum Press, New York. pp. 593-604.

Slooff, W. and J.H. Canton. 1983. Comparison of the Susceptibility of 11 Freshwater Species to 8 Chemical Compounds. II. (Semi)Chronic Toxicity Tests. *Aquatic Toxicology.* 4(3):271-282.

Smith, L.R., T.M. Holsen, N.C. Ibay, R.M. Block and A.B. DeLeon. 1985. Studies on Acute Toxicity of Fluoride Ion to Stickleback, Fathead Minnow, and Rainbow Trout. *Chemosphere.* 14(9):1383-1389.

Sparks, R.E. and K.B. Anderson. 1977. Acute Toxicity of Potassuim to the Fingernail Clam, *Musculium transversum. Transactions. Illinois State Academy of Science.* 69(2):229.

Stancyk, S.E. and P.L. Shaffer. 1977. The Salinity Tolerance of *Ophiothrix angulata* (Say) Echinodermata: Ophiuroidea in Latitudinally Separate Populations. *Journal of Experimental Marine Biology and Ecology* 29:35-43.

Stanley, R.A. 1974. Toxicity of Heavy Metals and Salts to Eurasian Watermilfoil (*Myriophyllum spicatum L.*) *Archives of Environmental Contamination and Toxicology.* 2(4):331-341.

Stormer, J., F.B. Jansen and J.C. Rankin. 1996. Uptake of Nitrite, Nitrate, and Bromide in Rainbow Trout, *Oncorhynchus mykiss*: Effects on Ionic Balance. *Canadian Journal of Fisheries and Aquatic Sciences.* 53:1943-1950.

Suzuki, K. 1959. The Toxic Influence of Heavy Metal Salts Upon Mosquito Larvae. *Hokkaido University. Journal of the Faculty of Science Series.* 6(14):196-209.

Takei, Y. 1993. Role of Peptide Hormones in Fish Osmoregulation. In J.C. Rankin and F.B. Jensen, eds. *Fish Ecophysiology.* Chapman and Hall, London, England. pp. 136-160.

Tibbetts, P.J.C., I.J. Buchanan, L.J. Gawel and R. Large. 1992. A Comprehensive Determination of Produced Water Composition. In J.P. Ray and F.R. Engelhardt, eds. *Produced Water Technological/Environmental Issues and Solutions.* pp. 97-112.

Tietge, J.E., J.R. Hockett and J.M. Evans. 1997. Major Ion Toxicity of Six Produced Waters to Three Freshwater Species: Application of Ion Toxicity Models and TIE Procedures. *Environmental Toxicology and Chemistry.* 16(10):2002-2008.

Tietge, J.E., D.R. Mount and D.D. Gulley. 1994. *The GRI Freshwater STR Model and Computer Program: Overview, Validation, and Application.* GRI-94/0369. Gas Research Institute, Chicago, IL.

TNRCC. 1995. *Implementation of the Texas Natural Resource Conservation Commission Standards via Permitting.* RG-194. Texas Natural Resource Conservation Commission, Water Planning and Assessment Division, Dallas, TX.

Toler, L.G. 1980. *Some Chemical Characteristics of Mine Drainage in Illinois.* Report 80-16. U.S. Geological Survey. 47 p.

Trama, F.B. 1954. The Acute Toxicity of Some Common Salts of Sodium, Potassium, and Calcium to the Common Bluegill. *Proceedings of the Academy of Natural Sciences of Philadelphia.* 106:185-205.

Tsuji, S., Y. Tonogai and S. Kanoh. 1986. The Influence of Rearing Temperatures on the Toxicity of Various Environmental Pollutants for Killifish (*Oryzias latipes*). *Eisei Kagaku /Journal of Hygienic Chemistry.* 32(1):46-53.

Tucker, M., J.S. Qi and R. MacGregor. 1997. Ionic Imbalance Causes Effluent Toxicity. *Industrial Wastewater.* 5(5):57-62.

Turoboyski, L. 1960. Attempt to Determine the Influence of High Doses of some Chemical Compounds upon Carp Fry. *Roczniki Nauk Rolniczych.* 75B(3):401-445.

