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EFFECT OF CARBAMATE PESTICIDES ON Cd AND Zn BIOACCUMULATION IN
A BIVALVE

A Thesis

Presented to

The Faculty of the Department of Environmental Studies

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Christopher J. Wellise

May 1999

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ABSTRACT

EFFECT OF DITHIOCARBAMATE PESTICIDES ON THE ASSIMILATION EFFICIENCY AND DISSOLVED INFLUX OF CADMIUM AND ZINC IN THE BIOINDICATOR *Potamocorbula amurensis*

by Christopher J. Wellise

This study set out to quantify the effects of carbamate (DDC) pesticides on the dissolved uptake and assimilation efficiency of the trace metals Cd and Zn in the bivalve, *Potamocorbula amurensis*, using radio tracer techniques. Experiments were carried out in a chemically defined media (Estuquill). Metal concentrations were 8.9×10^{-9} M for Cd and 1.8×10^{-8} M for Zn. The addition of 10^{-5} M concentrations of DDC increased the dissolved influx of Cd by ~25 times and Zn by ~10 times. Metals taken up as DDC complexes were lost at efflux rates similar to ionic forms. Cd assimilation from food was significantly lower in the DDC treatment than in the control (46% and 68% respectively), but the assimilation of Zn was not significantly affected ($40 \pm 3\%$ for the control and $47 \pm 5\%$ for the DDC exposure). Subcellular distributions of Cd and Zn indicated that metals taken up as DDC complexes, partition similar to ionic forms.

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Dedicated to my wife Monique, for her constant support and patience.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	viii
LIST OF FIGURES.....	x
I. INTRODUCTION.....	1
II. RELATED RESEARCH.....	11
III. MATERIALS AND METHODS.....	17
IV. RESULTS	
DISSOLVED INFLUX.....	55
EFFLUX FROM DISSOLVED PHASE.....	59
ASSIMILATION EFFICIENCY.....	77
SUBCELLULAR Cd AND Zn DISTRIBUTIONS.....	84
V. DISCUSSION.....	92
VI. RECOMMENDATIONS.....	99
VII. APPENDICES.....	101
VIII. REFERENCES.....	147

LIST OF TABLES

Table 1.	The Toxic Potential of Trace Elements	2
Table 2.	Selectivity Factors of Divalent Cations for Chelex Resin	23
Table 3.	Major Salt Composition in Estuquill Media	25
Table 4.	Major Nutrient Concentrations in Estuquill Media	26
Table 5.	Chelex 100 Resin Purification Procedures	26
Table 6.	Dissolved Influx Media pH Adjustments	30
Table 7.	Assimilation Efficiency Media pH Adjustments	31
Table 8.	Final Trace Metal Concentrations; Estuquill vs. Aquil	32
Table 9.	Distribution of Cd and Zn Species in Control Exposure (DI)	34
Table 10.	Distribution of Cd and Zn Species in DDC Exposure (DI)	34
Table 11.	Distribution of Cd and Zn Species in Control Exposure (AE)	35
Table 12.	Distribution of Cd and Zn Species in DDC Exposure (AE)	35
Table 13.	Total Radioactivity of Clams After Dissolved Influx	56
Table 14.	Radioactivity of 5 mL Aliquots of DI Exposure Media	59
Table 15.	Distribution of ^{109}Cd Activity in Clams During Efflux	66
Table 16.	Distribution of ^{65}Zn Activity in Clams During Efflux	67
Table 17.	Summary of Efflux Rate Constants of Cd and Zn	74
Table 18.	AE Calculations; A Summary of Two Approaches	77
Table 19.	Statistical Variables Obtained From <i>t</i> -test Analysis of Mean AE Values	84

Table 20.	Percentages of Bioavailable Metal, Control vs. DDC Treatments	92
Table 21.	A Comparison of Physiological Loss Constants to Previous Studies	94
Table 22.	A Comparison of Assimilation Efficiencies to Previous Studies	97

