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I am submitting herewith a thesis written by Terry L. Phipps entitled "Derivation of Site-Specific Water Quality Criteria for Selected Metals." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

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DERIVATION OF SITE-SPECIFIC WATER QUALITY CRITERIA FOR SELECTED METALS

A Thesis
Presented for the
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Terry L. Phipps
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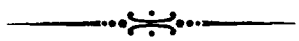
DEDICATION

This thesis is dedicated to my husband

Larry Phipps

for his strength of spirit



and to my parents

Harry and Audrie LaTowsky

for giving me roots and wings

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ABSTRACT

Site-specific permit limits for copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) were determined for four outfalls at the Paducah Gaseous Diffusion Plant (PGDP) in Paducah, KY. These revised limits were derived using a procedure issued by the Kentucky Division of Water (KDOW). The KDOW's method is a cost-effective alternative to the water-effect ratio (WER) method for developing site-specific numeric criteria for metals. Both methods (KDOW's and WER) require toxicity testing and chemical-specific analyses. With the WER method, site water is spiked with the metal of interest so that a concentration-effect curve can be evaluated. No metal-spiking is required with the KDOW method; therefore, all metals of interest may be analyzed simultaneously. This difference makes the KDOW method much less expensive than the WER method. I estimated that the analytical costs required for the KDOW method were 10–20% of those required for the WER method.

Toxicity tests with *Ceriodaphnia dubia* and *Pimephales promelas* (fathead minnow) larvae showed that the concentrations of metals in the effluents were not great enough to be toxic to the test organisms. Also, the concentrations of Cu, Ni, Pb, and Zn in the outfalls were below the permit limits and federal numeric water quality criteria. For this reason, metals in the effluents at PGDP are not likely to cause compliance-related problems. Levels of total recoverable and dissolved Cu, Ni, Pb, and Zn were measured in the effluents. These data were used to calculate the final mean ratio of dissolved metal to total recoverable metal for each effluent. Applying the equation in the KDOW method, the measured total recoverable metal concentration is adjusted downward by the portion of the metal in the

effluent that is biologically relevant (dissolved metal); this value is then reported to determine compliance with numeric limits. By using the KDOW recalculation method, the total recoverable metal concentrations of Cu, Ni, Pb, and Zn in the effluents were reduced from 4.7% to 54.1%.

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
ANOVA	analysis of variance
CF	conversion factor
DOC	dissolved organic carbon
DOE	U.S. Department of Energy
EDTA	ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
GLM	general linear models
HDPE	high-density polyethylene
IC_{25}	inhibition concentration (25% reduction)
ICP/MS	inductively coupled plasma-mass spectrometry
KDOW	Kentucky Division of Water
KPDES	Kentucky Pollutant Discharge Elimination System
LC_{50}	lethal concentration (to 50% of tested organisms)
MDL	method detection limit
ORNL	Oak Ridge National Laboratory
PGDP	Paducah Gaseous Diffusion Plant
RPD	relative percent difference
SAS	Statistical Analyses System®
SD	standard deviation
TRM	total recoverable metal
TSS	total suspended solids
TUc	toxic units, chronic
WER	water-effect ratio
WQC	water quality criteria/criterion

PART 1

Metal Toxicity: An Overview

INTRODUCTION

Metals in public water systems have been a human health concern for nearly a century, but it wasn't until the 1940s and 1950s that research began addressing the effects of contaminants, such as metals, on aquatic organisms [1]. Unlike organic pollutants, metals are considered to be persistent in the environment: they are not subject to photolysis, combustion, or hydrolysis. The focus of this thesis concerns the effects of selected metal contamination in the aquatic ecosystem and the influence of water quality on the bioavailability and/or toxicity of metals to aquatic organisms. For clarification, the term "bioavailable" is used herein to indicate the dissolved form of a metal. It is the dissolved form of a metal that is considered to be the more biologically available form and, thus, the form that causes toxicity [2]. Six metals, including cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) were included in this study. Based on the Environmental Protection Agency's (EPA's) freshwater quality criteria for priority pollutants, Cd, Pb, and Cu are considerably more toxic than Zn, Ni, and trivalent Cr, in that order [2].

Four of the six metals (Cu, Cr, Ni, and Zn) included in this study are essential trace metals, as well as potentially toxic. Essential metals are those that are required for specific biochemical and metabolic processes in microorganisms, plants, and animals. These metals are important in oxidation-reduction reactions, are essential components of enzymes, and stabilize and activate many proteins [1,3–5]. Although microconcentrations of these metals are essential, higher concentrations can be toxic, if they exceed levels necessary for normal

survival and development [1,3,6].

METAL TOXICITY

Many studies conducted during the 1970s to early 1990s evaluated the acute toxicity of single-metal exposures to fish and daphnids [7–16]. In these studies, organisms were exposed to concentrations of a metal for 4 d or less, and mortality was the response measured. In most cases, the toxicity data from acute tests such as those were used to estimate a 48- or a 96-h LC_{50} value (i.e., the concentration of the metal that is lethal to 50% of the tested organisms in 48 or 96-h). Toxicity data derived from acute tests were used in establishing federal water quality criteria [17–21]. Most effluent discharges, however, contain numerous metals as well as other chemicals [3,6,13,16,22,23]. Some researchers questioned the ecological relevance of acute toxicity tests, on the basis that the results of these tests may not provide data that can be reliably extrapolated to long-term chronic exposures to much lower levels of contaminants [6,7,24]. Additionally, the toxicity of a metal in a mixture may differ from its individual toxicity. Also, the results of a toxicity test apply to the aquatic species tested, and these results may be affected by the chemical characteristics of the water tested. Thus, to be protective of the aquatic life in a body of water, the EPA recommends that the "best" approach is to develop criteria specific for that body of water.

The mechanisms of toxic action are better understood and more thoroughly documented for some aquatic organisms, particularly fish, than for other aquatic organisms [25–30]. In acute

exposures, the gills are important sites of toxic action, whereas at chronic exposures the kidney and liver are more susceptible organs [29]. The kidneys and the liver play important roles in the detoxification process, and daphnids do not have these organs. In daphnids, the mechanisms of toxic action are not as well studied, and are assumed to be more primitive than the mechanisms in fish [16]. This may explain why daphnids are more sensitive than fish to most metals. Chronic effects observed in fish and/or daphnids may include reduced growth, fecundity, and hatchability of eggs; the inhibition of spawning or sexual maturation; or an increase in deformities [8,31–34].

Contaminants can also accumulate in storage sites within aquatic organisms. The concentration of the contaminant may increase above the threshold concentration needed to cause an adverse biological response [23,35,36]. Most toxicity is the result of some degree of accumulation [36]. The toxicity of metals, however, may be modified by biomolecules such as metallothioneins, which can remove or inactivate metal ions within a cell [3,25,37]. The presence of Zn, Cu, or Cd induces the synthesis of some metallothioneins [3].

Exposure, Uptake, and Complexation

Aquatic organisms can be exposed to metals in water (i.e, in the water column or interstitial water), sediment, or food. In the aquatic environment, the form, or species, of a metal can affect its bioavailability. The metal may be dissolved (operationally described as capable of passing through an 0.45-μm filter), adsorbed to a biotic or abiotic component and suspended

in the water, deposited in the sediment, or incorporated (bioaccumulated) in organisms via ingestion of metal-contaminated food [35]. The mechanism for the uptake of metals from solution by aquatic organisms is relatively fast and depends upon the maintenance of a concentration gradient across cell membranes. In general, the rate of uptake is based on the concentration of free metal ion, not the total metal concentration [38]. The concentration of a metal inside an organism can be 10^3 to 10^4 times greater that its concentration in the surrounding medium, but the actual concentration will depend on the metal itself, the metal species, the organism, and environmental conditions [3].

Metals can become complexed (bound) to a ligand that can make metals either more toxic (via transport mechanisms) or less toxic, by changing their biological availability. Metals that complex tightly to ligands generally are not readily bioavailable and thus, are less toxic than their free-metal ion counterparts to aquatic biota [10,39]. By definition, a ligand is a small molecule that binds to another molecule [40]. There are many different types of ligands, organic and inorganic, as well as natural and man-made. Examples of ligands found in natural surface waters and municipal and industrial effluent discharges include glycine, ammonia, oxalate, humic or fulvic acids, hydroxide, carbonate, bicarbonate, chloride, and hydrogen sulfide [4,30,41]. Metals that accumulate at the cell surface can be bound by ligands. These ligands are then transferred by molecular carriers through the cell membrane. Though some metals such as Pb, Cu, and Zn form strong bonds with ligands, they can also undergo ligand exchange at different rates [4]. Zn, for example, exchanges ligands 10^3 times faster than Ni [4].

Ethylenediaminetetraacetic acid (EDTA) is a synthetic organic ligand that is a common component of household detergents, soaps, and cosmetics [42,43]. EDTA also is found in various industrial effluents [42,43]. This complexing agent can "deactivate" metal ions by forming strong complexes that reduce the metal's biological availability [43]. A study using daphnids found that the addition of EDTA decreased the acute toxicity of Cu, Zn, and Cd by as much as 99% [43]. EDTA complexes are relatively stable, and thus are not readily biodegraded. As concentrations of EDTA in natural waters increase, metals bound in sediments may be re-mobilized and can become bioavailable [42,43].

The toxicity of a metal may be related to its complexation capabilities [30]. These capabilities may explain why Cd is such a toxic metal. The complexation of Cd with organic matter is less stable than those of other metals (e.g., Cu or Pb). Because Cd does not form strong, stable complexes, a relatively large portion of Cd is bioavailable; thus, smaller concentrations of Cd can cause toxicity [30]. Zn, on the other hand, is relatively nontoxic compared to other metals, probably because it forms fairly stable complexes. Because of its complexation and adsorption capacities, much of the total amount of Zn present in the water may be relatively nontoxic [30].

A metal's toxicity (or the lack thereof) may also be influenced by its affinity for specific binding sites. Different metals have different affinities for different binding sites. For example, Cr has a strong affinity to bind at O donor sites, whereas Ni and Cu bind more readily at N donor sites [4]. Cd and Pb both bind strongly to sulfhydryl groups (–SH), which

are components of many proteins [4,6]. Pb also has a strong affinity for phosphate-containing ligands [6]. Zn can transition between ligands that contain O, N, or S as electron donors [4]. Both Zn and Pb form strong hydroxide (OH^-) complexes, and Cd competes with and can displace Zn by irreversibly binding to active sites in a number of Zn-containing metalloenzymes [6].

Biotic Factors That Can Affect Metal Toxicity

Biotic factors characteristic of the organism in question can affect the toxicity of a contaminant. Such factors include the type of organism (e.g, fish, daphnid, plant, etc.), the organisms's life stage or size, its general health and nutritional status, and exposure history (e.g., degree of acclimation) to a contaminant [1,3,6,44,45].

Species sensitivity

The sensitivity of aquatic organisms to a particular contaminant can vary considerably [8,10,12,19–23,31,46–48]. In some cases, the differences in sensitivities among species can be several orders of magnitude between the least sensitive and the most sensitive organism [17–21,47,48]. Knowledge about the different sensitivities of aquatic organisms is important when selecting test organisms to be used for particular assessments. A cladoceran, *(Ceriodaphnia dubia*) and the fathead minnow (*Pimephales promelas*) are commonly used to conduct freshwater toxicity tests [49]. In many cases, an effluent is evaluated for toxicity

using both of these aquatic organisms because they differ in sensitivity to contaminants [49]. In waters similar with respect to hardness and pH, *C. dubia* were (1) more sensitive to Cu, Ni, or Pb; (2) equally sensitive to Zn; or (3) less sensitive to Cd, compared to fathead minnows [16,31,47].

Life-stage sensitivity

The duration of short-term (i.e., 7–10 days) toxicity tests may not be great enough to reveal subtle effects of contaminants [50]. Slowly-developing effects may include (1) morphological changes, (2) a delay of sexual development, (3) behavioral changes, or (4) genetic modification [3]. Immature organisms (e.g., eggs, larvae, neonates) generally tend to be more susceptible than adult organisms to metals [32–35,51–54]. The difference in sensitivities due to organism age may be related to the degree of development of detoxification mechanisms [35]. Research has been conducted to evaluate the effects of low concentrations of metals over long durations (i.e., months or years) [32,34,51–56]. Mount and Stephan [34] conducted tests to evaluate the effects of Cu in soft water to the various life stages of the fathead minnow (*Pimephales promelas*). The concentration of Cu that increased mortality and reduced growth and sexual development in fish exposed as eggs was about one-eighth of the concentration causing 50% mortality of 6-week old larval fish. Other studies have shown that the embryonic stage was more susceptible than adult stages to Cu [32,34,55], Zn [51], Cd [33], Ni [53], Pb [11], and trivalent Cr [52,54].

Acclimation

A period of acclimation, either to the contaminant being studied or to the test conditions (i.e., hardness, pH, temperature, etc.) sometimes can influence the outcome of laboratory toxicity tests. Organisms used in laboratory tests generally have no previous exposure to the toxicant before testing [29]. The results of these "no prior exposure" laboratory tests may indicate a greater sensitivity to a contaminant than what might be seen under ambient conditions. Aquatic organisms living in water that receives industrial effluents may have become acclimated to low concentrations of a toxicant present in the effluents [57]. Consequently, these organisms may be capable of tolerating greater concentrations of the toxicant before experiencing adverse effects, compared to organisms used in standard laboratory studies [29]. Studies that report a correlation between toxicity and hardness sometimes do not report whether they acclimated the test organisms to the test water hardness, or report that the organisms were acclimated only briefly before being exposed to the test water [27,34,58,59]. Other studies have been conducted to determine if acclimation to test water hardness affects toxicity test results [14,51,60]. The latter researchers were concerned that if the test organisms were not acclimated to the hardness of the water used in the study, the interpretation of toxicity data might be biased. Acclimation did not seem to have a significant effect on the toxicity of Cu [60] or Zn [51] to fathead minnows.

In the study described in Chapter 2, the organisms were not previously exposed to the metals at ambient concentrations, nor where they acclimated to the various water hardness

concentrations of the effluents. However, the test organisms were acclimated to the test temperature beforehand.

Abiotic Factors That Can Affect Metal Toxicity

Various environmental abiotic factors can affect the bioavailability and/or toxicity of metals. Such factors include temperature, pH, dissolved oxygen, hardness, redox potential, the types and concentrations of suspended and organic matter, salinity, and the presence and binding capacity of chelators [3,12,42,45]. In addition to abiotic factors, the concentration of the chemical entering the aquatic system, the duration and type of input (i.e., acute, chronic, intermittent, or continuous discharge), the ability of the system to resist pH changes (e.g., buffering capacity), and the location of the system relative to the release of the chemical all influence the vulnerability of the aquatic system [35]. These factors can strongly influence the concentration, transport, transformation, and disposition of the chemical in the aquatic ecosystem [35]. From the literature, pH and water hardness markedly affect the form and toxicity of most metals [7,14,18,27,28,45,51,61,62]. Although numerous abiotic factors can affect metal toxicity, not all of these factors were considered in the study described in Chapter 2. For my study, a portion of each effluent sample was analyzed for pH, hardness, alkalinity, conductivity, and concentrations of dissolved organic carbon (DOC) and total suspended solids (TSS). The temperature and dissolved oxygen concentration of the samples were not directly relevant to the bioavailability of the metals, because they did not reflect the ambient conditions.

pH

The chemical or physical form of a metal (i.e., the metal species) is affected by pH, or more precisely, by hydrogen ion (H^+) activity [7,27–29,47,61–65]. Some metal species are more toxic than others. In an acidic environment (pH < 4), the ionic form of the metal generally predominates and is considered to be the more toxic form. As the pH increases, carbonate, oxide, hydroxide, and sulfide complexes of the metals tend to predominate [63,64]. The free metal ion and hydroxide complexes tend to be more toxic than other metal species, whereas the metal-carbonate complexes tend to be less toxic [30,42,45,63]. Erickson et al. [27] found that on the basis of total copper, an increase in pH reduced the toxicity. However, on the basis of cupric ion activity, Cu toxicity increased with increasing pH.

Although pH influences the form of the metal present, not all metals are affected by pH in the same way. For example, at pH 3.0, nearly all of the Ni present is in the free ionic form (Ni^{2+}), but at a pH of 7.2, only about 20% of the Ni present exists in the form of Ni^{2+} [64]. The relatively small percentage of Ni available in the "toxic" form may explain why, when compared to the other metals such as Cd, Ni is relatively nontoxic to fish [4,31].

Most natural fresh waters have a pH in the range 6–10 [35], and within this pH range, approximately 85% of the Cd present exists primarily as free Cd ions (Cd^{2+}) [15,64]. The predominance of Cd in its most toxic (ionic) form makes it one of the most hazardous metals. The toxicity of Cd is believed to be due to the fact that it is similar in size to Ca^{2+} and can

effectively out-compete Ca^{2+} for Ca binding sites [4].

From pH 4 to 7, the majority of Zn present in fresh water is in solution (i.e., in the ionic form) [30,62]. At a pH > 7, less-toxic particulate forms of Zn, such as zinc carbonate ($ZnCO_3$) and zinc hydroxide [$Zn(OH_2)$] may prevail [62]. The toxic form of Pb (Pb^{2+}) predominates at pH < 7. Pb is less toxic in the form of hydroxide or carbonate complexes [4,30]. In natural waters, however, Pb is not readily converted to its more toxic forms [18]. At a pH < 6, free Cu^{2+} predominates, but Cu-carbonate complexes are more abundant when the pH is 6 to 9.3 [17]. The predominant form of Cr at pH 7 is the hydroxide, $Cr(OH)_2^+$, which is not very toxic [4]. Cr exists primarily as trivalent Cr (Cr^{3+}) only when the pH is < 4 [4]. Since the pH of most natural fresh waters is in the range of 6–10, there is almost no Cr^{3+} biologically available. This may explain why Cr^{3+} is one of the least toxic metals.

The solubility of many metals is also affected greatly by pH. Most metals are most soluble in the ionic form [18,42,64,66]. Hydroxide-metal complexes are minimally soluble at a pH of 9 to 12; sulfide-metal complexes are essentially insoluble at pH ≥ 7 [42]. Metal carbonate complexes tend to precipitate at high pH (> 8) [42]. The solubility of metal-carbonate complexes depends strongly on the presence of CO_2, since resolubilization occurs as solid-phase carbonates undergo dissolution [42,67].

Schubauer-Berigan, et al. (1993) evaluated the pH-dependent toxicity of five metals using four different species of aquatic organisms [47], and found pH-dependent changes in toxicity.

However, at a certain pH, different metals were more toxic to some organisms than others. For example, Cd and Zn were more toxic to *C. dubia* at pH 8.3. At an intermediate pH of 7.3, Zn was more toxic to *P. promelas,* and there was no pH-dependent toxicity of Cd to *P. promelas*. Cu and Pb were most toxic to all organisms at pH 6.3, and Ni was most toxic at pH 8.3 [47]. The results of different studies are sometimes contradictory. Mount [28] found that Zn was more toxic to *P. promelas* at a pH of approximately 9 than at a pH of 5. Lloyd and Herbert [68] suggest that precipitated Zn bound to the gills is solubilized a reduction in pH at the gill surface. CO_2, a product of respiration, is excreted at the gill surface, and decreases the pH by reaction to water, with the production of carbonic acid [68].

Other studies have investigated how pH interacts with other chemical factors such as water hardness, to modify the toxicity of metals [7,9,11,14,46,62]. The interaction of pH and hardness for two metals—Cu and Zn—was evaluated using *Ceriodaphnia dubia* [7]. *Ceriodaphnia* reproduction was reduced most strongly by Cu and Zn in a water with hardness of 100 mg/L and a pH of 6; Cu and Zn were less toxic when pH was > 6, for all hardness concentrations tested [7].