U.S.EPA. 1989. *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures.* EPA/600/3-88/036. United States Environmental Protection Agency, Office of Research and Development.

U.S.EPA. 1991a. *Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Procedures (Second Edition).* EPA/600/6-91/003. United States Environmental Protection Agency, Office of Research and Development.

U.S.EPA. 1991b. *Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I.* EPA/600/6-91/005. United States Environmental Protection Agency, Office of Research and Development.

U.S.EPA. 1993. *Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures.* EPA/200/R-92/080. United States Environmental Protection Agency, Office of Research and Development.

U.S.EPA. 1996. *Regions 9 and 10 Guidance for Implementing Whole Effluent Toxicity Testing Programs.* Final, May 31, 1996.

Valentich, J.D., K. Karnaky, Jr. and W.M. Moran. 1995. Phenotypic Expression and Natrivretic Petide-Activated Chloride Secretion in Cultured Shark (*Squales acanthias*) Rectal Gland Epithelial Cells. In C.M. Wood and T.J. Shuttleworth, eds. *Cellular and Molecular Approaches to Fish Ionic Regulation.* Academic Press, San Diego, California. pp. 173-205.

Wallen, I. E., W.C. Greer and R. Lasater. 1957. Toxicitiy to *Gambusia affinis* of Certain Pure Chemicals in Turbid Waters. *Sewage and Industrial Wastes* 29(6):695-711.

Weber, C.I., ed. 1993. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Edition.* EPA/600/4-90/027F. United States Environmental Protection Agency, Office of Research and Development.

Wetzel, R.G. 1983. *Limnology,* Second ed. Saunders College Publishing. Philadelphia, PA.

Williams, W.D. and H.F. Wan. 1972. Some Distinctive Features of Australian Inland Waters. *Water Research* 6:829-836.

Wright, D.A. and A.W. Davison. 1975. The Accumulation of Fluoride by Marine and Intertidal Animals. *Environmental Pollution.* 8:1-13.

Appendix A

GLOSSARY OF TERMS

GLOSSARY OF TERMS

Acoelomate - Referring to bilateral metazoans having no open cavity between the ectoderm and endoderm. Acoelomate animals include Platyhelminthes and Nemertea (also known as Rhynchocoela).

Acute Toxicity Test - A toxicity test involving short term exposure of an organism to a potential toxicant. The exposure is generally not more than 96 hours, and the effect is measured by mortality.

Anion - An ion with a net negative electrical charge.

C_{18} - An isotope of carbon, which can be used to extract some types of materials from solutions.

Cation - An ion with a net positive electrical charge.

Chronic Toxicity Test - A toxicity test involving an organism that is exposed to a potential toxicant for a long period of time, from weeks to years, depending on the normal development and life span of the organism. The effects of the toxicant are commonly measured in terms other than mortality, such as growth rate or reproductive ability.

Coelomate - Referring to bilateral metazoans having a true coelom, which is a cavity that, during development, forms from a split in the mesoderm (middle layer of cells in embryos), and is bounded by a peritoneum. Coelomate animals include Annelida, Mollusca, Arthropoda, Echinodermata, and Chordata.

Compound - A material that is made up of atoms of two or more elements chemically bound together.

Donnan Equilibrium - The passive distribution of permeant ions across a semi-permeable membrane. Cells contain ions which cannot cross the cell membrane (including proteins), as well as permeant ions that can cross the membrane. The distribution of the permeant ions is determined by the concentration gradient of both the permeant and impermeant ions. This results in an unequal distribution across the cell membrane.

Effective Concentration (EC) - The concentration of test material that is expected to cause an effect other than death to a certain percentage of test organisms (such as EC_{50}, EC_{10}). It is used in conjunction with other terms to describe the expected effect.

Effluent - A complex mixture produced as a waste material, such as an industrial discharge or sewage, that may be released into the environment.

Euryhaline - Accustomed to, or tolerant of, a wide range of salinity.

Glomerular Filtration Rate (GFR)- Rate at which a substance is filtered from the plasma in the nephronic glomerulus. GFR is calculated by multiplying the concentration of a substance in the urine (often radio-labeled inulin is used as a marker) by the rate of urine flow, and dividing the product by the plasma concentration.