LIST OF FIGURES

Figure 1.	Dithiocarbamates and Some of Their Degradation Products	7
Figure 2.	Experimental Exposure Pathways in <i>P. amurensis</i>	9
Figure 3.	Potential Transport Mechanisms of Cd Across a Cell Membrane	13
Figure 4.	Estuquill Mixing Curve	22
Figure 5.	Chelex 100 Purification Apparatus	28
Figure 6.	San Francisco Bay Map Showing the Clam Collection Site	37
Figure 7.	Clam Collection Procedure	38
Figure 8.	<i>P. amurensis</i>	39
Figure 9.	Depuration Chamber	48
Figure 10.	Feces Collection Vessel	49
Figure 11.	Procedure for Obtaining Subcellular Fractions	52
Figure 12.	Dissolved Influx of Cd and Zn	58
Figure 13.	Whole Clam Loss of Cd	60
Figure 14.	Whole Clam Loss of Zn	61
Figure 15.	Whole Clam Efflux Rates of Cd From 2.85-21 Days	63
Figure 16.	Whole Clam Efflux Rates of Zn From 2.85-21 Days	64
Figure 17.	Change in Weight Condition During Efflux	69
Figure 18.	Change in Activity During the Loss of Cd	70
Figure 19.	Change in Activity During the Loss of Zn	71
Figure 20.	Estimated Tissue Concentration of Cd During Efflux (0-6 Days)	72

Figure 21.	Estimated Tissue Concentration of Zn During Efflux (0-6 Days)	73
Figure 22.	Actual Decline in Tissue Activities From ^{109}Cd (0-6 Days)	75
Figure 23.	Actual Decline in Tissue Activities From ^{65}Zn (0-6 Days)	76
Figure 24.	Defecation Rate of Cd During Assimilation	79
Figure 25.	Defecation Rate of Zn During Assimilation	80
Figure 26.	Assimilation Efficiency of Cd	82
Figure 27.	Assimilation Efficiency of Zn	83
Figure 28.	Relative Subcellular Distribution of Cd and Zn from DI Experiment at $t=0$	85
Figure 29.	Change in Concentrations of Cd and Zn in Individual Subcellular Compartments from $t=0$ to $t=6$ Days for DI Experiment	87
Figure 30.	Relative Subcellular Distribution of Cd and Zn from AE Experiment at $t=0$ Days	89
Figure 31.	Relative Subcellular Distribution of Cd and Zn from AE Experiment at $t=2$ Days	90
Figure 32.	Change in Concentrations of Cd and Zn in Individual Subcellular Compartments from $t=0$ to $t=2$ Days for AE Experiment	91

I. INTRODUCTION

The goal of this research was to add to the limited body of knowledge that exists on the interaction of trace metals and organic pollutants. Specifically, this study set out to quantify the effects of carbamate pesticides on the uptake of trace metals to an aquatic bivalve found prevalently throughout the San Francisco Bay Estuary. The San Francisco Bay is a complex estuarine system that is vulnerable to a vast array of potential pollutants. Industrial inputs of trace metals, as well as the runoff of pesticides from agricultural land in the Central Valley can pose unique threats to the Bay's inhabitants. The potentially synergistic interactions that these two major classes of pollutants may undergo are rarely addressed.

The study of trace metals in the marine environment has been the focus of much research in aquatic ecology. Although essential for proper cellular function, many metals can be toxic to aquatic organisms in concentrations higher than those typically found in nature. Trace metals can generally be categorized into two major groups, essential and non-essential. Essential metals play a critical role in the metabolism and enzymatic activity of plant and animal cells, where they function as micronutrients. The essential group includes cobalt, copper, chromium, iron, manganese, nickel, molybdenum, selenium, tin and zinc (Rand and Petrocelli 1985). In addition to their critical roles in cellular activity, members of the essential group can result in a threat to the health of aquatic organisms when concentrations exceed the relatively limited range of tolerance of most species. Some important non-essential trace metals that have been identified as toxic

to aquatic species include thallium, mercury, lead, cadmium and barium. Nieboer and Richardson (1980) have further classified elements of toxicological importance into three categories (Class A, Class B and a borderline class) that reflect their chemical and biological reactivities. Class A are considered least toxic, while the ions in the Borderline category lie somewhere between A and the more toxic elements found in Class B (Nieboer and Richardson 1980). Table 1 lists elements in the various categories.

Unlike synthetic pollutants, metals are unique in the sense that humans do not manufacture but rather redistribute these potential contaminants. Anthropogenic processes rapidly accelerate the rates of redistribution of metals from the lithosphere to

Table 1. - Toxic Potential of Trace Elements.