Hardness

Many studies have shown that water hardness [i.e., the concentrations of cations such as calcium (Ca) and, to a lessor extent, magnesium (Mg), ferrous iron (Fe^{2+}), strontium (Sr^{2+}), and manganous (Mn^{2+}) ions], affects the toxicity of metals in aqueous solutions [7,11,25,42,

17–22,27,28,30,31,33,34,38,47,54,55,58,60,63,69,70]. The effect of hardness appears to be related to competition between hardness ions (Ca, Mg) and other metals (such as Cu or Zn) for Ca binding sites on metabolically active surfaces. If the sites are occupied by Ca, normal membrane functions prevail. However, if these sites are occupied by metal ions other than Ca^{2+}, membrane function can be disrupted [27,62].

The effect of hardness on metal toxicity depends in part on the metal itself [60] or the metal species [17,27]. Also, water hardness may affect acute and chronic toxicity differently [17]. For example, increasing hardness from 58 to 115 mg/L reduced acute toxicity of Cu to daphnids by nearly 20%, but had little effect on chronic toxicity of Cu [60].

Other studies report that a relationship exists between the bioaccumulation of metals and water hardness [37,60]. Dixon and Sprague (1981) found that juvenile rainbow trout accumulate Cu faster in soft water than in harder water [37]. A study by Winner (1984) reported little difference in the bioaccumulation of Cd or Zn in soft water versus hard water [59].

Effects of temperature, dissolved oxygen, and redox conditions on metal toxicity

Water temperature and the concentration of dissolved oxygen can strongly influence the physiological responses of organisms to metals [42] and affect metal speciation [41]. Temperature, for example, influences the rate of biochemical and physiological processes

(metabolic rates approximately double for each 10 °C rise in temperature) [71], and the solubility of gases such as O_2 and CO_2 [44]. Thus, the rate of metal uptake for most metals increases as temperature increases [29]. For most aquatic animals, the gills are the site of gas exchange, releasing CO_2 to the water and passing O_2 to the blood. If the water temperature increases, the rate of respiration increases and a greater volume of water is passed by the gills per unit time [29,42]. This is an important transport process for soluble metals, notably Pb and Zn [29].

Temperature also affects the solubility of gases such as oxygen (O_2) and carbon dioxide (CO_2), with warmer water holding smaller concentrations of these gases than cooler water [38,65,72,73]. In oxygenated waters, the concentrations of metals can be up to 10 times lower than the concentrations of metals found in anaerobic sediments [42]. Much of the acidity of natural waters is due to the presence of CO_2, and an increase in soluble CO_2 can result in a decrease in pH [72]. Consequently, as the pH drops more of the metals present in the water body will solubilize.

Chemical transformations of metals are strongly affected by redox potential [38,66,74,75]. In a redox reaction, there is a transfer of electrons, such that the original reducing agent becomes oxidized, and the original oxidizing agent becomes reduced [38]. Under more reducing conditions, such as those that occur in organic-rich sediments, the redox potential becomes more electronegative, and metals can be released in soluble forms from sediments [38,64,76]. In general, metals are cations and can readily give up electrons. When oxygen

is available, it is the preferred terminal electron acceptor. However, as conditions become increasingly anaerobic, the redox potential declines [38]. Nitrate than usually serves as the predominant electron acceptor, until it has been consumed. Eventually, sulfate (SO_4^{2-}) serves as the terminal electron acceptor, and is reduced to sulfide (S^{2-}) [38]. Metal-sulfide complexes are relatively insoluble, and readily precipitate from solution [38]. In reducing environments, the Cr^{3+} can become oxidized to Cr^{6+}, which is considerably much more toxic than Cr^{3+} [21].

Although temperature, dissolved oxygen, and redox potential can all affect metal toxicity and metal bioavailability, these factors were not included in the study described in Chapter 2. Conditions of the toxicity tests that were conducted require the control of exposure variability such that any toxic affects observed can be attributed to toxicant concentration, rather than to changes in physical or chemical parameters. The temperature of the toxicity tests was kept at 25 ± 1 °C, and the concentration of dissolved oxygen was maintained at approximately 7 to 9 mg/L.

Organic matter

The binding of a metal to dissolved or particulate organic matter also can alter its biological availability [39] and reduce the metal's toxicity [15]. Organic matter (ligands) occurring in natural waters includes compounds such as amino acids, sugars, polysaccharides, fatty acids, organic phosphorus compounds, and aromatic compounds containing carboxyl (–COOH) or

hydroxyl (–OH) functional groups [41]. Inorganic complexation agents such as bicarbonate (HCO_3^-) ions are generally more abundant than organic ligands, but many organic ligands have stronger affinities for metals [38]. Dissolved organic carbon (DOC) can decrease the uptake of some metals. However, the uptake of other metals, such as Cd, may actually be increased by DOC [40]. An organic–metal complex may become more lipid-soluble, thus increasing its transfer through cell membranes [39]. In a study using fathead minnows, the addition of DOC (added as commercially-available humic acid) decreased toxicity based on total Cu concentrations, but increased toxicity on the basis of Cu^{2+} activity [27].

Metal Toxicity in Mixtures

The toxicity of a metal tested singly and in mixtures may vary depending on the metal-metal interactions and the organism tested [58,77,78]. Metal mixtures were not considered in the development of water quality criteria [17–21]. In a mixture, the toxicity of a metal may increase (synergism), decrease (antagonism), or remain similar to its individual toxicity (additive) [58,77–81]. Results of two studies found that Ni, Zn, Cu [58], and Cd [77] were generally more toxic in mixtures than individually. Other studies found that the toxicity of a Zn-Cu mixture was either additive [78,81], synergistic [80], or antagonistic [77,79], depending on the organism tested.

Masnado, et al. (1995), evaluated the toxicity of various mixtures of Cd, Cr(VI), Cu, Ni, and Zn, including a solution that contained the five metals in concentrations equivalent to

proposed effluent limits [22]. The five-metal mixture was more toxic to *C. dubia* than to larval fathead minnows at all concentrations tested, including the proposed effluent concentrations [22].

CONCLUSIONS

The toxicity of metals is governed by the physical and chemical characteristics of the water in which it was tested, with toxicity being increased or decreased by changes in parameters such as pH or hardness [6]. It is very difficult to distinguish between direct and indirect effects of the various biotic and abiotic factors that modify toxicity or the availability of metals. Therefore, if toxicity of a pollutant varies with water characteristics and species of aquatic organism, no one safe concentration may be applicable to all surface waters. For this reason, many researchers and regulators feel that a safe concentration should be based on site-specific criteria, established for each body of water and contaminant of concern [2,34,82,83]. For these reasons, many scientists and regulators recommend using a integrated approach in establishing and setting site-specific water quality standards [48,83]. This approach combines toxicity testing with chemical-specific analyses [10,15,48,83]. The resulting site-specific water quality criteria should be more protective of aquatic biota [83].

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PART 2

SITE-SPECIFIC METALS LIMITS

INTRODUCTION

In accordance with the Clean Water Act, the Environmental Protection Agency (EPA) established numeric water quality criteria (WQC) for toxic pollutants, including metals, to protect aquatic life in all, or almost all bodies of water [1]. The assumption was that if the aqueous concentration of a potential toxicant in a body of water was lower than the federal or state WQC, aquatic life in that body of water was unlikely to be adversely affected by the toxicant. Consequently, numeric criteria for potential toxicants were incorporated into discharge permit(s) for treated wastewaters (effluents).

Several issues were raised regarding the ecological relevance of the federal WQC. (1) Federal WQC for metals were developed using data from laboratory toxicity studies of single-metal exposures [2–6]. However, effluents generally contain materials in addition to metals. (2) The EPA used total recoverable metals concentrations to express numeric metals criteria, because this approach was considered to be more conservative and more protective of aquatic life than criteria based on dissolved metal concentrations [1]. However, EPA's present policy is that dissolved metals, rather than total recoverable metals, are the fraction of waterborne metals that are biologically available to aquatic organisms [7]. (3) The biological availability and/or toxicity of metals depends upon the chemical and physical form of the metal, as well as on the aquatic organism exposed. Numerous abiotic factors (such as pH, hardness, and dissolved and particulate matter) can influence the chemical or physical form of the metal, and with the exception of hardness, these factors were not incorporated into the development of

numeric WQC [2–6]. Thus, if the biological availability of a metal varies with effluent or ambient water chemistry, and the toxicity varies with the species of aquatic organism exposed, one "safe" concentration of metal (other than zero) may not be applicable to all surface waters. For a specific water body, the national criteria may be over- or under-protective. These arguments suggest that a more rational approach is that of developing water quality criteria specific to the site of interest.

In 1985, the EPA issued guidelines describing three procedures that could be used for deriving site-specific water quality criteria for metals [2,8]. Briefly, the *recalculation procedure* accounts for differences in metal sensitivity among resident aquatic species, compared to sensitivities of species of aquatic organisms used in the development of federal WQC. The *resident species procedure* accounts simultaneously for differences in sensitivity among resident aquatic species and differences that may be attributed to water quality. The *indicator species procedure*, also known as the water-effect ratio (WER) method, accounts for differences in the toxicity of a metal due to the physical and/or chemical characteristics of the site water versus the laboratory "control" water. Determining the WER is an approach that can be used by dischargers who want higher permit limits. Following this approach, the national water quality criterion would be adjusted by the ratio of toxicity in ambient site water to the toxicity in laboratory water [7].

According to the WER method, a series of concentrations of the metal of interest are prepared using site water and laboratory water. The metal-spiked waters are analyzed for

toxicity (at least three times) and analyzed for final (actual) metal concentration. Also, a WER must be determined individually for each metal of interest, and at each site of interest; that is, WERs calculated for one metal cannot be extrapolated to another metal, or from one site to another [7]. If a discharger wants higher permit limits for several metals, and at a number of different sites, the WER method can become very expensive.

As an alternative to the WER method, the Kentucky Division of Water (KDOW) issued *Procedures to Facilitate Alternative Metal Limits* [9]. The KDOW method also requires toxicity testing and chemical-specific analyses of the effluent. However, since no metal-spiking of the effluent is required in the KDOW method, all metals of interest in an effluent sample may be analyzed simultaneously. Unlike the WER method, which requires chemical analysis of multiple test solutions, the KDOW method requires chemical analysis of full-strength effluent samples only. Thus, the KDOW procedure is much less costly than the WER method. For my study, I estimated that the analytical costs required for the KDOW method were < 20% of the analytical costs that would be incurred using the WER method.

The KDOW method provides the discharger with an alternative means for measuring compliance to permit limits expressed as total recoverable metal [3]. With the KDOW method, a discharger may "adjust" (reduce) the measured metal concentration by the biologically relevant portion (dissolved) of the metal. Using the WER method, a discharger may get a higher metal permit limit, whereas with the KDOW method, a discharger may be able to report a lower metal concentration.

Purpose and Objective

The purpose of my research project was to evaluate whether the KDOW method is a viable alternative to the WER method for developing site-specific water quality criteria for selected metals. The objective was to use the KDOW method to develop site-specific water quality criteria for cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) in the effluents from four outfalls at a uranium enrichment facility in Paducah, KY. The toxicity of the effluents was estimated using bioassays with fathead minnow larvae (*Pimephales promelas*) and a freshwater microcrustacean (*Ceriodaphnia dubia*). The effluents were also characterized by making measurements of pH, conductivity, alkalinity, hardness, total suspended solids, and dissolved organic matter. The toxicity results and the physico-chemical data were used to meet the measurement endpoints for this study. These measurement endpoints included (1) determining the 25% inhibition concentration (IC_{25}) for each toxicity test conducted, (2) calculating the chronic toxicity unit (TUc) to evaluate effluent toxicity to *C. dubia* or *P. promelas* larvae, (3) measuring the total recoverable metal and dissolved metal concentrations for each of the selected metals in the outfalls sampled, and (4) calculating the mean final ratio of dissolved metal to total recoverable metal for each selected metal in each outfall. I used these data and the equation from the KDOW procedures document to calculate site-specific alternative total recoverable metals concentrations.

MATERIALS AND METHODS

Study Site

The Paducah Gaseous Diffusion Plant (PGDP) is owned by the Department of Energy (DOE), which leases the property to the United States Enrichment Corporation. The plant is located in the western part of the Ohio River basin. In 1952, the plant became an active uranium enrichment facility. The uranium enrichment gaseous diffusion cascade process involves more than 1800 steps to produce 2% ^{235}U. Most of the enriched uranium produced by this method is used in commercial reactors. Support facilities include a steam plant, electrical switchyards, cooling towers, chromium-reduction processes, and various maintenance and laboratory operations [10].

The Clean Water Act for the PGDP is administered by the Kentucky Division of Water through the Kentucky Pollutant Discharge Elimination System (KPDES). Four of the permitted outfalls selected for this study included Outfalls 001, 008, 009, and 010 (hereinafter referred to as Outfalls A, B, C, and D, respectively) (Figure 1). Most of the permitted outfalls (003 and 011–018) at PGDP are intermittently-flowing and rainfall dependent. Outfalls A, B, C, and D were selected because they are continuously-flowing, and the discharged effluents consist primarily of treated wastewaters. Also, between November, 1992 and February, 1996, three of the outfalls (B, C, and D) have exceeded the KPDES permit limits for Cd, Cu, Pb, or Zn on several occasions [11]. Additionally, since October, 1991, personnel

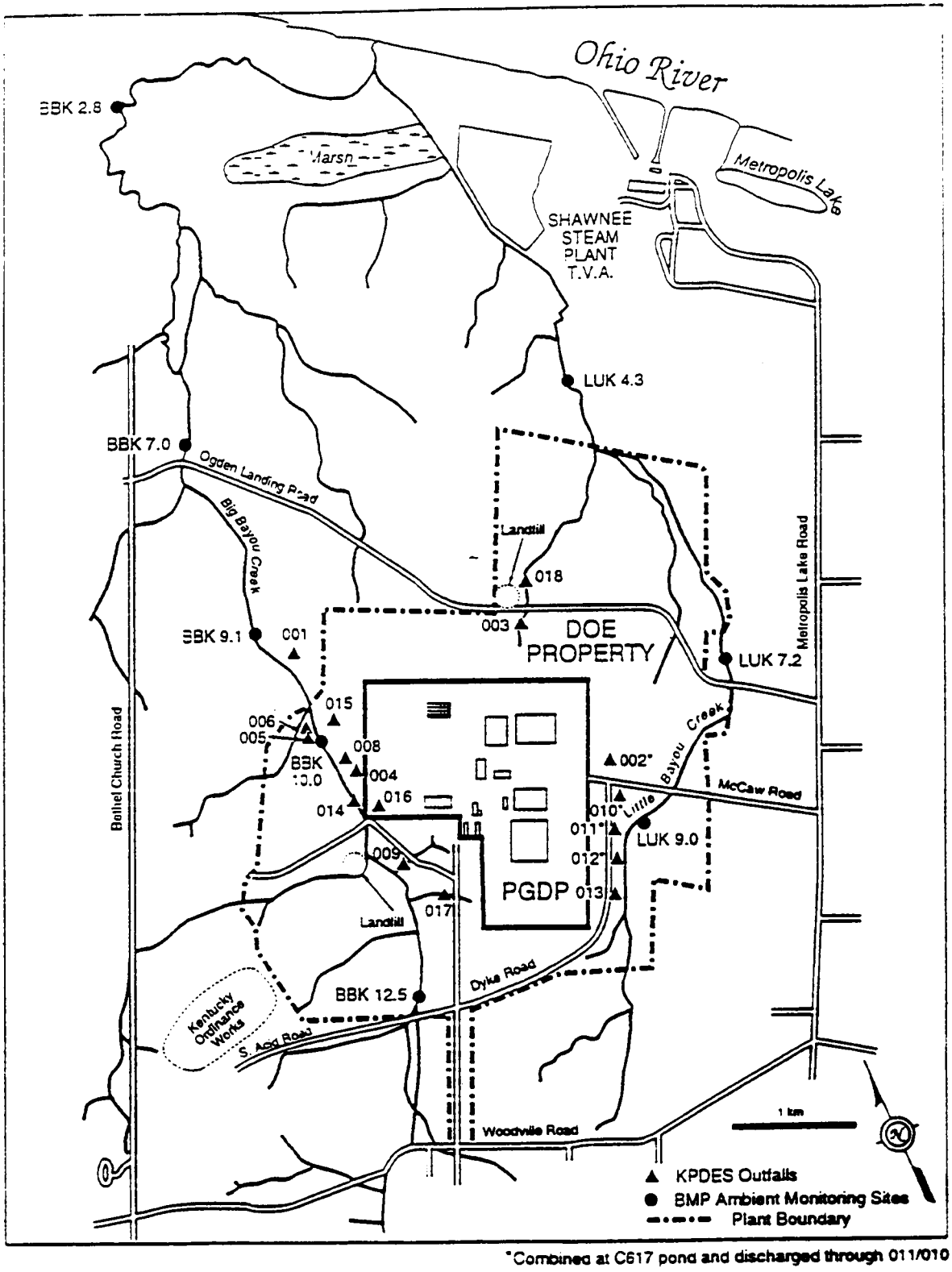

Figure 1. Location of outfalls at the Paducah Gaseous Diffusion Plant.

from the Environmental Sciences Division's Aquatic Toxicology Laboratory at Oak Ridge National Laboratory (ORNL) have evaluated the toxicity of the effluents from Outfalls A, B, C, and D quarterly using water fleas (*Ceriodaphnia dubia*) and/or fathead minnow (*Pimephales promelas*) larvae. The monitored outfalls were sometimes toxic to one or both of these test organisms [11]. The effluent samples used for the quarterly toxicity tests were not collected at the same time as samples used to measure compliance with metals limits; therefore, the toxic events cannot be correlated to incidents of metals exceedances.

Effluents from Outfalls A, B, and C discharge into Big Bayou Creek. Effluent from Outfall D is combined in a holding pond with the effluent from three other outfalls (002, 011, and 012); these combined effluents discharge continuously into Little Bayou Creek (Figure 1). Big Bayou Creek and Little Bayou Creek are tributaries to the Ohio River.

Test Animals and Test Conditions

Short-term, static-renewal toxicity tests with *P. promelas* and *C. dubia* were conducted according to standardized technical procedures [12] which were based on EPA methodology (Methods 1000.0, Fathead Minnow Larval Survival and Growth Test, and 1002.0, *C. dubia* Survival and Reproduction Test) [13]. Both test methods have been shown to be effective in assessing the toxicity of a variety of pollutants [14–22].

Individual *C. dubia* (≤ 24 h old) were placed into each of 10 replicate 30-mL disposable plastic beakers containing at least 15 mL of test water or control water. Five effluent dilutions were used: 6%, 12%, 25%, 50%, and 100%. The test beakers were randomized at the beginning of each 6- or 7-d test period. A test was terminated on the sixth day if at least 60% of the control organisms had produced three broods; otherwise, the test lasted for 7 d. Each day of the test, organisms were transferred to fresh test solution and the survival and the number of young produced were recorded. Any offspring and/or dead test organisms were discarded. The daphnids were fed 50 μL of a yeast-Cerophyl®-digested trout chow mixture and 100 μL of a suspension of green alga cells (*Selenastrum capricornutum*) daily after renewal of the test water. For each test, the measured responses were survival and the mean number of young produced per female.

Ten larval fathead minnows (≤ 48 h old) were randomly placed into each of four replicate 600-mL glass beakers containing at least 250-mL of test water or control water. The minnow tests used the same effluent dilution series as the *C. dubia* tests. The minnow larvae were fed 100 μL (approximately 600–900) of newly hatched brine shrimp (*Artemia salina*) twice daily, once at least 2 h before the test water was renewed and again 5–7 h after water renewal. The test water was siphoned out of the beakers daily and fresh test water was added. The number of minnow larvae surviving in each test beaker was recorded daily; any dead larva were discarded. At the end of the 7-d test period, the larvae were dried at 100 ± 5 °C and weighed to measure mean final weight.