Hypersaline - Having a relatively greater salinity than another solution. A solution may be hypersaline to one solution, but hyposaline (relatively lower salinity) to another solution.

Hypertonic - Having a higher ionic concentration than the surrounding environment.

Hypotonic - Having a lower ionic concentration than the surrounding environment.

Inhibition Concentration - Concentration, derived though linear interpolation methods, that represents a given reduction in organism performance. Use of an IC_{25} is common; typical performance endpoints include growth and reproduction.

Ion - An atom that has had electrons either removed or added to it, producing a positively or negatively charged particle.

Median Effective Concentration (EC_{50}) - The concentration of test material that is expected to cause an effect other than mortality in 50% of the organisms tested.

Median Lethal Concentration (LC_{50}) - The concentration of test material that is expected to cause mortality in one-half, or 50% of the organisms tested.

NPDES - National Pollutant Discharge Elimination System. A permit process created under Section 402 of the Clean Water Act that allows EPA to issue permits for the discharge of waste water into navigable waterways. The permits often require identification and measurements of the whole effluent toxicity (WET) on a site-specific basis, commonly attained through toxicity testing.

Osmolality - The concentration of an osmotic solution, in molal concentration of ions in solution.

Pseudocoelomate - Referring to bilateral metazoans having an open cavity between the ectoderm and endoderm, that is derived from the embryonic blastocoel and is not bounded by a peritoneum. Pseudocoelomate animals include Rotifera (rotifers) and Nematoda (round worms).

Stenohaline - Unaccustomed to, and unable to tolerate, large changes in water salinity.

WET - Whole Effluent Toxicity. Referring to the toxicity measured in an intact (unaltered) effluent solution. WET tests are often required under the NPDES permit program.

Appendix B

BIBLIOGRAPHY
(Additional Readings in Ion Toxicity)

BIBLIOGRAPHY

Abel, T.H. and F. Barlocher. 1984. Effects of Cadmium on Aquatic Hyphomycetes. *Applied and Environmental Microbiology*. 48(2):245-251.

Abraham, T.J., K.Y.M. Salih and J. Chacko. 1986. Effects of Heavy Metals on the Filtration Rate of Bivalve *Villorita cyprinoides* (Hanley) Var. Cochinensis. *Indian Journal of Marine Science*. 15(3):195-196.

Adelman, I.R., L.L. Smith, Jr. and G.D. Siesennop. 1976. Acute Toxicity of Sodium Chloride, Pentachlorophenol, Guthion, and Hexavalent Chromium to Fathead Minnows *(Pimephales promelas)* and Goldfish (*Carassius auratus*). *Journal of the Fish Research Board of Canada*. 33(2):203-208.

Amiard, J.C. 1976a. Experimental Study of the Acute Toxicity of Cobalt, Strontium, and Silver Salts in Some Crustacea and their Larvae and In Some Teleostei. *Reveue Internationale d'Oceanographic Medicale.* 43:79-95.

Amiard, J.C. 1976b. Phototactic Variations in Crustacean Larvae Due to Diverse Metallic Pollutants Demonstrated by a Sublethal Toxicity Test. *Marine Biology.* 34(3):239-245.

Anderson, B.G. 1944. The Toxicity Thresholds of Various Substances Found in Industrial Wastes As Determined by the Use of *Daphnia magna*. *Sewage Works Journal.* 16(6):1156-1165.

Anderson, B.G. 1948. The Apparent Thresholds of Toxicity to *Daphnia magna* for Chlorides of Various Metals When Added to Lake Erie Water. *Transactions of the American Fisheries Society*. 78:96-113.

Anderson, K.B. 1977. *Musculium transversum in the Illinois River and an Acute Potassium Bioassay Method for the Species*. Master's Thesis, Western Illinois University, Macomb, IL., Govt. Rep. Announce. Index 79(5), U.S. NITS PB-288 088.

Baird, D.J., I. Barber, M. Bradley, A.M.V.M. Soares and P.Calow. 1991. A Comparitive Study of Genotype Sensitivity to Acute Toxic Stress Using Clones of *Daphnia magna Straus. Ecotoxicology and Environmental Safety.* 21(3):257-265.