Class	Trace Element
A	Li^+ , Na^+ , K^+ , Cs^+ , Ca^{2+} , Ba^{2+} , Sr^{2+} , Mg^{2+} , Be^{2+} , La^{2+} , Y^{3+} , Gd^{3+} , Lu^{3+} , Sc^{3+} , Al^{3+}
Borderline	Mn^{2+} , V^{2+} , Ti^{2+} , Zn^{2+} , Ni^{2+} , Fe^{2+} , Co^{2+} , Cd^{2+} , Cu^{2+} , Sn^{2+} , Cr^{3+} , Fe^{3+} , Ga^{3+} , As^{3+} , Sn^{3+} , Pb^{4+} , In^{4+} , Sb^{4+}
B	Ag^+ , Au^+ , Tl^+ , Cu^+ , Hg^{2+} , Pd^{2+} , Bi^{2+} , Tl^{2+} , Pb^{2+}

Source: Nieboer and Richardson (1980).

various terrestrial, aquatic and atmospheric environments. These problems have been exacerbated by exponential population growth and increased global industrialization near coastal regions. Measurements indicate that concentrations of metals lethal to aquatic organisms are commonly present in surface waters and sediments near to industrial sites and population centers (Rand and Petrocelli 1985). Municipal outflow, mining, industrial effluent and urban runoff often result in concentrations hazardous to aquatic organisms.

Estuaries like the San Francisco Bay are particularly vulnerable to metal accumulation due to alterations in freshwater input, the destruction of natural marshlands, and their use as receiving waters for waste disposal (Nichols et al. 1986).

The toxicity of trace metals to aquatic biota may result in various effects ranging from sublethal responses such as slowed growth and impaired gonadal maturation to more severe effects including asynchronous reproduction, inability to feed, and death (Rand and Petrocelli 1985; Paez-Osuna, Frias-Espericueta and Ouna-Lopez 1995; Krishnakumar, Castillas and Varanasi 1994).

Studies that attempt to assess the effects of various forms of contamination on marine populations often use indicator organisms, whose presence or state of health provide a measure of ecological conditions. Bivalves are frequently used as indicators when assessing contamination from metals and other pollutants in the marine environment. Their relative abundance, ability to bioaccumulate toxic elements, and important position in the estuarine food chain has lead to the extensive use of mussels, clams and oysters when assessing the effects of trace metals (Gold-Bouchot et al. 1995; Krishnakumar, Castillas and Varanasi 1994; Boyden 1977; Paez-Osuna, Frias-Espericueta and Ouna-Lopez 1995; Wang, Fisher and Luoma 1996; Thompson et al. 1996).

A second class of pollutant that requires attention when assessing the health of aquatic ecosystems are the pesticides. Their use expanded rapidly, especially after World War II, when the discovery of DDT initiated the development of synthetic pesticides (Rand and Petrocelli 1985). Used to control insects, fungi, plants, molds and other

threats to humans, crops, and the environment, pesticides have been manufactured in several chemical forms with a wide range of toxicities. The major groups include: chlorinated hydrocarbons, organophosphates, carbamates, pyrethrins, dithiocarbamates, nitrogen-containing compounds, hexachlorobenzene, and the phenoxy acids. The primary use of the chlorinated hydrocarbons, organophosphates, carbamates and pyrethrins is to fight insects; the dithiocarbamates, nitrogen compounds and hexachlorobenzene are used as fungicides. The remaining chemicals, the phenoxy acids, are herbicides (Rand and Petrocelli 1985).

Because pesticides and their degradation products can persist in the environment, and are easily mobilized by runoff, aquatic environments adjacent to agricultural fields, golf courses, urban lawns and pesticide production facilities are especially vulnerable to disruption.

As previously mentioned, dithiocarbamates (DCs) are a class of pesticides used primarily as a fungicide. DCs are applied extensively and have been used since the mid-1930s to control a large number of different organisms including bacteria, fungi, nematodes and molluscs (Thorn and Ludwig 1962). In addition to their agricultural applications, the dithiocarbamates are used as vulcanization accelerators and antioxidants in the rubber industry where they activate thiazoles in the latex curing systems, and slimicides to control algal and bacterial growth in the large water cooling systems of sugar, pulp and paper mills (Thorn and Ludwig 1962). The chelating properties of DCs are also well known and have led to their use as heavy metal scavengers in waste water and in human medicine, as in the case of lead and nickel poisoning (Van Leeuwen et al.

1985). They are produced and used on a large scale. Dutch production alone was estimated at 14,000 metric tonnes per year in 1985 (Van Leeuwin et al. 1985).