As a condition of the KDOW method, the failure of two toxicity tests at any one of the four outfalls would require that a toxicity identification evaluation be implemented to identify the constituent(s) that caused the toxicity. To determine if any observed toxicity to *P. promelas* or *C. dubia* could be due to metals in the effluents, I treated a subsample of each full-strength effluent sample (and laboratory control water) with ethylenediaminetetraacetic acid (EDTA). Adding EDTA to chelate metals in the effluent samples should reduce toxicity, if toxicity was caused by a divalent cationic metal. Metals that form strong stable complexes with EDTA include Cd, Cu, Pb, Ni, and Zn [23]. The EDTA-treated control water served as a negative control. The final EDTA test concentrations were 2 mg/L for *P. promelas* and 1.5 mg/L for *C. dubia*. The selection of the EDTA concentrations was based on the results of the negative control toxicity tests with EDTA-treated control water. Also, the concentrations of metals present in the laboratory control water should be lower than the concentrations of metals present in the effluent samples; therefore, the EDTA test concentrations needed to be great enough to chelate the metals in the effluent samples, but low enough to not cause toxicity to the control organisms. EPA data also indicated that EDTA is approximately 50 times more toxic to *C. dubia* than to *P. promelas* [23]; therefore, a lower EDTA test concentration was used for the *C. dubia* toxicity tests than for the *P. promelas* tests. The EDTA-treated effluents and control water samples were tested for toxicity using the methods described previously. However, because of the limited amount of effluent.sample available, the EDTA toxicity tests with fathead minnow larvae used only two replicates, with 10 larvae per replicate, and the daphnid tests used five replicates, with one neonate per replicate. The tests with the EDTA-treated effluents and control water were always conducted concurrently with

those of the nontreated effluents.

Ceriodaphnia dubia were obtained from cultures maintained by the Aquatic Toxicology Laboratory at ORNL. The *P. promelas* larvae were obtained from Aquatic BioSystems, Fort Collins, Co. All toxicity tests were performed by personnel from the Aquatic Toxicology Laboratory at ORNL. A test temperature of 25 ± 1 °C and an automatically-controlled photoperiod of 16 h light:8 h dark were maintained for all daphnid and minnow tests.

Effluent Samples

About 12 L of effluent was collected from Outfalls A, B, C, and D every other month beginning in October, 1996, and ending in August, 1997. Following guidelines from the toxicity test methods, three effluent samples were collected from each of the outfalls for use during each of the six test periods [13]; thus, a total of 18 effluent samples were collected from each outfall. The sample-collection activities are summarized in Table A.1 in Appendix A.

Time-proportional composite samples were collected. I used automatic composite samplers in an attempt to collect effluent samples that were representative of the water-quality characteristics of each outfall. With this method, a 120-mL effluent sample was collected every 15 min; a total of 96 samples from each outfall were collected within a 24-h time period. However, grab samples were collected when the compositing equipment

malfunctioned. Flow data corresponding to each effluent sample were also collected using a primary flow monitoring device (consisting of a flow meter and a flume) that was installed in each outfall. These devices continuously recorded effluent flow in cubic feet per second. I converted these readings to millions of gallons per day (mgd) for use in the analyses.

The waters discharged from Outfalls A, B, C, and D are predominantly treated effluents, and personnel from the compliance division at PGDP presumed that the water quality characteristics (i.e., pH, hardness, alkalinity, etc.) of each outfall were generally consistent through time. As part of my research project, I also examined the variability of the effluent characteristics in each of the outfalls on a test-to-test basis.

Control Water

Diluted mineral water (25% of full strength) was prepared by adding Perrier® to deionized distilled water. The water was augmented with trace metals and aerated for at least 24 h before use to remove excess carbon dioxide [12]. The mean hardness and alkalinity values of batches of laboratory water used in my studies were 80–100 and 77–97 mg/L as $CaCO_3$, respectively, and the pH ranged from 8.04–8.38 SU. The water was used for testing with the control organisms, preparing dilutions of the effluents, and for *C. dubia* cultures.

Physico-Chemical Measurements

Subsamples of each effluent sample were analyzed for alkalinity, hardness, pH, conductivity, total suspended solids (TSS), and dissolved organic carbon (DOC). Dissolved oxygen and temperature of the effluent samples did not reflect ambient conditions so these measurements were not included in subsequent statistical analyses. Standard EPA-recommended procedures were used to measure pH, conductivity, dissolved oxygen, alkalinity, and hardness [24]; American Public Health Association (APHA) method 2540D was used for measurements of total suspended solids (TSS) [25].

Effluent samples for DOC analysis were collected in amber glass bottles with Teflon®-lined caps. The bottles were washed thoroughly and baked at 500 °C before use. Effluent samples were filtered through 0.45-μm (pore-size) pre-rinsed glass fiber filters (Gelman GF/F). The filters were also incinerated at 500 °C before use. The DOC samples were filtered < 48 h after being collected; they were then immediately acidified with 37% HCl to pH < 2 and stored in the dark at 4 ± 2 °C. Before analysis, the acidified samples were sparged for 4 min with purified oxygen to remove inorganic carbon. Dissolved (non-purgeable) organic carbon left in solution was measured using a Shimadzu Total Organic Carbon Analyzer (model TO-5000). Because of low concentrations of carbon (generally < 4 mg/L) in the samples, a platinum high-sensitivity catalyst was used to provide more complete combustion. Organic carbon was combusted and oxidized at 680 °C to CO_2; the CO_2 was then quantified by non-dispersive infrared gas analysis [26].

Metals

The six metals of interest—Cd, Cu, Cr, Pb, Ni, and Zn—were analyzed simultaneously using inductively coupled plasma/mass spectrometry (ICP/MS; EPA Method 200.8) [27]. The minimum detection limits were 0.1 μg/L for Cd, Pb, and Ni, and 1.0 μg/L for Cr, Cu, and Zn. Samples for analysis of dissolved metals were filtered using 0.45-μm (pore-size) sterilized, pre-rinsed disposable filter units [28]. After collection, the effluent samples for total recoverable and dissolved metals analyses were acidified with high-purity grade 35% nitric acid (HNO_3) to pH < 2. The ICP/MS measurements were made by General Engineering Laboratories, Charleston, SC.

Statistical Analyses

For each toxicity test, I calculated the concentration of effluent causing a 25% reduction in *P. promelas* weight or *C. dubia* reproduction, relative to a control. The 25% inhibition concentration (IC_{25}) was calculated using a linear interpolation method [29]. If the IC_{25} was < 100%, then I calculated the effluent's chronic toxicity unit (TUc) ($TUc = 100/IC_{25}$) to provide an estimate of the effluent's toxicity to minnows or daphnids, and to evaluate compliance with PGDP's permit limits for toxicity. The compliance endpoint for each outfall is expressed as a TUc. According to the permit, a TUc > 1 could result in a noncompliance; the greater the TUc, the more toxic the effluent. A Statistical Analyses System (SAS®) analysis of variance (ANOVA) method [general linear models (GLM)] [30] was used to

determine whether *C. dubia* mean reproduction or *P. promelas* mean weight in the full-strength effluent or the control water differed significantly from mean reproduction or mean weight in the EDTA-treated effluent or the EDTA-treated control water, respectively.

SAS® was also used to calculate the means, ranges, and standard deviations of the physico-chemical data and the metals data. I used ANOVA to determine whether the individual physico-chemical characteristics (i.e., pH, hardness, DOC, etc.) of each outfall differed significantly on a test-by-test basis. If the results of the ANOVA were significant, I used Tukey's Studentized Range Test to determine which test periods, within a given outfall, differed from the others [32]. Unless otherwise noted, all statements of significance were based on $\alpha \leq 0.05$. Because pH is not a concentration, but rather a measure of hydrogen-ion activity, the pH data were transformed to the hydrogen-ion concentration before the statistical analyses were performed [32]. The results (i.e., means and ranges) were back transformed to express pH values in the conventional form after means had been computed.

I used multivariate stepwise regression analysis to examine (1) variation in the dissolved concentration of Cu, Ni, Pb, and Zn, and (2) variation in the dissolved metal:total recoverable metal ratio of Cu, Ni, Pb, and Zn, in relation to the physico-chemical parameters. A significance level of $\alpha \leq 0.15$ was selected as the basis of deciding whether an individual parameter was included in the model. I used two approaches for conducting the regression analyses. In the first, I examined variation on the basis of each individual outfall. In the second, I examined variation on the basis of all outfalls combined.

For each effluent sample, a pair of random variables (a dissolved metal concentration and a total recoverable metal concentration) was determined. I used three methods to estimate the ratio of dissolved metal to total recoverable metal for each combination of outfall and metal. In the first method, I assumed that the joint distribution of the two random variables was normally distributed (bivariate normal distribution). In second method, I assumed the distribution to be a bivariate normal for $\log_{10}$-transformed dissolved- and total-recoverable metal observations. In the third method, I assumed that the ratio of dissolved metal to total recoverable metal followed a normal distribution, with no transformation. I also tested for normality of the individual distributions of the dissolved metal and total recoverable metal variables (both transformed and non-transformed). The results from the three methods revealed little practical difference in deriving the dissolved metal:total recoverable metal ratio. Thus, for simplicity, I assumed the metals data had a bivariate normal distribution.

Data Pruning

For each effluent sample, I obtained observations on a pair of variables (total recoverable metal, and dissolved metal). Fifty percent of the dissolved Cu, Ni, Pb, and Zn data collected on October 18, 1996, were rejected because the dissolved metals values exceeded the corresponding total recoverable metal values, by 130% to > 1000%. I could find no clear explanation for the unacceptably high concentrations of dissolved metals in the effluent samples collected on this date; the discrepancies might have been due to contamination of the samples during field collection, or during sample preparation. Because subsequent statistical

analyses focused on the dissolved metal:total recoverable metal ratio for the various metals, rejection of the dissolved metals data from October 18 also required exclusion of the corresponding total recoverable metals data. Thus, fully half of the metals data collected on this date were rejected. To be conservative, all metals data from this date were rejected as being suspect. For this reason, the corresponding physico-chemical data collected on October 18, 1996, were also excluded.

Twenty-nine paired observations for Pb were excluded also, due to the large number of non-detect values for this metal. Pb was not detected in 62% of the effluent samples collected in February, so, all Pb data from February were excluded from further statistical analysis. Of the effluent samples collected in June, the number of observations on paired variables (total recoverable metal and dissolved metal) in which Pb was below detection in one of the two variables was 42%. In other words, Pb may have been detected in the sample for total recoverable metal, and not detected in the corresponding sample for dissolved metal, making the paired observations unusable for statistical analysis. Due to these "below detection" problems, all Pb data from June were excluded from further statistical analysis. In addition, values for total recoverable and dissolved Pb in the August 11, 1997 effluent sample from Outfall C were excluded; dissolved Pb in this outfall, during August, 1997, was not detected.

Two values for total recoverable Cu in effluent samples from Outfall C were excluded as outliers. In the February 14 and April 25, 1997 effluent samples, total recoverable Cu was 23.9 μg/L and 16.0 μg/L, respectively. For comparison, the range of total recoverable Cu

measured in the effluent from Outfall C was 2.49–8.03 μg/L. The two suspect Cu values (and their corresponding dissolved Cu values) were excluded as outliers for two reasons: (1) the two suspect total recoverable Cu concentrations were much greater (50–66%) than the other total recoverable Cu concentrations measured in Outfall C, and (2) the ratio of dissolved Cu to total recoverable Cu in Outfall C on these two dates was 0.16 and 0.30, respectively. By comparison, the ratio of dissolved Cu to total recoverable Cu in Outfall C for other sample dates ranged from 0.6–1.2.

If all metals data collected for this study were usable, then the final dissolved metal to total recoverable metal ratios would be calculated using 18 paired observations for each metal. However, because some of the metals data were excluded from further use, the final mean dissolved metal to total recoverable metal ratios for Cu in Outfalls A, B, and D, and the final mean ratios for Ni and Zn in Outfalls A, B, C, and D were determined using 17 paired observations. Because two additional Cu values were excluded at Outfall C, the final Cu ratio was calculated from 15 paired observations. Final dissolved to total recoverable ratios for Pb in Outfalls A, B, and D were based on 11 paired observations; the final Pb ratio in Outfall C was based on 10 paired observations. Although the final mean metal ratios were calculated using less than the maximum number of paired observations possible, the number of observations used to calculate the ratios exceeded the minimum number required in the KDOW method (i.e., 6 paired observations).

RESULTS

Pimephales promelas survival and mean weight data and *C. dubia* survival and mean reproduction data are summarized, by test period and outfall, in Tables B.1 and B.2, respectively (Appendix B). The results of ANOVAs to determine whether mean weight or mean reproduction in the EDTA-treated effluent sample was significantly different from mean weight or reproduction in the non-treated effluent sample are also shown in Tables B.1 and B.2. The individual physico-chemical characteristics of each effluent sample are summarized, by outfall, in Table D.1 (Appendix D). Table D.2 (Appendix D) gives the mean physico-chemical characteristics of the outfalls, by test period, and the results of the ANOVA used to determine whether individual physico-chemical parameters, within a given outfall, differed significantly on a test-by-test basis. Total recoverable and dissolved metals data are summarized in Table E.1 (Appendix E), by outfall and sample date. Alternative (calculated) total recoverable metal concentrations for Cu, Ni, Pb, and Zn are given in Table F.1 (Appendix F), by outfall.

Toxicity Test Results

Effluents from Outfalls C and D were not toxic to *P. promelas* larvae or *C. dubia* in any of the six tests. Effluent from Outfall A was not toxic to *P. promelas* in any test, but in the test conducted in October, 1996, mean *C. dubia* reproduction in the effluent from Outfall A (14.3 offspring/female) was significantly lower than mean reproduction in the control (28.3

offspring/female). For this effluent, the IC_{25} was 11.6% and the TUc was 8.6. The EDTA-treated effluent from Outfall A did not significantly improve *C. dubia* reproduction ($P = 0.44$) (Table B.2); thus, something other than a divalent metal probably contributed to the effluent's toxicity to *C. dubia* in the October test. During the October test period, the DOC concentration in effluent from Outfall A was two to three times greater than the mean DOC concentration in the effluents from the other three outfalls. I also compared the concentrations of metals present in the October effluent samples from Outfall A to metals concentrations in effluents from the other outfalls. Based on this comparison, the metals concentrations in Outfall A in October did not appear to be high enough to have been toxic. This information suggests that the toxicity in Outfall A might have been caused by the presence of an organic compound.

Effluent from Outfall B was acutely toxic to *C. dubia* and *P. promelas* larvae in tests conducted during February, 1997. All of the daphnids in the 100% concentration of the effluent died, and all the minnows in the 50% and 100% concentrations died. Complete mortality of both test species also occurred in the EDTA-treated effluent sample from Outfall B. Mortality occurred < 24 h after the tests had been started, and coincided with a rapid decline in the pH of the effluent. At the time the test was started the pH was 6.89, but pH declined to 3.88 about 24 h later. The drop in pH was suspected to be related to excess loading of sodium thiosulfate at an outfall that discharged directly into Outfall B. However, the observed toxicity was, in part, an artifact of the test method. The temperature of the toxicity tests was maintained at 25 ± 1 °C, which is very close to the optimal growth

temperature of 28–30 °C for *Thiobacillus,* an aerobic bacterium that derives energy from the oxidation of reduced sulfur compounds [33]. The combination of an optimal growth temperature and an abundant energy source (thiosulfate) created conditions that led to the rapid decline in pH in the test solutions. A more complete explanation for the suspected cause of the toxicity is described in the *Ecotoxicology Highlights* (Appendix C). A follow-up acute toxicity test with fresh effluent from Outfall B was conducted during February, 1997. This test showed that the effluent was no longer toxic to minnows. An acute test with *Ceriodaphnia* was not conducted.

In August, 1997, three of the four fathead minnow tests were invalid due to unacceptably low survival (< 80%) in the controls. For my study, the minnow larvae were also received from the supplier the same day as the toxicity tests were started. However, for the August test, the minnow larvae had to be shipped to the laboratory a day earlier and held, because of a strike by a commercial shipper. The fathead minnow larvae used in the August tests were noticeably stressed: they were relatively inactive, and the level of mortality in these larvae was high. However, because of scheduling conflicts and time constrains, I decided to use the minnows to initiate the August tests anyway. Minnow survival in the full-strength effluent concentrations from Outfalls B, C, and D was similar to survival in the corresponding controls. In Outfalls B, C, and D, control survival was 77.5%, 77.5%, and 70%, respectively; minnow survival in the full-strength effluent concentration from these outfalls was 70%, 80%, and 70%, respectively. In addition, mean minnow weight in the EDTA-treated effluent from Outfalls C and D did not differ significantly from mean minnow weight in the nontreated

effluent from these outfalls. Also, *C. dubia* survival in the effluents from Outfalls B, C, and D in August was 100%, and the calculated IC_{25} for these outfalls was > 100%. These results indicate that the minnow mortality in the August tests probably was not caused by toxicants in the effluents.

As noted previously, ANOVA was used to determine whether mean *C. dubia* reproduction or *P. promelas* weight in full-strength effluent or control water differed significantly from reproduction or weight in the EDTA-treated effluent or the EDTA-treated control water, respectively. The ANOVA results did not reveal large differences in mean reproduction or mean weight in response to the EDTA treatments. However, the addition of EDTA to control water reduced *C. dubia* reproduction < 1% to 30% in five of the six toxicity tests and mean weight of fathead minnow larvae in the EDTA-treated control-water tests was 3–24% lower in three of the six tests, and 4–63% greater in three tests. The results of ANOVA comparing mean weights of *P. promelas* or mean reproduction of *C. dubia* in the full-strength, untreated effluents to mean weight or reproduction in the EDTA-treated effluents did not reveal significant differences in 89% of the toxicity tests. EDTA-treated effluent from Outfall B did lower *P. promelas* weight in October, 1996, and significantly increased mean weight in August, 1997 (Table B.1). EDTA-treated effluent from Outfall A resulted in increased mean *C. dubia* reproduction in February, 1997, and EDTA-treated effluent from Outfall D significantly reduced *C. dubia* reproduction in the October and December, 1996 (Table B.2). These results suggest that the final concentration of EDTA in the control water test (1.5 mg/L) may have been slightly too high.

Physico-Chemical Results

Factors such as pH, hardness, and dissolved and particulate organic matter can alter the biological availability (i.e., the solubility) of metals in an aquatic system and, thus, modify the toxicity, as mentioned previously [34–38]. For most metals, (1) toxicity increases as pH decreases; (2) toxicity decreases as hardness increases; and (3) the presence of organic ligands (such as EDTA) can reduce toxicity by formation of metal–ligand complexes. An increase in water temperature or a decrease in dissolved oxygen concentration can increase respiration rates and, thus, increase the transport of soluble metals through the gills [15,17,19,35,39–44].

The mean physico-chemical characteristics of effluents from Outfalls A, B, C, and D are summarized in Table 1. Effluent from Outfall A had greater mean hardness (65–72%), conductivity (61–66%), and flow (122–161%), and higher concentrations of DOC (28–46%), compared to the other outfalls. With the exception of DOC, TSS, and flow, the mean physico-chemical characteristics of the effluents from Outfalls C and D were similar. The percent differences in Outfalls C and D ranged from < 1% (pH) to 17% (alkalinity). Mean pH, alkalinity, hardness, and TSS in Outfall B were lower, compared to the other outfalls.