Baker, M.D., C.I. Mayfield and W.E. Inniss. 1983. Toxicity of pH, Heavy Metals, and Bisulfate to a Freshwater Green Alga. *Chemosphere*. 12(1):35-44.

Batterton, J.C., G.M. Boush and F. Matsumura. 1972. Inhibition of Sodium Chloride Tolerance by the Green Alga *Anacystis nidulans. Science*. 176:1141-1143.

Bringmann, G. and R. Kühn. 1977a. Limiting Values for the Damaging Action of Water Pollutants to Bacteria (*Pseudomonas putida*) and Green Algae (*Scenedesmus quadricauda*). *Zeitschrift fuer Wasser- und Abwasser Forschung*. 10(3/4):87-98.

Bringmann, G. and R. Kühn. 1977b. Results of the Damaging Effect of Water Pollutants on *Daphnia magna. Zeitschrift fuer Wasser- und Abwasser Forschung.* 13(5):170-173.

Bringmann, G. and R. Kühn. 1978a. Limiting Values for the Noxious Effects of Water Pollutant Material to Blue Algae (*Microcystis aeruginosa*) and Green Algae *Scenedesmus quadricauda. Vom Wasser.* 50:45-60.

Bringmann, G. and R. Kühn. 1978b. Testing of Substances for Their Toxicity Threshold: Model Organisms *Microcystis* (*Diplocystis*) *aeruginosa* and *Scenedesmus quadricauda. Mittielunge. Internationale Verienigug fuer Theoretische und Angewande Limnologe.* 21:275-284.

Bringmann, G. and R. Kühn. 1980. Comparison of the Toxicity Thresholds of Water Pollutants to Bacteria, Algae, and Protozoa in the Cell Multiplication Inhibition Test. *Water Research.* 14(3):231-241.

Bringmann, G., R. Kühn and A. Winter. 1980. Determination of Biological Damage From Water Pollutants to Protozoa, III. Saprozoic Flagellates. *Zeitschrift fuer Wasser- und Abwasser Forschung.* 13(5):170-173.

Calapaj, G.G. 1973. Richere Di Laboratorio Sull'Inquinamento Chimico Dei Mitili Nota I: Radiostronzio, Radiocesio, Mercurio Inorganico, Cromo Esavelente. *Igiene Moderna.* 66(3):243-270.

Cherry, D.S., J.H. Rodgers, Jr., R.L. Graney and J.Cairns, Jr. 1980. Dynamics and Control of the Asiatic Clam in the New River, Virginia. *Bulletin of the Virginia Water Resources Research Center. 123.*

Davis, K.B., N.C. Parker and M.A. Suttle. 1982. Plasma Corticosteroids and Chlorides in Striped Bass Exposed to Tricaine Methanesulfonate, Quinaldine, Etomidate, and Salt. *Progressive Fish-Culture.* 44(4):205-207.

Deneer, J.W., W. Seinen and J.L.M. Hermens. 1988. Growth of *Daphnia magna* Exposed to Mixtures of Chemicals with Diverse Modes of Action. *Ecotoxicology and Environmental Safety.* 15(1):72-77.

Ellis, M.M. 1937. Detection and Measurement of Stream Pollution. In: *Bulletin. Bureau of Fisheries No. 22.* U.S. Department of Commerce, Washington, D.C. pp. 365-437.

Ewell, W.S., J.W. Gorsuch, R.O. Kringle, K.A. Robillard and R.C. Spiegel. 1986. Simultaneous Evaluation of the Acute Effects of Chemicals on Seven Aquatic Species. *Environmental Toxicology and Chemistry.* 5(9):831-840.

Fehler, S.W.G. and R.J. Light. 1972. Biosynthesis of Methylheptadacanes in *Anabaena variabilis.* In Vitro Incorporation of S-[Methyl-14C] Adenosylmethionine. *Biochemistry.* 11(13):2411-2416.

Frahm, J.P. 1975. Toxitoleranzversuche an Wassermoosen. (Toxicology Tolerance Studies Utilizing Periphyton). *Gewasser Und Abwasser.* 57/58:59-66.