The dithiocarbamates (DCs) are classified into two main groups: dimethyldithiocarbamates (DDCs) and ethylenebisdithiocarbamates (EBDCs). Fungicides in the dimethyldithiocarbamate category include ferbam, thiram and ziram (State of California 1992). All except ferbam are salts or complexes with a metal cation (Smart 1990). The EBDC class includes maneb, mancozeb, nabem and zineb (Smart 1990). The solubilities of the DCs vary widely. Nabem is very soluble at approximately 200g/L, while ferbam and thiram are less soluble at 130 and 30 mg/L respectively (Smart 1990). Zineb is the least soluble at approximately 10 mg/L (Smart 1990). Figure 1 shows the structures of some of the more widely used dithiocarbamates and their degradation products.

Degradation pathways of DCs involve successive oxidations and hydrolyses and may take from days to several months within aquatic environments (Smart 1990).

EBDCs have traditionally received more attention than DDCs due the potential conversion of EBDC to ethylene-thiourea (ETU) which is a potential carcinogen in humans and has been shown to be toxic to rats and mice (Nash and Beall 1980).

A number of potential pathways exist for DCs to enter natural waters. Facilities that manufacture these chemicals do little to purify their effluent before it reaches receiving waters (Van Leeuwin et al. 1985). In addition, the widespread use of these chemicals in agricultural applications may result in their introduction into waterways through runoff. In the United States, carbamates are used on a large scale, especially in the west. In 1992 alone, over 2.5 million pounds of carbamates were applied to

California's agricultural fields (State of California 1992).

Current analytical procedures are unable to detect DCs at their biologically relevant concentrations of 0.1 ug/l to 0.5 ug/l (Miller 1997). However, although no formal studies have quantified DC concentrations in natural waters, their extensive use and production near receiving waters suggests that these chemicals might be entering aquatic environments in significant amounts (Phinney and Bruland 1994). In addition, recent bioassays conducted on larval fathead minnows with Sacramento River water collected at a site near Rio Vista showed toxicity consistent with metallodithiocarbamates (Miller 1997).

The presence of trace metals and their deleterious effects on biota in estuarine environments such as the San Francisco Bay have been well documented (Boyden 1977; Luoma 1983; Krishna-Kumar, Castillas and Varanasi 1994; Gold Bouchot et al. 1995; Wang, Fisher and Luoma 1996). However, an extensive literature review revealed that few studies have addressed the potential effects of metallodithiocarbamates on multicellular organisms in the estuarine environment.

Laboratory experiments can seldom accurately represent the complexity of the polluted marine ecosystem in which plants and animals can be exposed to a vast array of chemicals that are present in varied concentrations. Experimental methods generally attempt to replicate conditions within natural waters with respect to an isolated contaminant such as an organic chemical or trace metal. This approach has the limitation of excluding potential synergistic relationships that may occur between contaminants.

Figure 1. Dithiocarbamates and Some of Their Degradation Products.

Figure 1.

Source: Van Leeuwen et al. (1985).

This research addressed this potentially synergistic effect on exotic bivalve species frequently utilized in assessing contaminant exposure in the San Francisco Bay, *Potamocorbula amurensis*. It was hypothesized by the author of this thesis that the introduction of DDC would enhance the uptake of the trace metals Cd and Zn in the bivalve *P. amurensis*, as well as increase the assimilation efficiency of these trace metals in the animals when they were fed algal cells exposed to Cd and Zn that had been complexed by DDC. The hypotheses were based on primarily on the results obtained in previous experiments that indicated that the presence of a chelator such as DDC may result in increases in metal uptake over a biological membrane (Phinney and Bruland 1994).

Figure 2 summarizes the processes to be studied in this thesis. Two major pathways of metal to clams were analyzed for this research, exposure from the dissolved phase and exposure from the particulate phase. Pathways A and C represent the exposure of metal through the dissolved phase, while B and D, represent metal assimilated from a food source (i.e. algae). Pathways C and D will included the addition of DDC in the exposure or culturing medium. Pathways A and C are referred to as dissolved uptake or uptake from the dissolved phase because in both cases the free metal ion or DDC-metal complex is present in its dissolved form in water. Pathways B and D represent the particulate pathway whereby exposure occurred through food consumption, in this case, the ingestion of algae. Dissolved exposure was measured by quantifying the dissolved influx of metals into the clam tissues. The terms dissolved influx (DI) and uptake from the dissolved phase are synonymous. The accumulation of metals from the particulate

A - Uptake of divalent metal cation Me^{+2} (in this case Cd^{+2} or Zn^{+2}) occurs directly from dissolved phase (dissolved influx (DI) from water; Control).