For some parameters, the range of values (i.e., minimum to maximum) varied considerably. For example, in Outfall A, hardness ranged from 70 to 390 mg/L (as $CaCO_3$) and conductivity ranged from 233 to 1485 μS/cm (Table 1). Much of the effluent exiting Outfall A comes from a lagoon. During August, 1997, the lagoon was drained for maintenance. Effluent from

Table 1. Mean (±SD) physico-chemical measurements, by outfall

Parameter	Outfall A	Outfall B	Outfall C	Outfall D
H^+ (as pH)[a]				
Mean ± SD	7.90 ± 0.51	7.24 ± 0.22	7.78 ± 0.13	7.71 ± 0.12
Range	7.60–9.14	6.89–7.59	7.45–7.96	7.48–7.98
Alkalinity[b]				
Mean ± SD	37.6 ± 6.5	32.1 ± 10.1	47.2 ± 14.5	39.9 ± 9.1
Range	29–49	17–56	15–73	24–59
Hardness[b]				
Mean ± SD	248.8 ± 90.0	68.7 ± 8.9	79.8 ± 14.7	86.8 ± 17.8
Range	70–390	54–90	62–106	66–130
Conductivity[c]				
Mean ± SD	935.5 ± 359.1	334.1 ± 150.1	364.2 ± 293.3	316.6 ± 87.3
Range	233–1485	230–878	201–1418	188–486
DOC[d]				
Mean ± SD	5.78 ± 2.11	4.15 ± 1.05	3.89 ± 0.93	3.08 ± 0.59
Range	2.04–9.14	2.65–5.84	2.69–5.49	2.24–4.00
TSS[e]				
Mean ± SD	21.8 ± 5.9	5.1 ± 2.5	10.0 ± 7.1	23.7 ± 14.7
Range	11.1–30.8	1.6–12.2	3.8–28.2	7.1–59.2
Flow[f]				
Mean ± SD	2.68 ± 1.07	0.64 ± 0.13	0.29 ± 0.17	0.45 ± 0.22
Range	1.30–4.29	0.43–0.93	0.14–0.63	0.08–0.73

Note: Data collected on 10/18/96 were not included.
[a]Expressed as the hydrogen-ion concentration (n = 17).
[b]Expressed as mg/L as $CaCO_3$ (n = 17).
[c]Expressed as μS/cm (n = 17).
[d]Dissolved organic carbon; expressed as mg C/L (n = 17).
[e]Total suspended solids; expressed as mg/L (n = 17).
[f]Million gallons per day (n = 15).

Outfall A then consisted primarily of groundwater. Mean hardness, conductivity, and DOC in the effluent from Outfall A were considerably lower in August, compared to the other test periods (Table D.1).

Changes in the water quality characteristics of the effluents through time could affect the ratio of dissolved metal to total recoverable metal. Therefore, I used ANOVA to examined test-to-test differences in the mean physico-chemical characteristics, within a given outfall. If the results of the ANOVA were significant ($P \leq 0.05$), I used Tukey's Studentized Range Test to determine which test period(s) differed significantly from the others. Within a given outfall and parameter, I found several significant test-to-test differences ($P > 0.05$) (Table D.2). In general, the physico-chemical parameters in Outfall A were more variable than those for the other outfalls, and the parameters in Outfall B were least variable. In Outfall A, all parameters differed statistically on a test-to-test basis; however, mean hardness, DOC, and conductivity were the most variable. In Outfall A, mean hardness ranged from 80.7 mg/L as $CaCO_3$ in August, to 360.0 mg/L as $CaCO_3$ in October; DOC ranged from 2.26 mg/L to 9.14 mg/L, and conductivity ranged from 290 μS/cm to 1460 μS/cm (Table D.2). In Outfall B, only pH varied significantly from one test period to another. I found significant test-to-test differences in mean alkalinity and hardness in Outfall C, and effluent from Outfall D varied in conductivity, hardness, and flow (Table D.2).

Metals Results

Over the course of the study, a total of 18 effluent samples from each outfall were collected and analyzed for total recoverable and dissolved metals. More than 65% of the total number of measured metal concentrations were estimated quantities [i.e., the values were greater than the method detection limit (MDL), but lower than the quantitation (i.e., reporting) limit]. The MDL is the minimum amount of an analyte that can be detected above the normal "noise" of the analytical instrument; it can be identified, but not quantified, with 99% confidence [27]. The MDLs for Cd, Pb, and Ni were each 0.1 μg/L, and the reporting limits for these three metals were 5 μg/L. The MDLs for Cr, Cu, and Zn were 1.0 μg/L, and the reporting limits for these three metals were 3, 5, and 10 μg/L, respectively.

All total recoverable and dissolved Cd and Cr values were estimated quantities. Final dissolved metal: total recoverable metal ratios were not determined due to the large number of total non-detectable results for Cd (> 60%) and Cr (> 75%). Table 2 summarizes the range of total recoverable Cd and Cr measured in all outfalls, and compares the values to the permit limits and the federal water quality criteria. From Table 2, all measurable Cd and Cr values were well below the permit limits and the federal water quality criteria (WQC).

Thirty-five percent of the total recoverable Cu values and 68% of the dissolved Cu values were estimated quantities. The total recoverable permit limits for Cu in Outfall A are 42 μg/L (daily maximum) and 26 μg/L (monthly average); the permit limits for Cu in Outfalls B, C,

Table 2. Comparison of measurable total recoverable cadmium and chromium to the permit limits and federal water quality criteria (WQC)

Metal	Total recoverable metal measured (μg/L)	Permit limits[a] (μg/L)		Federal WQC[b] (μg/L)	
		Daily maximum	Monthly average	Acute criterion	Chronic criterion
Cadmium	0.11–0.40	4	1	3.7	1.0
Chromium	1.07–3.93	16	11	550	180

[a]Total recoverable permit limits for Outfalls B, C, and D. Permit limits for Cd in Outfall A are 11 μg/L (daily max.) and 2 μg/L (monthly avg.), and are based on hexavalent Cr [Cr(VI)].

[b]The federal water quality criteria (WQC) for Cd and trivalent Cr are expressed as the dissolved metal concentration, based on a total hardness of 100 mg $CaCO_3$/L [45].

and D are 18 μg/L (daily maximum) and 12 μg/L (monthly average). For comparison, the federal water quality acute and chronic criteria for dissolved Cu are 17 μg/L and 11 μg/L, respectively, based on a hardness of 100 mg/L as $CaCO_3$ [45]. Between November, 1992 and February, 1996, effluents from Outfalls B, C, and D each exceeded the permit limits for Cu five times [11]. Excluding the two very high total recoverable Cu values (16.0 μg/L and 23.9 μg/L) in Outfall C, all measured concentrations of total recoverable and dissolved Cu were below the permit limits and the federal WQC. Across all outfalls, the total recoverable Cu concentrations measured in the effluent samples ranged from 2.40 μg/L to 9.67; dissolved Cu concentrations ranged from 1.59 μg/L to 7.24 μg/L. Mean (±SD) total recoverable and dissolved Cu concentrations, by outfall, are given in Figure 2. Outfall A had greater mean

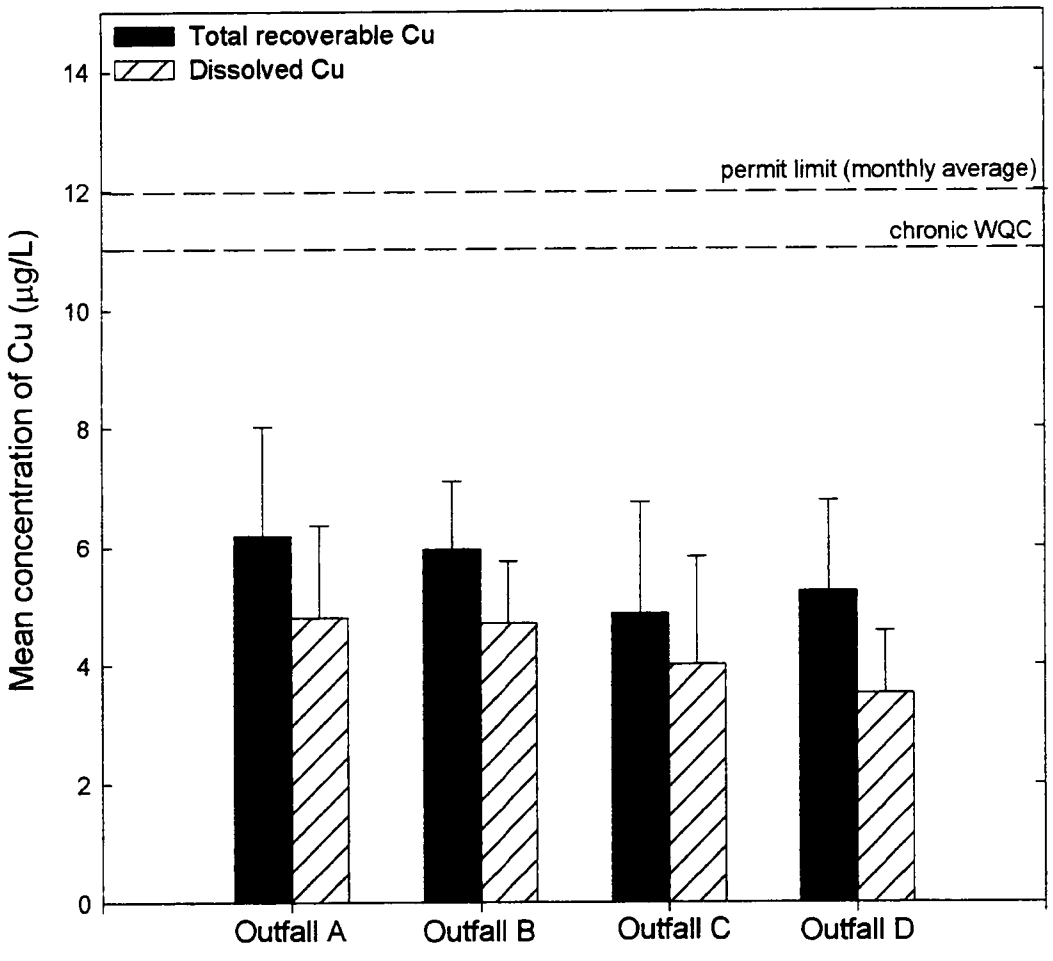

Figure 2. Mean (+SD) total recoverable and dissolved copper, by outfall. For comparison, the permit limits for Outfall A are 42 µg/L (daily maximum) and 26 µg/L (monthly average); permit limits for Cu in Outfalls B, C, and D are 18 µg/L (daily maximum) and 12 µg/L (monthly average). The chronic federal water quality criterion (WQC) is expressed as the dissolved metal, based on a hardness of 100 mg $CaCO_3$/L.

concentrations of total recoverable and dissolved Cu (24% and 31%, respectively), than the other outfalls.

All measurable Pb concentrations were estimated quantities. Fifty-eight Pb values (29 total recoverable + 29 dissolved Pb values) had to be excluded from the analyses, because one or both of the paired values were "non-detects." The total recoverable permit limits for Pb in Outfall A are 262 μg/L (daily maximum) and 10 μg/L (monthly average); the permit limits for Pb in Outfalls B, C, and D are 82 μg/L (daily maximum) and 3 μg/L (monthly average). Across all outfalls, the measured concentrations of total recoverable Pb ranged from 0.25 μg/L to 2.71μg/L (in Outfall A); measurable dissolved Pb ranged from 0.10 μg/L to 0.88 μg/L. Between November, 1992 and February, 1996, the permit limits for Pb were exceeded twice at Outfall B, three times at Outfall C, and five times at Outfall D [11]. The federal WQC for dissolved Pb are 65 μg/L (acute criterion) and 2.5 μg/L (chronic criterion), based on a total hardness of 100 mg $CaCO_3$/L [45]. All measured concentrations of total recoverable and dissolved Pb were below the applicable permit limits and the WQC. Mean (±SD) total recoverable and dissolved Pb concentrations are given in Figure 3, by outfall. Among outfalls, the mean concentrations of total recoverable Pb differed by 7–28%; mean dissolved Pb concentrations differed by 5–14%.

More than 80% of the values for total recoverable Ni and dissolved Ni were lower than the reporting limit (5.0 μg/L) for the analytical method and thus were estimated quantities. The permit limits for total recoverable Ni in Outfall A are 3079 μg/L (daily maximum) and 342

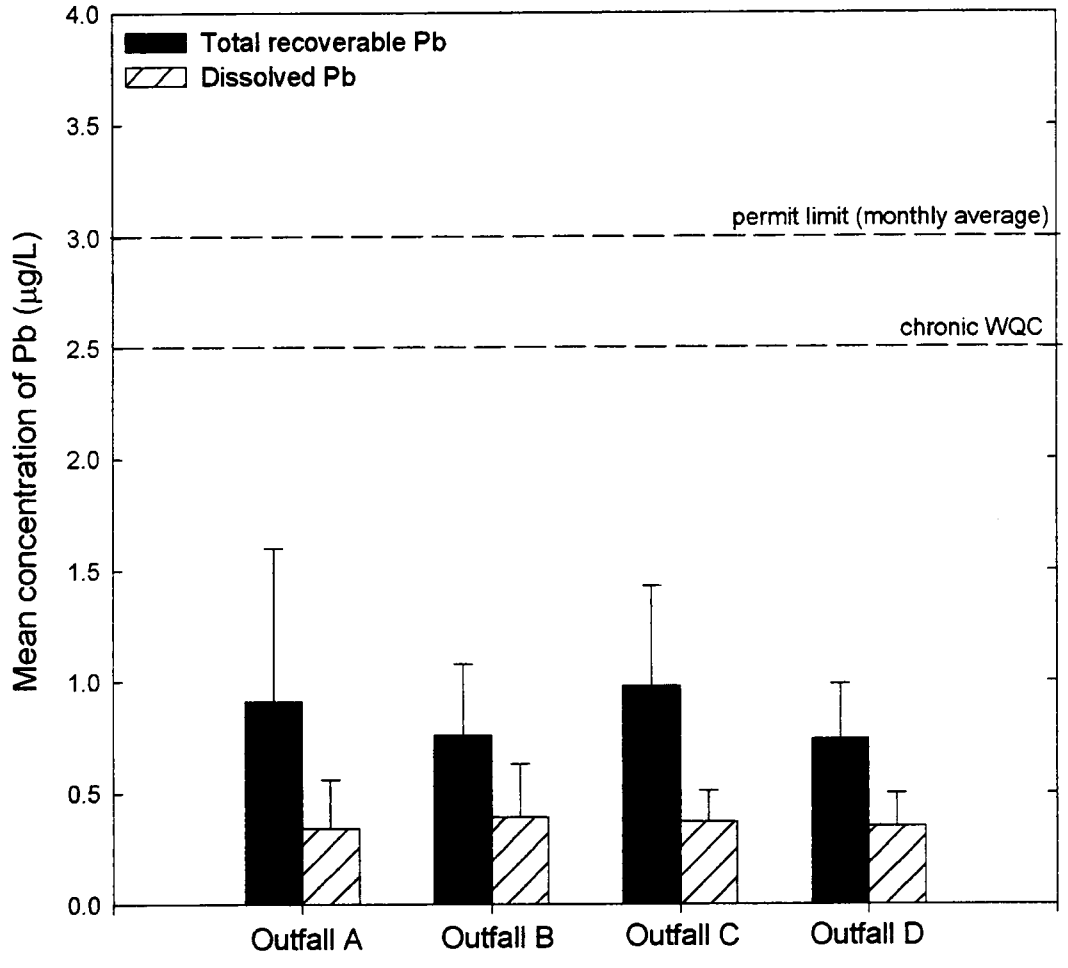

Figure 3. Mean (+SD) total recoverable and dissolved lead, by outfall. For comparison, the permit limits for Outfall A are 262 µg/L (daily maximum) and 10 µg/L (monthly average); permit limits for Pb in Outfalls B, C, and D are 82 µg/L (daily maximum) and 3 µg/L (monthly average). The chronic federal water quality criterion (WQC) is expressed as the dissolved metal, based on a hardness of 100 mg $CaCO_3$/L.

μg/L (monthly average); the permit limits for Ni in Outfalls B, C, and D are 1418 μg/L (daily maximum) and 158 μg/L (monthly average). Between November, 1992 and February, 1996, the permit limits for Ni were not exceeded at any of the four outfalls [11]. The WQC for dissolved Ni are 1400 μg/L (acute criterion) and 160 μg/L (chronic criterion), based on a hardness of 100 mg $CaCO_3$/L [45]. Across all outfalls, the total recoverable Ni concentrations ranged from 0.82 μg/L to 10.70 μg/L; dissolved Ni concentrations ranged from 0.28 μg/L to 9.50 μg/L. Mean (±SD) concentrations of total recoverable and dissolved concentrations Ni are given in Figure 4, by outfall. Mean concentrations of total recoverable Ni in all outfalls differed by 61–188%; mean concentrations of dissolved Ni differed by 58–116%. Mean concentrations of total recoverable and dissolved Ni were greatest in Outfall A, and lowest in Outfalls C and D; however, all measured values for Ni were well below the permit limits and the federal WQC.

The total number of Zn concentrations that were estimated values were 14.7% (total recoverable) and 25.0% (dissolved). The permit limits for total recoverable Zn in Outfall A are 254 μg/L (daily maximum) and 230 μg/L (monthly average); the permit limits for Zn in Outfalls B, C, and D are 117 μg/L (daily maximum) and 106 μg/L (monthly average). Between November, 1992 and February, 1996, the permit limits for Zn were exceeded three times in Outfall C and twice in Outfall D [11]. The federal WQC for dissolved Zn are 120 μg/L (acute criterion) and 110 μg/L (chronic criterion), based on a hardness of 100 mg $CaCO_3$/L [45]. Across all outfalls, total recoverable Zn concentrations ranged from 4.75 μg/L to 67.3 μg/L; dissolved Zn concentrations ranged from 5.75 μg/L to 63.6 μg/L. Mean

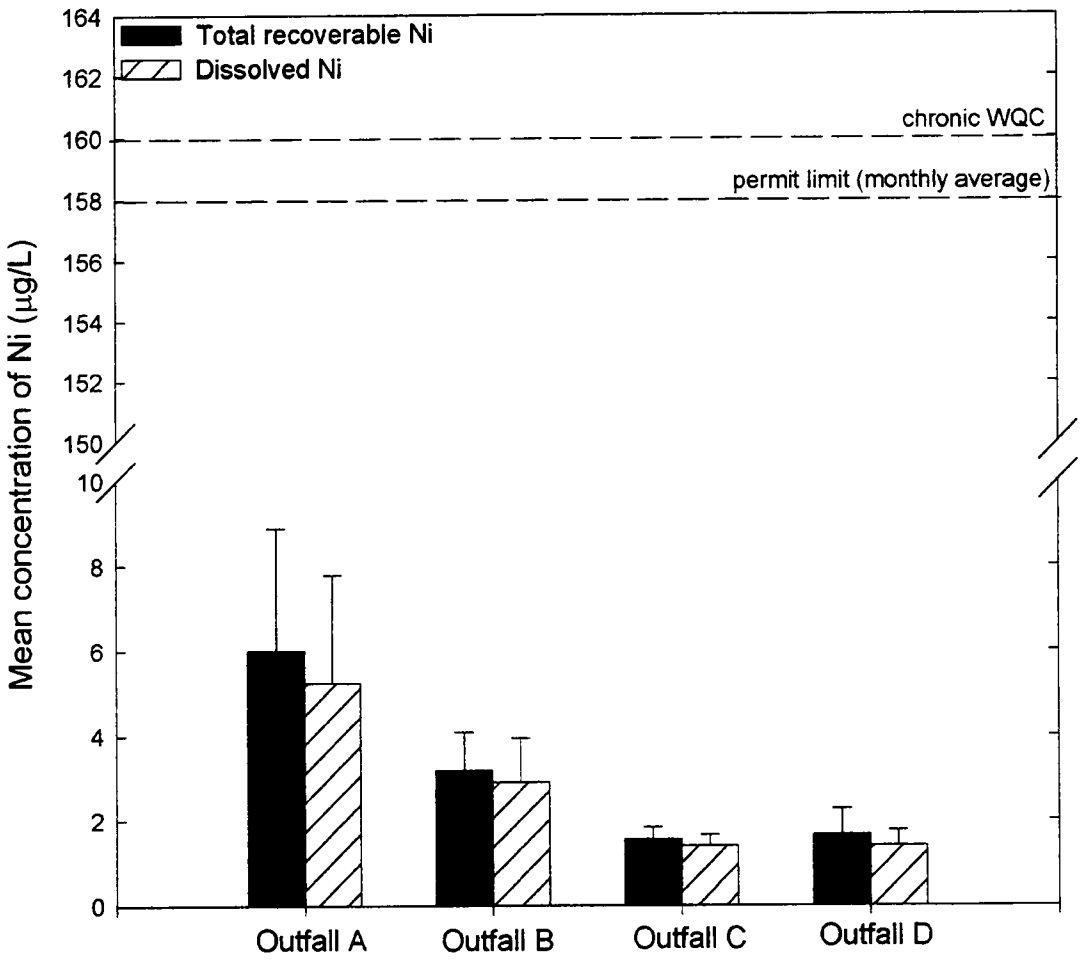

Figure 4. Mean (+SD) total recoverable and dissolved nickel, by outfall. For comparison, the permit limits for Outfall A are 3079 µg/L (daily maximum) and 342 µg/L (monthly average); permit limits for Ni in Outfalls B, C, and D are 1418 µg/L (daily maximum) and 158 µg/L (monthly average). The chronic federal water quality criterion (WQC) is expressed as the dissolved metal, based on a hardness of 100 mg $CaCO_3$/L.