Freeman, L. and I. Fowler. 1953. Toxicity of Combinations of Certain Inorganic Compounds to *Daphnia magna Straus. Sewage and Industrial Wastes.* 25(10):1191-1195.

Gannon, J.E. and S.A. Gannon. 1975. Observations on the Narcotization of Crustacean Zooplankton. *Crustaceana (Leiden).* 28(2):220-224.

Grantham, C.K. and J.P. Sloan. 1975. *Toxicity Study Drilling Fluid Chemicals on Aquatic Life.* EPA 560/1-75-004, Conference Proceedings on Environmental Aspects of Chemical Use in Well-Drilling Operations, Research Triangle Inst, RTP, N.C., PB-246 947.

Harry, H.W. and D.V. Aldrich. 1963. The Distress Syndrome in *Taphius glabratus* (Say) as a Reaction to Toxic Concentrations of Inorganic Ions. *Malacologia.* 1(2):283-289.

Hinton, M.J. and A.G. Eversole. 1978. Toxicity of Ten Commonly Used Chemicals to American Eels. *Proceedings of the World Maricultural Society.* 10:599-604.

Iwai, T., T. Oto and K. Tamura. 1974. The Toxicity of Nitrite on Ayu-Fry, *Plecoglossus altivelis*, and the Chlorinity in the Culturing Water. *Bulletin. Faculty of Fisheries. Mie University.* 1:43-51.

Jones, J.R.E. 1941. A Study of the Relative Toxicity of Anions, with *Polycelis nigra* as Test Animal. *Journal of Experimental Biology.* 18:170-181.

Kanta, S. and T.A. Sarma. 1980. Biochemical Studies on Sporulation in Blue-Green Algae II. Factors Affecting Glycogen Accumulation. *Zietschrift fuer Allegmeine Mikrobiologie. Morphologie, Physiologie, Genetik, und Oekologie der Mikrorganismen.* 20(7):459-463.

Khatib, S.A. 1990. Response of a Freshwater Bivalve *Indonaia caeruleus* to Sodium Chloride. *Environmental Ecology.* 8(2):697-701

Kühn, R. et. al. 1988. Schadstoffwirkungen von Umweltchemmikalien im Daphnien-Reproduktions-Test als Grudlage fr die Bewertung der Umweltgefhrlichkeit in Aquatischen System. *Forschungsbericht* . 10603052, Mrz.

Kosinova, N.R. and A.M. Alesan'Yan. 1978. Investigation of Reversability of Effects of Strontium-90 and Metaphos on *Cyprinus carpio. Radiobiology.* 18(4):180-184; Translated From *Radiobiologiya.* 18(4):630-634.

Kouril, J., M. Dvorak and I. Prikryl. 1985. Sensitivity of the Early Fry of Common Carp and Grass Carp to NaCl and Formalin in Preventative Baths. *Aquatic Science and Fisheries Abstracts.* 15(11)23842-1Q15; *Bulletin. Vzykumenko Ustavu Ryb. Hydrobiol. Vodnany.* 20(4):22-32 (1984).

Linden, E., B.E. Bengtsson, O. Svanberg and G. Sundstrom. 1979. The Acute Toxicity of 78 Chemicals and Pesticide Formulations Against Two Brackish Water Organisms, the Bleak (*Alburnus alburnus*) and the Harpaticoid *Nitocras pinipes. Chemosphere.* 8(11/12):843-851.

Loeb, H.A. and W.H. Kelly. 1963. *Acute Oral Toxicity of 1,496 Chemicals Force-Fed to Carp.* U.S. Fish and Wildlife Service, Special Science Report-Fish. No. 471, Washington, D.C.

Mezhnin, F.L. 1973. Histopathological Changes in Organs and Tissues of *Lebistes reticulatus* During Acute Experimental Intoxication with Polychloropinene, Phenol, and *Trudy In Stituta Biologii Vnutrennikh Vod Akademii Nauk SSSR.* 24: pp. 53-66.