B - Dissolved Me is taken up by phytoplankton then accumulated by clam (assimilation from food (AE); control) .

C - Me is complexed by DDC then complex is taken up by clam in dissolved phase (dissolved influx (DI) from water; experimental DDC treatment).

D - Phytoplankton is exposed to $Me[DDC]_2$ complex then taken up by clams (assimilation from food (AE); experimental DDC treatment).

Figure 2. Experimental Exposure Pathways in *P. amurensis*.

phase was characterized by measuring the assimilation efficiency (AE) (expressed as a percentage) of metal by clams. The metals in the AE experiment were associated with algal cultures grown in media that had spiked with metal. In the case of pathway D in figure 2, the media also contained DDC. The efflux of metals from exposed clams was also characterized. However an efflux experiment was only conducted on the clams that were exposed to metals from the dissolved pathway (pathways A and C).

Because the DDC-metal complexes are neutrally charged, and lipophilic in nature, it is possible that these complexes could enter, and be mainly retained in, the phospholipid bilayer of the cells of the exposed animal. This is important because it has been shown that metals bound in this tissue (membrane) fraction do not pose as great of threat to predatory organisms as metals sequestered in the cytosol (Wallace and Lopez 1997). Therefore, the final portion of this study involved characterizing the subcellular partitioning of metals in the clams from both the dissolved influx exposure and the assimilation efficiency exposure, and comparing the control groups to those treated with DDC. It was hypothesized that a greater fraction of both Cd and Zn would be found in the tissue (debris) fraction of the cells, which contained the cell membranes.

The hypotheses of this thesis are outlined below:

- 1. The addition of DDC will enhance the dissolve uptake of Cd and Zn from water.***
- 2. The addition of DDC to the algal culturing media, will enhance the assimilation efficiency of Cd and Zn from food.***
- 3. A greater fraction of both Cd and Zn will be found in the subcellular debris fraction of the clams from both the dissolved influx and assimilation efficiency experiments.***

II. RELATED RESEARCH

Traditionally, the free ion model of the uptake of trace metals to aquatic biota has been considered the dominant mechanism for cellular trace metal accumulation (Blust, Baillieu and Decleir 1995, Luoma 1983). This is believed to be true because the free ion species of metals possess the highest free energy and are apt to react more readily with the carrier molecules that are associated with transporting metals across the cellular interface (Luoma 1983). However, research has shown that in the presence of low molecular weight organic ligands (e.g. dithiocarbamates) passive diffusion of trace metal-organic complexes through membrane tissue may substantially contribute to cellular trace metal concentrations (Phinney and Bruland 1994, Vasseur et al. 1988). Therefore, when assessing the exposure of biota to trace metals, consideration should be given to the potential presence of substances which may increase actual doses.

The research conducted for this thesis is based on the chelating properties of DCs and their ability to form complexes with trace metals. The complexing properties derive from the two donor sulfur atoms which determine the nature of the metals that can be bound (Hulanicki 1967).

In a recent study, Phinney and Bruland (1997) demonstrated that the fungicides ziram and maneb, complexes of carbamates with zinc and manganese respectively,

possess the ability to undergo exchange reactions where Cu and Pb ions replace sites previously occupied by Zn or Mn (Phinney and Bruland 1997). These complexes are neutrally charged and lipophilic in nature, and have the ability to diffuse across the lipid bilayer of phytoplankton cells and into the cytosol (Phinney and Bruland 1994). Figure 3 depicts some of the potential transport mechanisms of the trace metal Cd and various complexing agents, including dithiocarbamates, at a biological / environmental interface.

After transport through the phospholipid bilayer, two prospects for potential disruption to biota exist. First, the metallodithiocarbamate complex may disassociate, resulting in the addition of free trace metal ion to the cell. Second, the negatively charged carbamate molecule may then bind with various intracellular binding sites or trace metals (micro nutrients), thereby rendering them non-bioavailable and eventually excreted by the organism. It is this second property that has lead to the use of DDC as a chelator to treat nickel, cadmium and lead poisoning in humans.