(±SD) concentrations of total recoverable and dissolved Zn are given, by outfall, in Figure 5. Mean concentrations of total recoverable and dissolved Zn in Outfall D were 79% and 64% greater, respectively, than those in Outfall A. All measured values for Zn were below the permit limits and the federal WQC.

Dissolved Metal to Total Recoverable Metal Ratios

Total recoverable and dissolved metals data for Cu, Ni, Pb, and Zn measured in the effluent from Outfalls A, B, C, and D were used to calculate final mean dissolved metal to total recoverable metal ratios. Final ratios for Cd and Cr were not calculated because of the large number of non-detectable values for these two metals. Mean Cu ratios ranged from 0.67 to 0.82, the range for Ni ratios was 0.84–0.91, the range of Pb ratios was 0.37–0.51, and the range of Zn ratios was 0.74–0.94 (Table 3).

Alternative Total Recoverable Metal Calculations

I calculated alternative total recoverable metal concentrations for Cu, Ni, Pb, and Zn for each outfall using the measured total recoverable metal concentrations, the final ratios of dissolved metal to total recoverable metal, and the following equation from the KDOW guidance document [9]

$$\begin{matrix} Metal \\ concentration \\ reported \end{matrix} = [TRM \times DM{:}TRM] \times \frac{1}{EPA\ CF},$$

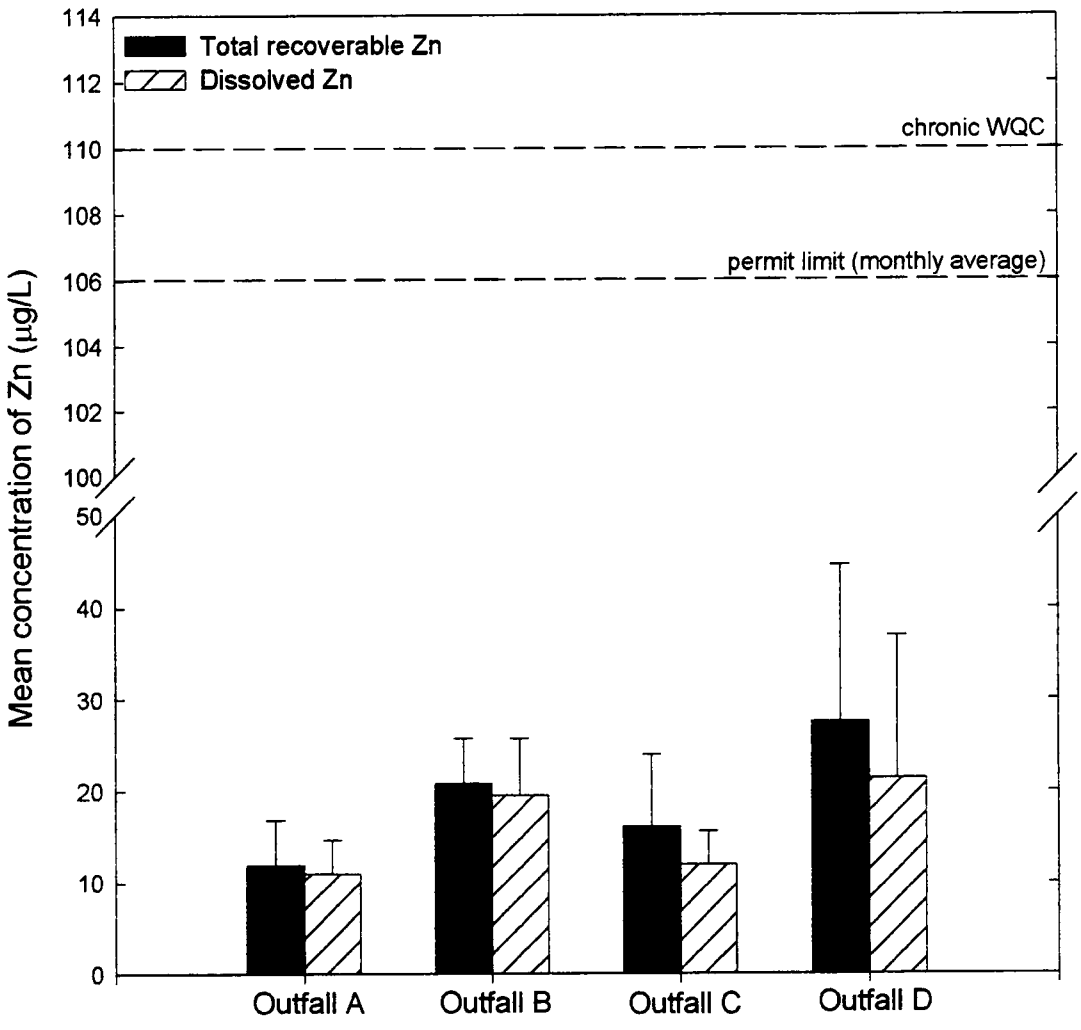

Figure 5. Mean (+SD) total recoverable and dissolved zinc, by outfall. For comparison, the permit limits for Outfall A are 254 mg/L (daily maximum) and 230 mg/L (monthly average); permit limits for Zn in Outfalls B, C, and D are 117 mg/L (daily maximum) and 106 mg/L (monthly average). The chronic federal water quality criterion (WQC) is expressed as the dissolved metal, based on a hardness of 100 mg $CaCO_3$/L.

Table 3. Ratios of dissolved metal to total recoverable metal, by outfall and metal

Metal	Outfall A	Outfall B	Outfall C	Outfall D
Copper				
Mean	0.78	0.79	0.82	0.67
95% C.L.	0.71–0.85	0.74–0.84	0.68–0.97	0.56–0.81
n	17	17	15	17
Nickel				
Mean	0.87	0.91	0.90	0.84
95% C.L.	0.82–0.93	0.82–1.01	0.82–0.98	0.75–0.97
n	17	17	17	17
Lead				
Mean	0.37	0.51	0.38	0.46
95% C.L.	0.23–0.65	0.38–0.63	0.26–0.56	0.33–0.62
n	11	11	10	11
Zinc				
Mean	0.92	0.94	0.74	0.78
95% C.L.	0.84–1.03	0.85–1.03	0.64–0.90	0.67–0.86
n	17	17	17	17

where TRM is the amount of total recoverable metal measured in the effluent sample, DM:TRM is the mean ratio of dissolved metal to total recoverable metal, and CF is the EPA's freshwater criteria conversion factor.

Freshwater criteria conversion factors for dissolved metals were developed by the EPA and published in 1995 in the Federal Register [45]. Metal complexes cannot be considered nontoxic, but are generally less toxic than dissolved, non-complexed metals [45]. The EPA conversion factors are hardness-dependent (100 mg/L as $CaCO_3$) and are applied to total

recoverable metal concentrations to express water quality criteria as dissolved metal. The EPA freshwater criteria conversion factors for Cu, Ni, and Zn are 0.960, 0.997, and 0.986, respectively [45]. Although the conversion factor for Pb is also hardness-dependent, the EPA requires the use of site-specific hardness data to derive conversion factors for Pb. Mean hardness concentrations (cf. Table 1) were used in the following EPA equation to calculate Pb conversion factors for each outfall (Table 4) [45]

$$\textit{Lead conversion factor} = 1.46203 - [(\ln \textit{hardness})(0.145712)]$$

Representative alternative total recoverable Cu, Ni, Pb, and Zn concentrations for each outfall were calculated and compared to measured total recoverable metal concentrations (Table F.1, Appendix F). The magnitude of the difference between the measured total recoverable metal concentration and the recalculated metal concentration were also included in Table F.1. For Cu, the percent reductions in calculated total recoverable metal concentrations compared to the measured metal concentration ranged from 14.5% (Outfall C) to 30.3% (Outfall D). The percent reduction for Ni ranged from 8.8% to 15.8%; for Zn, the range was 4.7% to 25.0%. The largest percent reductions occurred for Pb in all outfalls: 39.5% (Outfall B) to 54.1% (Outfall C).

The alternative (calculated) total recoverable metal concentration provides the discharger with a "cushion" for meeting water quality permit limits for total recoverable metals. For example,when the KDOW calculation method is used, the maximum concentration of total

Table 4. Hardness-dependent lead conversion factors, by outfall

Outfall	Mean hardness[a]	Conversion factor
A	248.8	0.658
B	68.7	0.846
C	79.8	0.824
D	86.8	0.812

[a]Expressed as mg $CaCO_3$/L. Mean hardness values from Table 1.

recoverable Cu in Outfall B that would not exceed the monthly average permit limit of 12 μg/L would be 14.58 μg/L.

Multivariate Regression Analyses

I used multivariate regression analysis to examine (1) variation in the dissolved concentration of Cu, Ni, Pb, and Zn, and (2) variation in the dissolved metal to total recoverable metal ratio of Cu, Ni, Pb, and Zn, in relation to the physico-chemical parameters. A significance level of $\alpha \leq 0.15$ was selected as the basis of determining whether an individual parameter was included in the model. I used two approaches for conducting the regression analyses. In the first, I examined variation in dissolved metal (or in the metal ratio) on the basis of each outfall separately. In the second, I examined variation on the basis of all outfalls combined.

Table 5 summarizes the results of the regression analyses of variation in the dissolved metal concentrations, by individual outfall, and by all outfalls combined. Only the physico-chemical parameters that explained at least 30% of the variation were included in the table. Using the first approach, conductivity explained 78% of the variation in dissolved Cu in Outfall A; DOC explained 59% and 39% of the variation in dissolved Cu in Outfalls B and C, respectively (Table 5). For variation in dissolved Ni, pH explained 33% and hardness explained 51% of the variation in Outfall A; conductivity explained 32% of the variation in Outfall D. Conductivity in Outfall D accounted for 64% of the variation in dissolved Pb. The variation in dissolved Zn was best explained by DOC (47%) in Outfall B and conductivity (43%) in Outfall C.

In the second approach, DOC explained 46% of the variation in dissolved Cu for all outfalls combined. Sixty-two percent of the variation in dissolved Ni for all outfalls was attributed to variation in hardness. No physico-chemical parameter explained as much as 30% of the variation in dissolved Pb or Zn, for all outfalls combined. The results of the regression analysis indicate that, in general, variation in the dissolved metal concentration(s) was best explained by DOC and conductivity.

Results of the regression analysis of variation in the dissolved metal to total recoverable metal ratios are summarized in Table 6. Only the physico-chemical parameters that explained at least 30% of the variation were included in the Table 6. Thirty-nine percent (39%) of the variation in dissolved Cu:total recoverable Cu ratio in Outfall A was attributed to conductivity

Table 5. Results of multivariate regression analysis of variation in the dissolved metal concentration of Cu, Ni, Pb, and Zn, by metal[a]

Metal	Partial R^2	Parameter	Outfall
Cu	0.78	Conductivity	A
	0.59	Dissolved organic carbon (DOC)	B
	0.39	Dissolved organic carbon (DOC)	C
	0.46	Dissolved organic carbon (DOC)	Outfalls combined
Ni	0.33	pH	A
	0.51	Hardness	A
	0.32	Conductivity	D
	0.62	Hardness	Outfalls combined
Pb	0.64	Conductivity	D
Zn	0.47	Dissolved organic carbon (DOC)	B
	0.43	Conductivity	C

[a]Variation in dissolved metal concentrations was examined for each outfall separately, and for all outfalls combined. Only parameters explaining at least 30% of the variation were included in the table.

Table 6. Results of multivariate regression analysis of variation in the dissolved metal to total recoverable metal ratio of Cu, Ni, Pb, and Zn, by metal[a]

Metal	Partial R^2	Parameter	Outfall
Cu	0.39	Conductivity	A
Ni	0.35	Total suspended solids (TSS)	C
Pb	0.48	Total suspended solids (TSS)	C
	0.41	Dissolved organic carbon (DOC)	D
Zn	0.49	Total suspended solids (TSS)	C

[a]Variation in metal ratios was examined for each outfall separately, and for all outfalls combined. Only parameters explaining at least 30% of the variation were included in the table.

(Table 6). TSS in Outfall C explained 35% of the dissolved:total recoverable ratio for Ni, 48% of the ratio for Pb, and 49% of the ratio for Zn (Table 6). DOC explained an additional 41% of the variation in the dissolved:total recoverable ratio for Pb in Outfall D. For all outfalls combined, no physico-chemical parameter explained as much as 30% of the variation in the dissolved metal:total recoverable metal ratio for Cu, Ni, Pb, or Zn. The results of the regression analyses indicate that the concentration of total suspended solids best explained the overall variation in the metal ratio(s).

QUALITY ASSURANCE/QUALITY CONTROL

The quality assurance objective for all data collected was to obtain measurements that were reproducible, precise, accurate, and representative. The sampling, testing, and chemical analysis of effluent samples were performed following standardized procedures to ensure an appropriate level of quality. Quality control samples included filter blanks, field and laboratory splits, matrix spikes, equipment rinse blanks, process blanks, duplicates, and certified reference standards. All blanks, unless otherwise specified, consisted of deionized distilled water.

Toxicity Data

The validity of the toxicity tests is based on data for survival, weight, and/or reproduction of the control organisms. The acceptability criteria for the *C. dubia* tests are ≥ 80% survival in

the controls and an average of ≥ 15 young per surviving female in the controls [13]. The acceptability criteria for the *P. promelas* tests are ≥ 80% survival in the controls and a mean dry weight of ≥ 0.25 mg/larvae for the surviving control organisms [13]. For each test species and each of the four outfalls, a separate negative control was used in each test period. All control tests with *C. dubia* met the test acceptability criteria. With the exception of the three minnow tests in August, all control tests with *P. promelas* met the test acceptability criteria.

Physico-Chemical Data

All instruments used for measuring pH and conductivity were calibrated or the calibration status was verified before use using certified reference standards. Analytical balances were verified before use with a certified weight. TSS analyses were performed in duplicate, and the relative percent difference (RPD) was calculated as follows to evaluate the precision of the analytical method:

$$RPD = \frac{(|A - B|)}{(A + B)/2} \times 100 ,$$

where A is the value of the first measurement, and B is the value of the second measurement. The RPD for the TSS samples ranged from 0% to 25%; the average RPD was 2.7%.

The Shimadzu Total Organic Carbon Analyzer used to measure DOC was calibrated before use with standards prepared from a stock solution of 1000 mg/L potassium hydrogen phthalate. Process blanks and bottle blanks were analyzed with each batch of DOC samples. Concentrations of carbon in the process blanks and bottle blanks ranged from 0–0.05 mg C/L and 0.09–0.91 mg C/L, respectively. Seven split samples were analyzed for DOC. The RPD for the split DOC samples ranged from 2.6% to 14.0%.

Metals Data

Inductively coupled plasma/mass spectrometry (ICP/MS) was used to analyze the effluent samples for metals. This analytical method has the advantage of being relatively sensitive for most metals. However, different elements with a mass-to-charge ratio similar to the element(s) of interest can interfere with the ability of ICP/MS to accurately detect and quantify the element(s) of interest [46]. A large portion (> 65%) of the total metal concentrations were estimated quantities: all metals data for Cd, Cr, and Pb; and > 80% of the total Ni values. When concentrations are estimated quantities (i.e., concentrations are greater than the method detection limit, but less than the reporting limit), it can be difficult to accurately quantify the element(s) of interest. This difficulty may account for the large standard deviations in mean metal concentrations and the mean dissolved metal:total recoverable metal ratios. Lower detection limits for metals probably would have resulted in better-quantified data. A revised ICP/MS method lists detection limits that ranged from 8.7% (for Cu) to 330% (for Ni) lower than those used in this study [46].

Because metals data were used to estimate effluent concentrations and determine final dissolved:total recoverable metal ratios, efforts were made to identify and quantify potential sources of contamination. Water samples may become contaminated with metals through a number of ways which may subsequently inflate measured metal concentrations. EPA guidelines for collecting samples for trace metals using "clean" techniques address ways to control contamination [47]. These guidelines were intended for use in collecting ambient samples, but their use in effluent sampling should also reduce potential contamination. The cleaning and decontamination activities used in this study appeared to be sufficient to control metal contamination problems. Although no data were available to support this idea, metal contamination of the samples used to measure compliance with the permit limits may have contributed to past permit exceedances. For example, the permit limit for Zn had been exceeded five times (three times in Outfall C and twice in Outfall D) between November, 1992 and February, 1996. The monthly average permit limit for Zn in Outfalls C and D is 106 μg/L; the daily maximum limit is 117 μg/L. In my study, however, the majority (> 85%) of the total recoverable Zn values were < 28 μg/L.

Decontamination activities for my study included washing thoroughly the automatic sampler units, and replacing the pump and suction line tubing with new, clean silicone tubing before collecting the first (of three) samples per test period [28]. Stainless-steel tubing connectors were replaced with new high-density polyethylene (HDPE) connectors. Equipment rinse blanks were collected and analyzed for total recoverable metals. The automatic sampler unit bottles were washed thoroughly and soaked overnight in 20% nitric acid before use. The

bottles were rinsed thoroughly with deionized distilled water and a sample of this rinsate was analyzed for total recoverable metals. Samples of the water ("source water blank") used to collect equipment rinse blanks and sampler bottle blanks were also analyzed for total recoverable metals.

Analysis of the blanks indicated the presence of trace-level quantities of almost all metals (Figure 6). No Cr was detected in any of the blanks, and all measured metal concentrations were estimated quantities. The source water appeared to be a low-level source of metal contamination; however, it is unlikely that deionized distilled water contained concentrations of Cu and Zn at the levels indicated in Figure 6. Concentrations of metals in the laboratory process blanks indicated that some contamination of the samples occurred during sample preparation (digestion) and/or analysis.

Sterilized single-use filter units were used to process the effluent samples for dissolved metals analysis. Periodically, filter blanks were collected and analyzed for dissolved metals. Analysis of these blanks revealed one source of metal contamination, particularly Zn. Starting with the February samples, each filter unit was pre-rinsed with 100 mL of deionized distilled water. This simple procedure reduced Zn contamination by 79%.