Nell, J.A. and J.E. Holliday. 1986. Effects of Potassium and Copper on the Settling Rate of Sydney Rock Oyster (*Saccostrea commercialis*) Larvae. *Aquaculture.* 58(3-4):263-267.

Olson, P.R. 1971. *Accumulation of Calcium-45 in Developing Coho Salmon Eggs and Fry Reared in Varying Concentrations of Stable Calcium.* Rept. No. CONF-710501-29, Proceedings of the 3rd National Symposium on Radioecology, Oak Ridge, TN: U.S. NTIS RLO-225-T-7-4.

Palmer, C.M. and T.E. Maloney. 1955. Preliminary Screening for Potential Algicides. *Ohio Journal of Science* 55(1):1-8.

Rabe, R.. W. Nobel and A. Kohler. 1982. Effects of Sodium Chloride on Photosynthesis and Some Enzyme Activities of *Potamogeton alpinus. Aquatic Botany.* 14(2):159-165.

Rahimaian, H. 1972. *The Effect of Calcium on Growth and Morphogenesis of* Chlorella, Golenkinia, *and* Scenedesmus. PhD. Thesis, Washington State University, Pullman, WA.

Robison, W.R., S.H. Newton and M.V. Meisch. 1982. Effect of Selected Sodium Chloride Concentrations for Minimizing Mortality of Mosquitofish *Gambusia affinis* in Holding Tanks. *Mosquito News.* 42(3):440-442.

Roderer, G. 1979. Hemmung Der Cytokinese Und Bildung Von Riesenzellen Bei *Poterioochromonas malhamensis* Durch Organische Blieverbindungen Und Andere Agenzien. *Protoplasma.* 99(½):39-51.

Sestokas, J. and J. Cukerzis. 1972. Effect of Chemical Preparations on the Crayfish *Astacus astacus* and *Astacus leptodactylus. Lietuvos TSR Mokslu Akademijos Darbai, Serija C.* (4):119-124.

Sheplay, A.W. and T.J. Bradley. 1982. A Comparative Study of Magnesium Sulphate Tolerance in Saline-Water Mosquito Larvae. *Journal of Insect Physiology.* 28(7):641-646.

Stangenberg, M. 1975. The Influence of the Chemical Composition of Water in the Pike Perch (*Lucioperca lucioperca L.*) Fry From the Lake Gopio. *Limnologica.* 9(3):421-426.

Sunila, I. 1988. Acute Histological Responses of the Gill of the Mussel, *Mytilus edulis*, to Exposure by Environmental Pollutants. *Journal of Invertebrate Pathology.* 52(1):137-141.

Suzuki, Y., R. Nakamura and T. Ueda. 1972. Accumulation of Strontium and Calcium in Freshwater Fishes of Japan. *Journal of Radiation Research.* 13(4):199-207.

Threader, R.W. and A.H. Houston. 1983. Use of NaCl As a Reference Toxicant for Goldfish, *Carassius auratus. Canadian Journal of Fisheries and Aquatic Sciences.* 40(1):89-92

Van Horn, W.M., J.B. Anderson and M. Katz. 1949. The Effect of Kraft Pulp Mill Wastes on Some Aquatic Organisms. *Transactions of the American Fisheries Society.* 79:55-63

Van Leeuwen, C.J., M. Rijkeboer and G. Niebeek. 1986. Population Dynamics of *Daphnia magna* As Modified by Chronic Bromide Stress. *Hydrobiologica* 133(3):277-285.

Wang, W. 1986. Toxicity Tests of Aquatic Pollutants by Using Common Duckweed. *Environ. Pollut. Ser. B Chem. Phys.* 11(1):1-14.

Wester, P.W., J.H. Canton and J.A.M.A. Dormans. 1988. Pathological Effects in Freshwater Fish *Poecilia reticulata* (Guppy) and *Oryzias latipes* (Medaka) Following Methyl Bromide and Sodium Bromide Exposure. *Aquatic Toxicology.* 12(4):323-344.

Wooldridge, C.R. and D.P. Wooldridge. 1969. Internal Damage in an Aquatic Beetle Exposed to Sublethal Concentrations of Inorganic Ions. *Annals of the Entomological Society of America Association.* 62(4):921-933.

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