Split samples for analysis of total recoverable and dissolved metals were analyzed to provide an estimate of variability of the sample collection and processing activities, as well as to evaluate the precision of the analytical method. The RPD of split samples for total recoverable

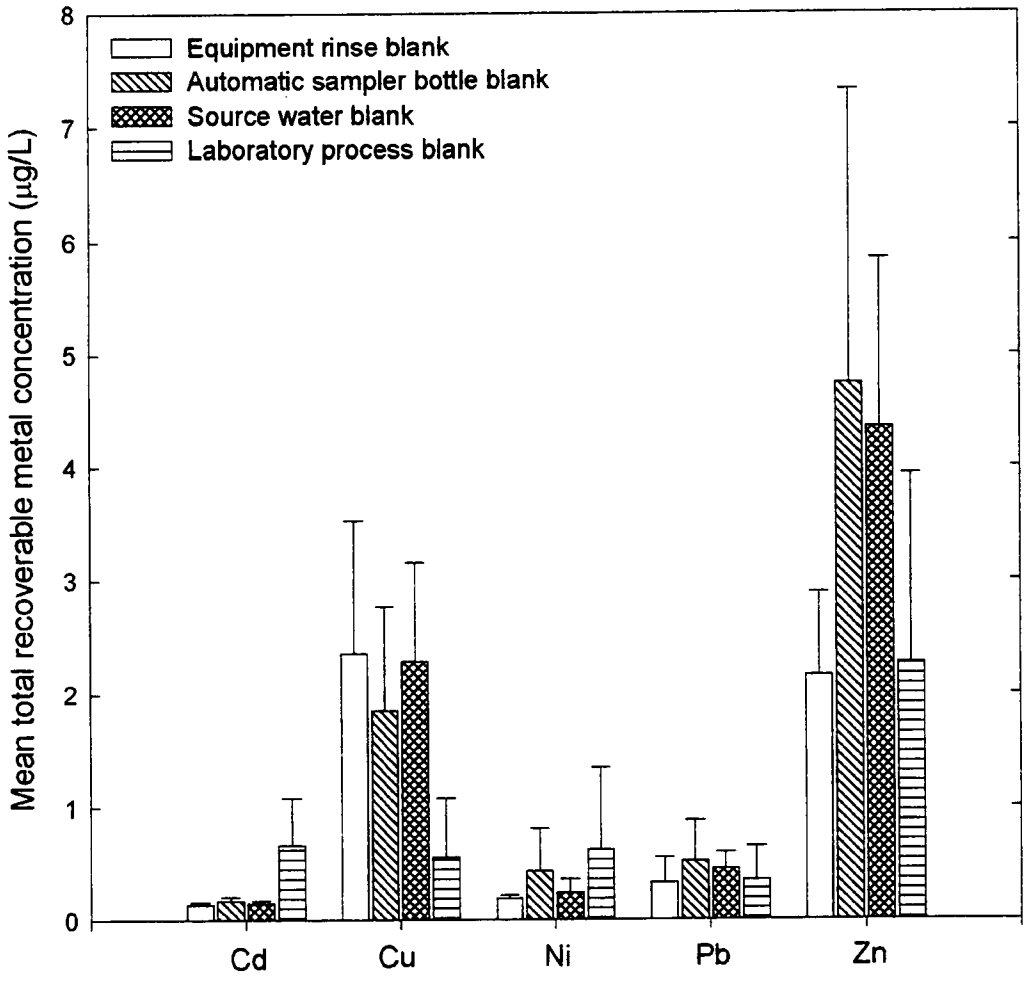

Figure 6. Concentrations of metals in quality control blanks.

metals analysis ranged from 0 to 82.8%; 59 of 78 RPD values were < 25%. The RPD of split samples for dissolved metals analysis ranged from 0 to 184%; 72 of 90 RPD values were <25%.

DISCUSSION

With the exception of the two high total recoverable Cu concentrations in Outfall C (16.0 and 23.9 μg/L), all metal values were below the permit limits and the federal WQC. Even using the KDOW alternative calculation method, these two Cu concentrations would have exceeded the monthly average permit limit for Cu (12 μg/L) at Outfall C. The largest total recoverable Ni concentration measured in Outfalls B, C, and D (6.26 μg/L) was < 4% of the monthly average permit limit (158 μg/L) and the chronic WQC (160 μg/L) in these outfalls. Total recoverable Zn concentrations were 36–71% lower than the monthly average permit limits and the chronic WQC. The largest concentration of Pb measured in Outfall B, C, and D was 2.49 μg/L (Outfall D). For comparison, the monthly average permit limit for Pb in these outfalls is 3.0 μg/L, and the chronic WQC is 2.5 μg/L. Applying the KDOW recalculation method, a Pb concentration of 2.49 μg/L measured in Outfall D would have been reported as 1.41 μg/L, a reduction of nearly 45%. Mean hardness in Outfall A is about three times greater than that in the other outfalls; consequently, the permit limits for metals in Outfall A are higher than those in the other outfalls. All measured concentrations of metals in the effluent samples from Outfall A were from 37% (Cu) to 97% (Ni) lower than the applicable monthly average permit limit.

I found no evidence suggesting that the concentrations of metals present in the effluent samples that I collected were toxic to *C. dubia* or *P. promelas*. None of the effluent samples evaluated for toxicity in this study contained a mixture of the six metals at concentrations equal to the permit limits. Thus, the toxicity of metals at these concentrations could not be evaluated. However, based on the metals data that I obtained, it seems unlikely that the effluents from these outfalls would contain metals at concentrations equal to the discharge limits. Thus, the metals assessed in this study are unlikely to cause compliance-related problems in the effluents from Outfalls A (001), B (008), C (009), or D (010) at PGDP.

As discussed previously, the biological availability and/or the toxicity of a metal may be influenced by the physico-chemical characteristics of the site water. I used multivariate regression analysis to investigate whether a "cause-and-effect" relationship existed between the portion of a dissolved metal present in the effluent and the physical or chemical characteristics of that effluent. This information would be useful had the metals data revealed that the concentrations of metals present in the outfalls were likely to cause compliance-related problems. For example, if the results of the regression analysis showed there was a relationship between pH and the portion of dissolved Cu in an outfall, then personnel from PGDP could probably control the portion of dissolved Cu in that outfall by controlling pH in the outfall. The results of my regression analysis indicated that, in general, the concentrations of dissolved and particulate organic matter best explained the variation in mean dissolved metals concentrations, as well as the variation in the mean portion of dissolved metals in the outfalls. For dissolved metals, DOC explained approximately 40% of the variation.

Approximately 40% of the variation in the mean ratio of dissolved metal to total recoverable metal was attributed to either TSS or DOC. Also, conductivity explained a large portion (60–80%) of the variation in dissolved Cu in Outfall A, and dissolved Pb in Outfall D.

The portions of dissolved Cu, Ni, or Zn in the effluent samples collected for my study are in good agreement with the percentages that the EPA considers ecologically relevant [48]. The recommended EPA percentages were based on the best available data and can be expected to change as analytical methods improve. In Table 7, I compared the percentages of dissolved metals measured in my study to the percentages of dissolved metals the EPA considers most relevant in fresh water. The EPA data indicate that 90–95% of the Pb present in freshwater is in particulate form [48]. My results, however, indicate a lower percentage of particulate Pb in the outfalls, ranging from 44–74%. The difference may be due to the artificial nature of the chemical characteristics of the effluents. In more natural waters, the concentrations of hardness and alkalinity (measured as mg $CaCO_3$/L) tend to be in fairly close agreement. The extent of "chemical perturbation" was especially pronounced in effluent from Outfall A, where mean hardness and mean alkalinity values were 248.8 and 37.6 mg/L (measured as mg $CaCO_3$/L), respectively.

CONCLUSIONS

In summary, my study indicates that the KDOW method is a viable, cost-effective alternative to the water-effect ratio (WER) method for developing site-specific water quality criteria for

Table 7. Comparison of percent dissolved metals

Metal	Study values[a] (% dissolved)	EPA recommended values[b] (% dissolved)
Copper	56–97	52–95
Nickel	75–101	88–100
Zinc	64–103	31–103

[a]From Table 2.3.
[b]From memorandum issued by EPA Office of Water [48].

selected metals . The KDOW recalculation method allows a discharger to "adjust" (reduce) the measured metal concentration reported by the biologically relevant portion (dissolved) of the metal. Applying the KDOW method to my data, the magnitude of the difference between the total recoverable metal concentration measured and the metal concentration reported ranged from 4.7% to 54.1%. Using the WER method, a discharger may get higher permit limits, whereas with the KDOW method, a discharger may get to report a lower concentration of the metal. However, by using the KDOW method to develop site-specific metals limits instead of the WER method, a discharger may save up to 80% of the analytical costs associated with the WER method. Since the physical and chemical characteristics of the site water will affect the biological availability of a metal, as well as the metal's toxicity, the development of site-specific water quality criteria might be most applicable in situations where these characteristics do not vary much through time.

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APPENDICES

Table A.1. Summary of sample collection activities, by test period

Test period	Date and times of collection	Sample type[a]	Sample temperatures (°C)
October 1996	10/16/96; 0813–1026	CS/GS (A, D)	4.4–20.8
	10/18/96; 0845–0930	CS/GS (B,C)[b]	2.0–5.0
	10/21/96; 0750–0834	CS	3.1–4.8
December 1996	12/04/96; 0755–0828	CS	3.2–4.3
	12/06/96; 0753–0849	CS	2.3–2.6
	12/09/96; 0815–0840	CS	2.2–4.3
February 1997	02/12/97; 0729–0800	CS	1.4–2.5
	02/14/97; 0835–0928	CS	1.8–3.2
	02/17/97; 0843–0918	CS	1.2–4.8
April 1997	04/23/97; 0855–0935	CS	2.2–2.8
	04/25/97; 0800–0845	CS/GS (D)	2.6–13.1
	04/28/97; 0804–0833	CS/GS (D)	2.8–13.1
June 1997	06/18/97; 0838–0935	CS/GS (C)	6.2–23.7
	06/20/97; 0816–0900	CS	5.6–11.5
	06/23/97; 0832–0902	CS	5.7–9.0
August 1997	08/06/97; 0755–0845	CS	6.2–10.5
	08/08/97; 0815–0900	CS	4.6–6.3
	08/11/97; 0752–0847	CS	6.6–10.3

[a]CS = 24-h time-proportional composite sample; GS = grab sample; outfalls for which grab samples were collected are given in parentheses.

[b]Mixed grab/composite sample.

Table B.1. Results of fathead minnow toxicity tests, by outfall and test period

(NOTE: Mean weight in the 100% effluent concentration was compared to mean weight in the EDTA-treated effluent from the same outfall. Mean weights with the same symbol (♦ or †) did not differ significantly different from each other[a].)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
10/17–24/96	Control	0	90.0	0.36		0.05
	Control + EDTA	0	95.0	0.33		0.02
	Outfall A	6	87.5	0.56		0.14
		12	87.5	0.55		0.05
		25	97.5	0.77		0.05
		50	100.0	0.77		0.03
		100	95.0	0.77	♦	0.05
	Outfall A + EDTA	100	95.0	0.73	♦	0.03
	Control	0	82.5	0.37		0.05
	Outfall B	6	87.2[c]	0.54[c]		0.09
		12	87.5	0.56		0.06
		25	100.0	0.63		0.04
		50	87.5	0.61		0.05
		100	82.5	0.52	♦	0.06
	Outfall B + EDTA	100	50.0	0.26	†	0.07
	Control	0	90.0	0.44		0.08
	Outfall C	6	95.0	0.57		0.03
		12	100.0	0.62		0.03
		25	90.0	0.60		0.08
		50	95.0	0.64		0.06
		100	90.0	0.51	♦	0.08
	Outfall C + EDTA	100	100.0	0.67	♦	0.03
	Control	0	85.0	0.45		0.06
	Outfall D	6	87.5	0.51		0.06
		12	85.0	0.48		0.14
		25	77.5	0.49		0.06

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
		50	87.5	0.54		0.07
		100	87.5	0.57	♦	0.03
	Outfall D + EDTA	100	95.0	0.50	♦	0.05
12/5–12/96	Control	0	100.0	0.39		0.02
	Control + EDTA	0	100.0	0.44		0.02
	Outfall A	6	97.5	0.43		0.04
		12	95.0	0.43		0.02
		25	87.5	0.42		0.06
		50	87.5	0.42		0.03
		100	90.0	0.47	♦	0.05
	Outfall A + EDTA	100	95.0	0.49	♦	0.01
	Control	0	95.0	0.37		0.03
	Outfall B	6	95.0	0.40		0.01
		12	90.0	0.41		0.02
		25	95.0	0.44		0.02
		50	97.5	0.46		0.05
		100	87.5	0.37	♦	0.05
	Outfall B + EDTA	100	75.0	0.35	♦	0.06
	Control	0	87.5	0.34		0.03
	Outfall C	6	92.5	0.40		0.01
		12	95.0	0.39		0.04
		25	90.0	0.39		0.06
		50	92.5	0.41		0.02
		100	95.0	0.41	♦	0.04
	Outfall C + EDTA	100	95.0	0.46	♦	0.01
	Control	0	97.5	0.39		0.04
	Outfall D	6	95.0	0.44		0.03
		12	95.0	0.46		0.05

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
		25	95.0	0.44		0.02
		50	92.5	0.43		0.04
		100	95.0	0.48	♦	0.05
	Outfall D + EDTA	100	80.0	0.39	♦	0.04
2/13–20/97	Control	0	97.5	0.41		0.05
	Control + EDTA	0	100.0	0.39		0.00
	Outfall A	6	95.0	0.48		0.04
		12	100.0	0.51		0.03
		25	97.5	0.52		0.04
		50	100.0	0.51		0.03
		100	100.0	0.57	♦	0.04
	Outfall A + EDTA	100	65.0	0.47	♦	0.10
	Control	0	95.0	0.40		0.01
	Outfall B	6	100.0	0.54		0.02
		12	100.0	0.56		0.05
		25	97.5	0.55		0.04
		50	0.0	0.00		0.00
		100	0.0	0.00	NC[d]	0.00
	Outfall B + EDTA	100	0.0	0.00	NC[d]	0.00
	Control	0	95.0	0.38		0.02
	Outfall C	6	97.5	0.47		0.04
		12	97.5	0.47		0.05
		25	95.0	0.48		0.04
		50	100.0	0.47		0.02
		100	97.5	0.52	♦	0.02
	Outfall C + EDTA	100	100.0	0.57	♦	0.01

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
	Control	0	97.5	0.41		0.03
	Outfall D	6	70.0	0.39		0.11
		12	92.5	0.54		0.06
		25	87.5	0.51		0.07
		50	62.5	0.38		0.11
		100	71.8[c]	0.45[c]	♦	0.08
	Outfall D + EDTA	100	50.0	0.32	♦	0.32
4/24–5/1/97	Control	0	100.0	0.43		0.01
	Control + EDTA	0	100.0	0.42		0.00
	Outfall A	6	100.0	0.45		0.03
		12	97.5	0.50		0.02
		25	90.0	0.51		0.03
		50	100.0	0.54		0.02
		100	97.5	0.54	♦	0.03
	Outfall A + EDTA	100	100.0	0.56	♦	0.01
	Control	0	100.0	0.34		0.02
	Outfall B	6	97.5	0.46		0.02
		12	100.0	0.47		0.04
		25	100.0	0.47		0.01
		50	92.5	0.46		0.03
		100	100.0	0.46	♦	0.04
	Outfall B + EDTA	100	70.0	0.38	♦	0.13
	Control	0	92.5	0.42		0.04
	Outfall C	6	100.0	0.52		0.03
		12	97.4[c]	0.52[c]		0.01
		25	100.0	0.54		0.03
		50	100.0	0.57		0.03

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
		100	95.0	0.55	♦	0.02
	Outfall C + EDTA	100	100.0	0.57	♦	0.05
	Control	0	100.0	0.43		0.04
	Outfall D	6	97.5	0.46		0.03
		12	94.9[c]	0.48[c]		0.05
		25	95.0	0.52		0.03
		50	97.5	0.55		0.03
		100	95.0	0.50	♦	0.03
	Outfall D + EDTA	100	100.0	0.50	♦	0.01
6/19–26/97	Control	0	92.5	0.44		0.05
	Control + EDTA	0	80.0	0.34		0.06
	Outfall A	6	90.0	0.41		0.03
		12	77.5	0.41		0.07
		25	77.5	0.37		0.05
		50	87.5	0.43		0.06
		100	82.5	0.39	♦	0.07
	Outfall A + EDTA	100	80.0	0.52	♦	0.03
	Control	0	82.5	0.39		0.04
	Outfall B	6	77.5	0.38		0.05
		12	95.0	0.51		0.05
		25	75.0	0.44		0.05
		50	70.0	0.43		0.07
		100	80.0	0.40	♦	0.05
	Outfall B + EDTA	100	80.0	0.40	♦	0.04
	Control	0	97.5	0.48		0.04
	Outfall C	6	92.5	0.49		0.06
		12	80.0	0.46		0.08
		25	80.0	0.45		0.13

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
		50	85.0	0.48		0.09
		100	72.5	0.40	♦	0.13
	Outfall C + EDTA	100	75.0	0.40	♦	0.07
	Control	0	95.0	0.46		0.04
	Outfall D	6	90.0	0.42		0.04
		12	87.5	0.49		0.13
		25	70.0	0.43		0.11
		50	72.5	0.46		0.09
		100	67.5	0.40	♦	0.12
	Outfall D + EDTA	100	40.0	0.23	♦	0.00
8/7–14/97	Control	0	92.5	0.36		0.05
	Control + EDTA	0	100.0	0.47		0.04
	Outfall A	6	80.0	0.39		0.08
		12	100.0	0.38		0.04
		25	80.0	0.35		0.07
		50	80.0	0.36		0.04
		100	80.0	0.35	♦	0.06
	Outfall A + EDTA	100	75.0	0.46	♦	0.12
	Control	0	77.5[e]	0.29		0.08
	Outfall B	6	82.5	0.30		0.03
		12	80.0	0.30		0.11
		25	72.5	0.30		0.09
		50	52.5	0.22		0.09
		100	70.0	0.32	†	0.05
	Outfall B + EDTA	100	80.0	0.51	♦	0.05
	Control	0	77.5[e]	0.28		0.09
	Outfall C	6	87.5	0.37		0.06
		12	92.5	0.45		0.03

Table B.1 (continued)

Test period	Outfall	Effluent concentration (%)	Mean Survival[b] (%)	Weight (mg/larvae)[b] Mean		SD
		25	92.5	0.57		0.04
		50	82.5	0.48		0.09
		100	80.0	0.42	♦	0.09
	Outfall C + EDTA	100	55.5	0.30	♦	0.10
	Control	0	70.0[c]	0.22		0.04
	Outfall D	6	82.5	0.34		0.11
		12	70.0	0.30		0.02
		25	87.5	0.37		0.17
		50	85.0	0.42		0.04
		100	70.0	0.31	♦	0.11
	Outfall D + EDTA	100	100.0	0.63	♦	0.10

[a] Analysis of variance (ANOVA) was used to compare mean weight in nontreated effluent to mean weight in the EDTA-treated effluent from an outfall, by test period ($\alpha \leq 0.05$).

[b] Mean survival and final dry weight are based on the number of larvae used to initiate the test (i.e., 40).

[c] Mean survival and weight were adjusted to account for test organisms reported missing or killed.

[d] NC = no ANOVA conducted; all test organisms died.

[e] Test invalid based on unacceptable survival (< 80%).

Table B.2. Results of *Ceriodaphnia* toxicity tests, by outfall and test period

(NOTE: Mean reproduction in the 100% effluent concentration was compared to reproduction in the EDTA-treated effluent from the same outfall. Mean reproduction values with the same symbol (♦ or †) did not differ significantly different from each other[a].)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b]		
				Mean		SD
10/17–24/96	Control	0	100	28.3		3.3
	Control + EDTA	0	80	20.6		7.6
	Outfall A	6	100	24.9		6.1
		12	100	20.4		1.1
		25	100	21.5		3.0
		50	100	16.5		3.5
		100	90	14.3	♦	3.4
	Outfall A + EDTA	100	100	16.0	♦	4.9
	Control	0	100	27.7		2.5
	Outfall B	6	100	25.7		7.5
		12	100	20.7		8.6
		25	100	25.0		9.0
		50	100	22.9		8.8
		100	100	22.9	♦	6.4
	Outfall B + EDTA	100	100	26.0	♦	3.9
	Control	0	90	23.1		12.1
	Outfall C	6	100	32.2		6.4
		12	100	30.6		7.0
		25	100	31.9		7.9
		50	100	33.1		4.8
		100	100	28.2	♦	6.2
	Outfall C + EDTA	100	100	27.2	♦	10.2
	Control	0	100	31.4		3.9
	Outfall D	6	100	31.3		2.4
		12	100	32.2		2.3
		25	100	32.0		2.3

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
		50	100	33.1		2.2
		100	100	27.9	♦	4.0
	Outfall D + EDTA	100	100	21.8	†	5.6
12/5–11/96	Control	0	100	30.3		2.8
	Control + EDTA	0	100	20.2		12.2
	Outfall A	6	100	30.4		3.4
		12	100	30.8		3.1
		25	100	33.2		6.8
		50	100	31.3		4.7
		100	100	29.5	♦	11.1
	Outfall A + EDTA	100	100	31.0	♦	1.7
	Control	0	90	27.8		10.3
	Outfall B	6	90	26.3		8.2
		12	90	27.2		11.5
		25	100	27.7		4.4
		50	90	27.0		5.5
		100	100	24.4	♦	5.7
	Outfall B + EDTA	100	100	24.8	♦	1.3
	Control	0	100[c]	28.5[c]		5.6
	Outfall C	6	100	29.1		2.8
		12	100	27.6		4.6
		25	100	29.4		5.6
		50	100	23.9		6.5
		100	100	28.4	♦	5.3
	Outfall C + EDTA	100	100	21.8	♦	9.7
	Control	0	100	28.6		4.9
	Outfall D	6	100	22.6		4.4
		12	100	25.2		4.2

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
		25	90	18.1		11.5
		50	100	21.7		6.9
		100	100	27.9	♦	4.9
	Outfall D + EDTA	100	100	18.2	†	4.1
2/13–20/97	Control	0	100	29.0		3.4
	Control + EDTA	0	100	28.2		7.3
	Outfall A	6	100	30.5		12.8
		12	100	28.6		3.8
		25	100[c]	34.0[c]		2.6
		50	100	34.2		3.5
		100	100	34.0	†	2.2
	Outfall A + EDTA	100	100	37.8	♦	2.8
	Control	0	100	30.2		2.9
	Outfall B	6	100	28.3		6.3
		12	90	17.6		13.0
		25	80	21.8		15.2
		50	100	32.1		5.2
		100	0	0.0	NC[d]	0.0
	Outfall B + EDTA	100	0	0.0	NC[d]	0.0
	Control	0	100	25.3		5.5
	Outfall C	6	90	24.1		6.8
		12	100	27.8		3.1
		25	90	26.6		8.5
		50	100	26.9		2.0
		100	100	26.9	♦	3.0
	Outfall C + EDTA	100	100	26.8	♦	2.8
	Control	0	100	29.7		2.5
	Outfall D	6	90	25.4		7.3

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
		12	100	26.4		7.6
		25	100	27.6		3.5
		50	100	26.6		3.2
		100	90	27.3	♦	3.5
	Outfall D + EDTA	100	100	27.8	♦	2.8
4/24–30/97	Control	0	100	23.0		8.5
	Control + EDTA	0	100	22.2		3.3
	Outfall A	6	100	25.1		6.1
		12	100	25.0		9.9
		25	100	29.5		3.0
		50	90	27.1		8.0
		100	100	29.2	♦	5.1
	Outfall A + EDTA	100	100	29.0	♦	2.0
	Control	0	100	26.4		4.2
	Outfall B	6	100	27.7		2.3
		12	100	26.0		9.2
		25	100	28.6		4.1
		50	100	26.1		3.5
		100	100	29.4	♦	2.7
	Outfall B + EDTA	100	100	26.6	♦	7.6
	Control	0	90	24.9		9.9
	Outfall C	6	100	29.0		2.1
		12	100	28.4		3.6
		25	100	30.3		2.8
		50	100	29.2		2.9
		100	90	27.8	♦	10.3
	Outfall C + EDTA	100	80	18.6	♦	10.6

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
	Control	0	100	28.1		1.4
	Outfall D	6	100	24.4		9.2
		12	100	25.4		9.5
		25	100	26.8		9.7
		50	100	26.0		9.3
		100	100	27.9	♦	4.2
	Outfall D + EDTA	100	100	28.0	♦	2.4
6/19–25/97	Control	0	90	25.4		10.1
	Control + EDTA	0	100	29.2		2.2
	Outfall A	6	80	22.1		12.1
		12	100	23.2		7.6
		25	90	24.6		7.0
		50	100	29.6		5.8
		100	88.9[c]	21.4[c]	♦	11.6
	Outfall A + EDTA	100	100	30.4	♦	3.9
	Control	0	100	31.0		1.7
	Outfall B	6	100	31.7		3.1
		12	90	28.8		8.9
		25	100	30.1		3.5
		50	100	29.0		5.8
		100	100	28.5	♦	4.0
	Outfall B + EDTA	100	100	25.0	♦	8.3
	Control	0	100	31.1		1.5
	Outfall C	6	100	30.5		5.6
		12	100	32.1		2.9
		25	90	29.9		6.0
		50	100	30.7		3.9

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
		100	100	31.7	♦	2.1
	Outfall C + EDTA	100	100	31.2	♦	4.8
	Control	0	100	29.9		7.1
	Outfall D	6	100	29.1		6.2
		12	90	26.5		10.6
		25	100	29.4		4.4
		50	100	30.0		6.4
		100	100	28.3	♦	4.2
	Outfall D + EDTA	100	100	31.2	♦	2.7
8/7–13/97	Control	0	100[c]	31.6[c]		1.6
	Control + EDTA	0	100	33.0		2.4
	Outfall A	6	90	26.8		8.5
		12	88.9[c]	28.7[c]		6.9
		25	100	30.3		3.3
		50	100	28.2		5.9
		100	100	26.7	♦	9.8
	Outfall A + EDTA	100	100	28.2	♦	0.8
	Control	0	100	25.9		6.5
	Outfall B	6	100	25.9		5.3
		12	90	27.5		7.3
		25	100	29.5		2.0
		50	100	29.2		4.0
		100	100	29.4	♦	3.7
	Outfall B + EDTA	100	100	26.8	♦	4.9
	Control	0	100	34.2		3.5
	Outfall C	6	100	32.2		2.7
		12	100	32.8		2.9
		25	100	30.9		3.2

Table B.2 (continued)

Test period	Outfall	Effluent concentration (%)	Survival[b] (%)	Offspring/female[b] Mean		SD
		50	100	32.5		5.6
		100	100	30.8	♦	3.6
	Outfall C + EDTA	100	100	28.6	♦	3.4
	Control	0	100	33.5		6.5
	Outfall D	6	100	31.9		3.0
		12	100	33.6		1.8
		25	100	31.3		3.1
		50	100	32.7		3.2
		100	100	28.0	♦	2.4
	Outfall D + EDTA	100	100	25.4	♦	8.5

[a] Analysis of variance (ANOVA) was used to compare mean reproduction in nontreated effluent to reproduction in the EDTA-treated effluent from an outfall, by test period ($\alpha \leq 0.05$).

[b] Survival and offspring/female are based on the number of neonates used to initiate the test (i.e., 10).

[c] Survival and offspring/female were adjusted to account for test organisms reported missing or killed.

[d] NC = no ANOVA conducted; all test organisms died.

Ecotoxicology Highlights

February 25, 1997

Close Call with an "Environmental Sulfur Bomb"

In a well-intentioned effort to protect the environment, a waste treatment plant at a DOE facility recently generated a temporary "environmental sulfur bomb" that could have resulted in a fish kill. A fortunate combination of season and weather prevented this undesirable outcome. In this edition of Ecotoxicology Highlights, we describe the evolution of the problem so as to provide a brief "lessons learned" report of potential value for waste treatment operations at other facilities.

Chlorine commonly is used to control fecal coliform bacteria in sewage but is very toxic to fish and other biota. Accordingly, after a wastewater has been treated with chlorine to control fecal coliforms, the residual or "left-over" chlorine must be removed before the wastewater is suitable for release to a receiving stream. Sodium thiosulfate is often used for this purpose: this chemical has little toxicity and is very efficient at eliminating toxicity due to residual chlorine. Historically, chlorine has been shown to be a major contributor to toxic conditions in various streams near DOE facilities in Oak Ridge and was implicated as the major factor in several fish kills. Thus, a strong effort was made to lower the concentrations of chlorine in various wastewaters. But because thiosulfate has little toxicity and is very efficient at eliminating chlorine toxicity, there is sometimes a tendency to add it to excess, to ensure complete removal of residual chlorine. A situation that occurred recently demonstrated that excess loading of thiosulfate, for the well-intentioned purpose of chlorine control, can create an interesting but environmentally undesirable situation.

In oxygenated waters, excess thiosulfate is readily used as a source of energy by certain species of naturally occurring bacteria. As these bacteria metabolize thiosulfate, they precipitate elemental sulfur as a metabolic waste. Elemental sulfur is not toxic but can accumulate as a white- or cream-colored "floc" upon substrates in the receiving stream. Overfeeding of thiosulfate for several days led to the formation of such a floc, over a 25- to 30-meter reach of stream. As long as the water temperature in the receiving stream was low, no problem was evident other than the accumulation of precipitated sulfur. However, when thiosulfate feed rates are reduced, bacteria that precipitated the sulfur "get hungry" and begin to metabolize the precipitated sulfur. In such secondary reactions, sulfuric acid is generated and pH can decline, to approximately 4.0 -- a level that is low enough to kill or injure stream fish and benthic invertebrates and release metals previously bound in stream sediments. Warmer temperatures accelerate the rate of sulfur metabolism by the bacteria. Thus, the accumulation of much precipitated sulfur on substrates over a fairly large area of stream bed, coupled with the onset of warmer spring weather, plus "starving" the thiosulfate bacteria by curtailing the rate of thiosulfate addition, collectively primed the stream for a big problem. Fortunately, Mother Nature intruded: before water temperatures increased enough to stimulate the metabolism of the sulfur that had been precipitated, a rainfall event dispersed the material harmlessly to below "critical mass" levels.

About 4.5 mg of sodium thiosulfate is sufficient to neutralize 1.0 mg of residual chlorine. Thiosulfate added in excess of this amount is wasteful and, as shown by the situation described above, may contribute to instream environmental problems. Chemicals other than sodium thiosulfate can be used to eliminate toxicity of residual chlorine (sodium bisulfite is an example), but it is wise to remember that even nontoxic chemicals may be environmentally active.

For additional information, please contact T. Phipps (p57@ornl.gov) or A. Stewart (aqs@ornl.gov).

Table D.1. Summary of physico-chemical measurements, by outfall and sample date

Sample date	pH[a]	Conductivity[b]	Alkalinity[c]	Hardness[c]	D.O.[d]	TSS[e]	DOC[f]	Flow[g]
				Outfall A				
10/16/96	8.59	1435	35	330	8.6	17.8	8.77	3.28
10/18/96	9.06	1324	33	342	8.1	36.6	--	4.92
10/21/96	9.14	1485	36	390	9.3	24.2	9.50	3.43
12/4/96	7.60	1133	35	340	8.4	27.6	7.61	2.80
12/6/96	7.88	918	49	256	8.5	30.8	5.83	2.38
12/9/96	7.76	969	41	254	8.6	12.4	5.59	--
2/12/97	7.75	1058	39	306	8.6	20.4	5.97	2.50
2/14/97	7.72	975	41	274	8.3	16.4	5.34	2.21
2/17/97	7.89	868	49	242	8.8	21.0	4.62	--
4/23/97	7.71	882	45	248	8.6	22.9	6.03	1.68
4/25/97	7.78	844	43	232	8.5	22.6	5.19	1.48
4/28/97	7.78	947	41	260	8.7	25.2	5.66	3.76
6/18/97	8.67	1149	34	270	8.6	22.2	7.52	4.19
6/20/97	8.82	1220	29	276	8.5	29.6	6.85	3.87
6/23/97	8.94	1150	30	310	8.5	29.3	6.97	4.29
8/6/97	7.92	398	34	98	8.6	14.4	2.64	1.30

Table D.1 (continued)

Sample date	pH[a]	Conductivity[b]	Alkalinity[c]	Hardness[c]	D.O.[d]	TSS[e]	DOC[f]	Flow[g]
8/8/97	7.68	240	30	70	8.6	23.4	2.04	1.48
8/11/97	7.85	233	29	74	8.3	11.1	2.10	1.50
				Outfall B				
10/16/96	7.44	293	28	60	8.2	1.6	4.72	0.43
10/18/96	7.53	316	39	74	7.9	9.4	--	1.50
10/21/96	7.59	323	56	90	8.6	3.6	3.40	0.51
12/4/96	7.41	267	37	62	8.3	8.2	5.63	0.51
12/6/96	7.54	261	48	68	8.2	12.2	5.84	0.78
12/9/96	7.07	242	25	60	8.6	3.4	3.10	--
2/12/97	6.89	438	30	66	8.4	6.6	4.57	0.59
2/14/97	7.03	878	30	70	8.6	6.3	4.12	0.93
2/17/97	6.98	349	25	76	8.6	3.0	2.98	--
4/23/97	7.23	361	39	78	8.8	5.5	5.84	0.57
4/25/97	7.47	363	35	76	8.3	5.0	4.52	0.50
4/28/97	7.52	314	42	76	8.5	6.6	4.86	0.80
6/18/97	7.28	283	34	60	8.5	5.0	5.02	0.66

Table D.1 (continued)

Sample date	pH[a]	Conductivity[b]	Alkalinity[c]	Hardness[c]	D.O.[d]	TSS[e]	DOC[f]	Flow[g]
6/20/97	7.22	255	28	54	8.5	4.0	3.50	0.60
6/23/97	7.41	230	29	62	8.2	4.4	3.16	0.59
8/6/97	7.49	291	22	66	8.7	3.5	3.38	0.70
8/8/97	7.08	283	17	72	8.6	4.0	3.31	0.71
8/11/97	7.41	249	20	72	8.3	3.0	2.65	0.67
				Outfall C				
10/16/96	7.61	201	32	62	8.5	10.6	3.54	0.14
10/18/96	7.71	209	35	60	7.8	17.8	--	0.33
10/21/96	7.96	262	55	82	8.9	5.4	2.87	0.32
12/4/96	7.85	305	66	88	8.3	4.6	3.88	0.16
12/6/96	7.95	244	53	78	8.2	13.4	4.66	0.60
12/9/96	7.87	265	51	76	8.8	3.8	2.90	--
2/12/97	7.87	449	73	106	8.9	28.2	3.39	0.18
2/14/97	7.80	1418	49	98	8.3	26.2	3.13	0.52
2/17/97	7.95	667	69	106	8.7	7.3	2.69	--
4/23/97	7.84	315	51	78	8.3	12.8	5.49	0.21

Table D.1 (continued)

Sample date	pH[a]	Conductivity[b]	Alkalinity[c]	Hardness[c]	D.O.[d]	TSS[e]	DOC[f]	Flow[g]
4/25/97	7.88	326	46	96	8.3	11.9	4.26	0.18
4/28/97	7.72	236	45	74	8.3	9.4	5.33	0.63
6/18/97	7.45	281	48	72	8.0	5.3	5.11	0.38
6/20/97	7.76	288	42	80	8.5	7.5	3.69	0.22
6/23/97	7.86	229	42	74	8.4	5.7	4.64	0.26
8/6/97	7.83	246	37	62	8.2	5.7	2.90	0.18
8/8/97	7.67	247	29	62	8.3	5.6	3.13	0.18
8/11/97	7.76	212	15	62	8.5	7.0	4.54	0.18
				Outfall D				
10/16/96	7.57	269	24	70	8.2	9.0	2.48	0.45
10/18/96	7.87	278	40	84	7.8	--	--	0.88
10/21/96	7.98	327	59	98	9.0	49.8	2.92	0.47
12/4/96	7.77	280	46	86	8.6	13.4	2.92	0.40
12/6/96	7.90	248	45	76	8.0	59.2	2.81	0.39
12/9/96	7.63	273	33	80	8.6	15.4	2.25	--
2/12/97	7.62	296	38	90	8.7	16.3	2.72	0.40

Table D.1 (continued)

Sample date	pH[a]	Conductivity[b]	Alkalinity[c]	Hardness[c]	D.O.[d]	TSS[e]	DOC[f]	Flow[g]
2/14/97	7.48	364	33	74	8.2	23.7	2.89	0.61
2/17/97	7.81	311	42	84	8.8	20.8	2.24	--
4/23/97	7.65	334	39	82	8.7	38.1	3.57	0.49
4/25/97	7.76	348	38	88	8.1	16.3	2.30	0.43
4/28/97	7.73	280	42	84	8.6	24.8	4.00	0.67
6/18/97	7.76	232	48	66	8.3	27.0	3.78	0.10
6/20/97	7.84	217	44	70	8.7	25.0	3.08	0.08
6/23/97	7.80	188	40	74	8.6	36.0	3.41	0.09
8/6/97	7.73	470	52	100	8.6	7.8	3.52	0.71
8/8/97	7.65	486	26	124	8.3	13.2	3.57	0.72
8/11/97	7.67	460	29	130	8.4	7.1	3.95	0.73

[a]Standard units.
[b]μS/cm; corrected to 25°C.
[c]mg/L as $CaCO_3$.
[d]Dissolved oxygen in samples adjusted to test temperature (mg/L).
[e]Total suspended solids (mg/L).
[f]Dissolved organic carbon; mg C/L.
[g]Million gallons per day.

Table D.2. Mean (±SD) physico-chemical characteristics of the outfalls, by test period, and results of Tukey's tests for temporal differences

(NOTE: Within a given outfall and parameter, mean values sharing the same symbol (★, ▲, ■, †) did not differ significantly from each other[a])

Outfall	Parameter	Test period					
		10/96	12/96	2/97	4/97	6/97	8/97
A	pH[b]	8.80 (0.39)	7.73 (0.14)	7.78 (0.09)	7.75 (0.04)	8.80 (0.14)	7.8 (0.12)
	Tukey result	★	▲	▲	▲	★	▲
	Alkalinity[c]	35.5 (0.7)	41.7 (7.0)	43.0 (5.3)	43.0 (2.0)	31.0 (2.6)	31.0 92.6)
	Tukey result	★,▲	★,▲	★	★	▲	▲
	Hardness[c]	360.0 (42.4)	283.3 (49.1)	274.0 (32.0)	246.7 (14.0)	285.3 (21.6)	80.7 (15.1)
	Tukey result	★	★,▲	★,▲	▲	★,▲	■
	Conductivity[d]	1460.0 (35.4)	1006.7 (112.3)	967.0 (95.3)	891.0 (52.1)	1173.0 (40.7)	290.3 (93.3)
	Tukey result	★	▲,■	▲,■	■	▲	†
	DOC[e]	9.14 (0.52)	6.34 (1.10)	5.31 (0.68)	5.63 (0.42)	7.11 (0.36)	2.26 (0.33)
	Tukey result	★	■	■	▲	▲	†
	TSS[f]	21.0 (4.5)	23.6 (9.8)	19.3 (2.5)	23.6 (1.4)	27.0 (4.2)	16.3 (6.4)
	Tukey result	★	★	★	★	★	★
	Flow[g]	3.36 (0.11)	2.59 (0.30)	2.36 (0.21)	2.31 (1.26)	4.12 (0.22)	1.43 (1.26)
	Tukey result	★,▲	★,▲	★,▲	▲	★	▲

Table D.2 (continued)

Outfall	Parameter	Test period 10/96	12/96	2/97	4/97	6/97	8/97
B	pH[b]	7.51 (0.11)	7.29 (0.24)	6.96 (0.07)	7.39 (0.16)	7.30 (0.10)	7.29 (0.22)
	Tukey result	★	★	▲	★	★	★, ▲
	Alkalinity[c]	42.0 (19.8)	36.7 (11.5)	28.3 (2.9)	38.7 (3.5)	30.3 (3.2)	19.7 (2.5)
	Tukey result	★	★	★	★	★	★
	Hardness[c]	75.0 (21.2)	63.3 (4.2)	70.7 (5.0)	76.7 (1.2)	58.7 (4.2)	70.0 (3.5)
	Tukey result	★	★	★	★	★	★
	Conductivity[d]	308.0 (21.2)	256.7 (13.1)	555.0 (283.2)	346.0 (27.7)	256.0 (26.5)	274.3 (22.3)
	Tukey result	★	★	★	★	★	★
	DOC[e]	4.06 (0.93)	4.86 (1.53)	3.89 (0.82)	5.07 (0.69)	3.89 (0.99)	3.11 (0.40)
	Tukey result	★	★	★	★	★	★
	TSS[f]	2.6 (1.4)	7.9 (4.4)	5.3 (2.0)	5.7 (0.8)	4.5 (0.5)	3.5 (0.5)
	Tukey result	★	★	★	★	★	★
	Flow[g]	0.47 (0.06)	0.65 (0.19)	0.76 (0.24)	0.62 (0.16)	0.62 (0.04)	0.69 (0.02)
	Tukey result	★	★	★	★	★	★
C	pH[b]	7.75 (0.25)	7.89 (0.05)	7.87 (0.08)	7.81 (0.08)	7.65 (0.21)	7.75 (0.08)
	Tukey result	★	★	★	★	★	★
	Alkalinity[c]	43.5 (16.3)	56.7 (8.1)	63.7 (12.9)	47.3 (3.2)	44.0 (3.5)	27.0 (11.1)
	Tukey result	★,▲,■	★,▲	★	★,▲,■	★, ▲, ■	■
	Hardness[c]	72.0 (14.1)	80.7 (6.4)	103.3 (4.6)	82.7 (11.7)	75.3 (4.2)	62.0 (0.0)
	Tukey result	▲	▲	★	★,▲	▲	▲

Table D.2 (continued)

Outfall	Parameter	Test period 10/96	12/96	2/97	4/97	6/97	8/97
	Conductivity[d]	231.5 (43.1)	271.3 (31.0)	844.7 (508.3)	292.3 (49.1)	266.0 (32.2)	235.0 (19.9)
	Tukey result	★	★	★	★	★	★
	DOC[e]	3.21 (0.47)	3.81 (0.35)	3.07 (0.35)	5.03 (0.67)	4.48 (0.72)	3.52 (0.89)
	Tukey result	★	★	★	★	★	★
	TSS[f]	8.0 (3.7)	7.3 (5.3)	20.6 (11.5)	11.4 (1.8)	6.2 (1.2)	6.1 (0.8)
	Tukey result	★	★	★	★	★	★
	Flow[g]	0.23 (0.13)	0.38 (0.31)	0.35 (0.24)	0.34 (0.25)	0.29 (0.08)	0.18 (0.00)
	Tukey result	★	★	★	★	★	★
D	pH[b]	7.73 (0.29)	7.75 (0.14)	7.62 (0.17)	7.71 (0.06)	7.80 (0.04)	7.68 (0.04)
	Tukey result	★	★	★	★	★	★
	Alkalinity[c]	41.5 (24.7)	41.3 (7.2)	37.7 (4.5)	39.7 (2.1)	44.0 (4.0)	35.7 (14.2)
	Tukey result	★	★	★	★	★	★
	Hardness[c]	84.0 (19.8)	80.7 (5.0)	82.7 (8.1)	84.7 (3.1)	70.0 (4.0)	118.0 (15.9)
	Tukey result	▲	▲	▲	▲	▲	★
	Conductivity[d]	298.0 (41.0)	267.0 (16.8)	323.7 (35.7)	320.7 (35.9)	212.3 (22.4)	472.0 (13.1)
	Tukey result	▲,■	▲,■	▲	▲	■	★
	DOC[e]	2.70 (0.31)	2.66 (0.36)	2.62 (0.34)	3.29 (0.88)	3.42 (0.35)	3.68 (0.24)
	Tukey result	★	★	★	★	★	★

Table D.2 (continued)

Outfall	Parameter	Test period					
		10/96	12/96	2/97	4/97	6/97	8/97
	TSS[f]	29.4 (28.9)	29.3 (25.9)	20.3 (3.7)	26.4 (11.0)	29.3 (5.9)	9.4 (3.3)
	Tukey result	★	★	★	★	★	★
	Flow[g]	0.46 (0.01)	0.40 (0.01)	0.51 (0.15)	0.53 (0.13)	0.09 (0.01)	0.72 (0.01)
	Tukey result	▲	▲	★,▲	★,▲	★	★

[a]Mean values were examined using ANOVA. If the results of the ANOVA were significant ($P \leq 0.05$), then Tukey's tests were performed to determine which test period, within a given outfall and parameter, differed from each other.

[b]Expressed as $-\log[H^+]$.

[c]Expresed as mg $CaCO_3$/L.

[d]Expressed as μS/cm, corrected to 25°C.

[e]Dissolved organic carbon, expressed as milligrams of carbon per liter.

[f]Total suspended solids, expressed as mg/L.

[g]Million gallons per day.

Table E.1. Summary of total recoverable metal (TRM) and dissolved metal (DM), by outfall and sample date
(Boxes enclose sets of values excluded from the statistical analyses and from the calculation of final ratios)

Sample date	Cadmium		Chromium		Copper		Nickel		Lead		Zinc	
	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)
Outfall A												
10/16/96	0.33	ND	ND	ND	9.28	7.24	6.09	5.35	0.43	0.27	4.8	5.8
10/18/96	ND	ND	ND	ND	8.64	16.1	6.65	5.15	0.62	0.49	9.7	12.9
10/21/96	0.13	ND	ND	ND	8.15	6.68	6.35	5.81	2.71	0.59	7.6	10.6
12/4/96	0.18	ND	ND	ND	6.84	5.67	10.70	9.50	0.75	0.50	9.8	9.8
12/6/96	0.17	0.25	ND	ND	5.08	4.50	9.70	8.38	0.75	0.23	11.0	11.2
12/9/96	ND	ND	ND	ND	7.81	5.56	8.74	8.74	0.81	0.23	13.3	11.6
2/12/97	ND	ND	1.12	ND	7.00	5.96	6.98	6.21	ND	ND	9.7	7.5
2/14/97	ND	0.16	ND	ND	5.38	5.59	7.15	6.47	ND	ND	16.3	13.6
2/17/97	0.11	ND	ND	ND	5.45	3.84	7.04	6.34	0.61	ND	14.1	9.1
4/23/97	ND	ND	3.93	ND	6.75	5.09	8.74	5.91	1.16	0.87	19.0	14.7
4/25/97	ND	ND	ND	ND	9.67	6.07	5.39	4.75	0.59	0.19	20.3	18.5
4/28/97	ND	ND	ND	ND	5.37	3.89	7.29	6.53	0.37	0.19	21.9	18.6
6/18/97	ND	ND	ND	ND	4.60	4.59	4.68	3.52	0.46	ND	7.3	7.2
6/20/97	ND	ND	ND	ND	5.62	5.66	3.92	3.77	0.25	0.76	8.4	12.0
6/23/97	ND	ND	2.94	ND	6.44	4.97	5.89	4.65	ND	ND	11.9	8.7
8/6/97	0.13	0.13	ND	ND	2.67	2.17	1.34	1.51	0.42	0.23	7.6	8.6
8/8/97	0.26	0.20	ND	ND	4.68	2.67	1.03	1.08	1.48	0.27	10.6	10.6
8/11/97	ND	0.11	1.08	ND	4.36	1.59	0.98	0.70	0.53	0.14	8.7	8.7

Table E.1 (continued)

Sample date	Cadmium		Chromium		Copper		Nickel		Lead		Zinc	
	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)
Mean	--	--	--	--	6.19	4.81	6.00	5.25	0.91	0.34	11.9	11.0
SD	--	--	--	--	1.83	1.56	2.89	2.55	0.69	0.22	4.95	3.64
					Outfall B							
10/16/96	ND	ND	ND	ND	6.21	5.46	2.54	2.57	0.51	0.53	17.3	17.1
10/18/96	0.38	0.36	ND	ND	80.3	112.0	3.62	3.55	3.20	4.19	58.5	75.7
10/21/96	0.20	0.50	ND	ND	4.04	3.05	2.15	2.30	1.07	0.73	17.0	15.5
12/4/96	0.15	ND	1.24	ND	6.70	5.40	2.87	2.75	0.62	0.20	31.4	26.4
12/6/96	0.29	0.22	ND	ND	7.52	5.60	3.83	3.81	1.01	0.40	24.3	24.1
12/9/96	ND	0.21	ND	ND	4.69	4.03	2.93	2.61	1.46	0.88	17.0	16.4
2/12/97	ND	ND	ND	ND	6.24	5.90	2.88	2.94	0.23	ND	17.4	15.2
2/14/97	0.14	ND	1.46	ND	6.30	4.40	3.11	2.84	ND	ND	25.8	23.7
2/17/97	ND	0.13	ND	ND	3.94	3.37	2.76	2.94	0.85	0.15	14.7	13.4
4/23/97	ND	ND	ND	ND	6.46	5.21	3.35	3.38	0.76	0.36	30.6	32.3
4/25/97	ND	ND	ND	ND	6.96	5.72	2.99	3.10	0.72	0.30	21.9	21.7
4/28/97	ND	ND	ND	ND	7.35	5.29	6.26	5.89	0.85	0,36	24.8	27.2
6/18/97	ND	ND	ND	1.21	7.46	6.39	2.78	2.73	0.59	0.65	18.5	19.8
6/20/97	ND	ND	ND	ND	5.90	3.40	2.54	0.28	0.72	0.68	16.4	7.8
6/23/97	0.17	ND	ND	ND	5.43	4.78	3.73	2.89	0.46	ND	16.7	24.8
8/6/97	0.26	0.23	ND	ND	6.16	4.97	2.80	2.65	0.48	0.25	20.1	16.9

Table E.1 (continued)

Sample date	Cadmium		Chromium		Copper		Nickel		Lead		Zinc	
	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)
8/8/97	0.18	ND	ND	ND	5.44	4.07	3.00	2.67	0.40	0.17	20.9	16.0
8/11/97	ND	ND	1.26	ND	4.47	3.09	3.47	3.00	0.46	0.10	18.7	14.1
Mean	--	--	--	--	5.96	4.71	3.18	2.90	0.76	0.39	20.8	19.6
SD	--	--	--	--	1.14	1.05	0.90	1.05	0.32	0.24	4.99	6.20
					Outfall C							
10/16/96	0.11	ND	ND	ND	2.98	1.81	1.29	1.09	1.11	0.39	14.2	11.1
10/18/96	0.27	0.17	ND	ND	7.10	83.8	2.29	2.18	1.52	3.50	17.5	59.9
10/21/96	ND	0.16	ND	ND	2.75	1.59	1.50	1.47	1.08	0.29	10.6	7.6
12/4/96	0.40	0.17	ND	ND	3.49	3.17	2.03	1.69	0.75	0.51	11.8	10.6
12/6/96	0.14	0.12	ND	ND	6.48	5.54	1.83	1.93	1.63	0.66	16.6	13.9
12/9/96	0.18	ND	ND	ND	2.49	4.53	1.58	1.43	0.49	0.44	10.1	9.8
2/12/97	ND	ND	1.42	ND	5.38	2.38	1.75	1.17	0.51	ND	22.6	8.7
2/14/97	ND	ND	2.19	ND	23.90	3.82	1.79	1.12	2.09	ND	41.9	21.5
2/17/97	0.11	ND	ND	ND	3.52	2.43	1.41	1.48	0.44	ND	17.5	14.7
4/23/97	ND	ND	ND	ND	5.50	3.47	1.41	1.37	1.43	0.22	14.4	11.5
4/25/97	ND	ND	ND	ND	16.00	4.74	1.30	1.19	1.18	0.32	19.3	10.8
4/28/97	ND	ND	ND	ND	7.77	5.80	1.68	1.63	1.37	0.37	20.1	16.7
6/18/97	ND	ND	ND	ND	7.07	4.79	1.25	1.21	0.91	0.12	9.5	9.4
6/20/97	ND	ND	ND	ND	5.57	6.83	1.34	1.53	0.79	0.38	10.1	12.0

Table E.1 (continued)

Sample date	Cadmium		Chromium		Copper		Nickel		Lead		Zinc	
	TRM (µg/L)	DM (µg/L)	TRM (µg/L)	DM (µg/L)	TRM (µg/L)	DM (µg/L)	TRM (µg/L)	DM (µg/L)	TRM (µg/L)	DM (µg/L)	TRM (µg/L)	DM (µg/L)
6/23/97	0.11	0.11	ND	ND	8.03	6.51	1.67	1.68	1.06	0.52	10.8	10.0
8/6/97	0.20	ND	ND	ND	3.53	2.31	1.55	1.33	0.38	0.33	8.9	8.4
8/8/97	ND	ND	ND	ND	3.36	2.83	0.85	0.80	0.42	0.19	18.5	10.2
8/11/97	ND	ND	1.82	ND	5.31	6.16	1.97	1.43	0.49	ND	16.8	16.5
Mean	--	--	--	--	4.88	4.01	1.54	1.39	0.98	0.37	16.1	12.0
SD	--	--	--	--	1.87	1.82	0.30	0.27	0.45	0.14	7.88	3.63
					Outfall D							
10/16/96	ND	0.13	ND	ND	3.83	2.31	1.16	1.25	0.56	0.51	12.5	11.3
10/18/96	0.11	0.12	ND	ND	6.47	3.87	2.08	1.64	1.22	0.27	22.7	14.3
10/21/96	0.34	0.20	ND	ND	4.42	2.34	1.75	1.46	0.79	0.46	12.2	9.6
12/4/96	ND	ND	1.68	ND	6.28	2.78	1.96	1.70	0.65	0.43	21.9	15.8
12/6/96	0.17	0.22	ND	ND	5.83	4.53	2.04	1.80	1.18	0.43	46.9	36.7
12/9/96	0.17	0.10	ND	ND	4.95	3.25	1.73	1.56	1.05	0.57	18.6	18.7
2/12/97	ND	ND	2.07	1.16	5.06	2.27	0.90	1.00	ND	ND	17.4	13.8
2/14/97	ND	ND	2.23	1.11	5.88	6.55	1.00	1.00	ND	0.43	67.3	63.6
2/17/97	0.15	0.16	1.73	ND	3.82	2.77	0.82	0.76	0.18	ND	18.7	14.1
4/23/97	ND	ND	1.40	ND	5.54	3.67	1.48	1.19	0.70	0.42	45.8	39.1
4/25/97	ND	ND	1.54	ND	4.30	3.36	1.26	1.18	0.39	0.23	18.9	17.0
4/28/97	ND	ND	2.24	ND	6.66	4.64	2.03	1.35	0.95	0.26	60.0	44.7

Table E.1 (continued)

Sample date	Cadmium		Chromium		Copper		Nickel		Lead		Zinc	
	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)	TRM (μg/L)	DM (μg/L)
6/18/97	ND	ND	ND	ND	2.85	4.24	2.20	1.84	0.67	0.37	19.8	10.4
6/20/97	ND	ND	1.07	ND	3.28	3.30	1.86	2.17	0.65	ND	12.3	9.8
6/23/97	0.27	ND	2.17	ND	8.64	3.35	3.15	1.76	2.49	ND	19.6	8.3
8/6/97	0.29	0.23	1.85	ND	4.69	2.87	2.42	1.47	0.89	0.16	28.8	12.0
8/8/97	ND	ND	2.63	1.39	7.63	3.85	1.12	1.09	0.51	0.19	17.4	13.1
8/11/97	ND	ND	2.56	1.52	5.57	3.67	1.20	1.13	0.52	0.17	30.4	25.2
Mean	--	--	--	--	5.25	3.52	1.65	1.39	0.74	0.35	27.56	21.37
SD	--	--	--	--	1.52	1.06	0.62	0.37	0.25	0.15	17.03	15.57

Note: Boxes enclose sets of values that were excluded from the statistical analyses and from the determination of the final DM:TRM ratios. All metals data from October 18, 1996 were excluded, as were all Pb data collected in February and June. Total recoverable and dissolved values for Pb collected on August 11, 1997 were excluded because dissolved Pb was not detectable. All Cu data collected on February 14, 1997 and April 25, 1997 from Outfall C were excluded.

Table F.1. Alternative total recoverable metal concentrations of copper, nickel, lead, and zinc, by outfall

Outfall	Metal	KPDES permit limits[a] (μg/L)		Measured TRM[b] (μg/L)		DM: TRM[c]	CF[d]	Alternative TRM concentration (μg/L)[e]		Percent reduction
		Daily maximum	Monthly average	Mean	Max.			Mean	Max.	
A	Cu	42	26	6.19	9.67	0.78	0.960	5.03	7.86	18.7
	Ni	3079	342	6.00	10.70	0.87	0.997	5.24	9.34	12.7
	Pb	262	10	0.91	2.71	0.37	0.658	0.51	1.52	43.9
	Zn	254	230	11.89	21.9	0.92	0.986	11.09	20.4	6.7
B	Cu	18	12	5.96	80.30	0.79	0.960	4.90	66.08	17.8
	Ni	1418	158	3.18	6.26	0.91	0.997	2.90	5.71	8.8
	Pb	82	3	0.76	3.20	0.51	0.846	0.46	1.93	39.5
	Zn	117	106	20.79	58.5	0.94	0.986	19.82	55.8	4.7
C	Cu	18	12	4.88	23.90	0.82	0.960	4.17	20.41	14.5
	Ni	1418	158	1.54	2.03	0.90	0.997	1.39	1.83	9.7
	Pb	82	3	0.98	2.09	0.38	0.824	0.45	0.96	54.1
	Zn	117	106	16.10	41.9	0.74	0.986	12.08	31.4	25.0

Table F.1 (continued)

Outfall	Metal	KPDES permit limits[a] (μg/L)		Measured TRM[b] (μg/L)		DM: TRM[c]	CF[d]	Alternative TRM concentration (μg/L)[e]		Percent reduction
		Daily maximum	Monthly average	Mean	Max.			Mean	Max.	
D	Cu	18	12	5.25	8.64	0.67	0.960	3.66	6.03	30.3
	Ni	1418	158	1.65	3.15	0.84	0.997	1.39	2.65	15.8
	Pb	82	3	0.74	2.49	0.46	0.812	0.42	1.41	43.2
	Zn	117	106	27.56	67.3	0.78	0.986	21.80	53.2	20.9

[a]Current Kentucky Pollutant Discharge Elimination System (KPDES) total recoverable metal permit limits.
[b]Mean and maximum total recoverable metal (TRM) values, from Table E.1, Appendix E.
[c]Ratio of dissolved metal:total recoverable metal (DM:TRM), from Table 3.
[d]Environmental Protection Agency's freshwater criteria conversion factors for dissolved metals.
[e]Alternative total recoverable metal concentration, calculated by means of the KDOW equation:

$$Metal\ concentration\ reported = [Measured\ TRM \times DM{:}TRM] \times \frac{1}{EPA\ CF},$$

VITA

Terry Phipps was born in Boston, Massachusetts. She attended public schools in Wilmington, Delaware and Tinton Falls, New Jersey, where she was graduated from Monmouth Regional High School in 1973. She attended classes at Brookdale Community College in Lincroft, New Jersey; Central Piedmont Community College in Charlotte, North Carolina; and Virginia Highlands Community College in Abingdon, Virginia. In 1988, she enrolled at East Tennessee State University, Johnson City, Tennessee where in May 1992 she was graduated magna cum laude with a Bachelor of Science in Environmental Health. While working as a consultant with an engineering and environmental firm, she entered the Master's program in Life Sciences at The University of Tennessee, Knoxville in January of 1993. She completed the course work in the environmental toxicology major and officially received the Master of Science degree in May 1998.

Ms. Phipps has worked for more than six years in the environmental field, primarily in water quality assessment. She is planning on applying her professional and academic background to the field of risk assessment.