Thus far, the bulk of the research on these substances has focused on the stability and breakdown of DCs and EBDCs to their potentially more toxic degradation products. Smart (1990) characterized the residues of dithiocarbamate fungicides in fruits and vegetables with focus on analytical methods used to detect the presence of their toxic degradation products. In toxicological experiments, Van Leeuwen et al. (1985) used guppies, water fleas and green algae to assess the toxicities of both DDC and EBDCs. They quantified the differences in toxicities from the two compounds and elucidated that it is probable that toxicity from both BCDs and DDCs results from the fact that either they or their degradation products undergo radical interchange reactions with SH- groups.

Figure 3. Uptake Mechanism of Cd at a Biological / Environmental Interface. Cd is entering the cell by two possible pathways: 1.) via complexation of the Cd²⁺ ion onto surface binding sites (in competition with Ca²⁺) channel FT, then being transported across the membrane or 2.) via diffusion of Cd(DDC)₂ lipophilic complex directly into the cytosol. After transport of the lipophilic complex, disassociation from the ligand can occur with subsequent complexation with intracellular ligands (ICL) and remain within the cell. The DDC⁻ ligand can become protonated within the cell and form a HDDC lipophilic complex. This complex can diffuse back across the cell membrane and into the extracellular solution until it achieves equilibrium with the intracellular species.

(Source: Phinney and Bruland 1994).

In a recent study, members of the Department of Pharmacology and Toxicology at the Swedish University of Agricultural Sciences, measured kinetics and body distribution of waterborne $^{65}\text{Zn(II)}$ and $^{109}\text{Cd(II)}$, as well as $^{203}\text{Hg(II)}$ and $\text{CH}_3^{203}\text{Hg}$ in *Chaoborus americanus* (Phantom Midge larvae) and the effects of complexing agents. One of the agents used in the experiment was DDC (Roulea, Block and Tjalve 1998). In the midge experiment, the addition of DDC did not have an, “important effect on the accumulation of $^{65}\text{Zn(II)}$ and $^{109}\text{Cd(II)}$ ” (Roulea, Block and Tjalve 1998). The authors of the study summarized that, “the lack of effect of DDC on $^{65}\text{Zn(II)}$ and $^{109}\text{Cd(II)}$ uptake might be related to their increased hardness compared to Hg(II) resulting in an increased stability of the first hydration sheat of Cd^{2+} and Zn^{2+} ions of which H_2O dipoles could not be successfully displaced by DDC at very low concentrations” (Roulea, Block and Tjalve 1998). Zn(II) and Cd(II) concentrations used in the study were equivalent to $(2 \text{ nmol L}^{-1})d$ and DDC was added at a ratio of 25:1 [chelator]/[metal] (Roulea, Block and Tjalve 1998).

Several studies have successfully characterized the assimilation of trace elements by marine biota. Fisher, Nolan and Fowler (1991) employed gamma-emitting radio tracers to assess the retention efficiency of ingested and excreted metals in the copepod *Anomalocera patersoni*. Results suggested that actinides would be rapidly defecated by the copepods that had ingested them in their food and that the sinking particles would serve to depurate surface waters of these elements while enriching deeper waters and sediments (Fisher, Nolan and Fowler 1991). Reinfelder and Fisher (1994) measured the assimilation efficiencies of nine elements in planktonic bivalve mollusc larvae fed radio

labeled phytoplankton cells. Results showed that assimilation efficiencies were directly correlated to the fraction of each element present in the cytosol of the ingested algal cells (Reinfelder and Fisher 1994).

Adult bivalves have also been employed in similar studies. Wang, Fisher and Luoma (1995) used the mussel *Mytilus edulis* in pulse chase feeding experiments, such as the study proposed here, to measure the assimilation of trace elements from ingested diatoms. Key elements of this study focused on determining the effect of food quantity on assimilation efficiency (AE) of metals, and assessing the importance of gut passage time and digestive partitioning on metal assimilation (Wang, Fisher and Luoma 1995). In a related study, an effort was made to determine assimilation efficiencies in *Crassostrea virginica* (an oyster), *Mytilus edulis* (a mussel), and *Macoma balthica* and *Mercenaria mercenaria* (two species of clam) (Reinfelder et al. 1997). The study used six metals (Ag, Am, Cd, Co, Se and Zn), that were all chosen because they all exhibit elevated concentrations in coastal waters (Reinfelder et al. 1997). Conclusions of the study showed that the food processing rates were specific to both the metal studies, and the bivalve species employed (Reinfelder et al. 1997).

Whenever live animals are used in laboratory experiments, the potential for the artificial environment to affect behavior that might be exhibited in nature exists. One series of experiments that addressed this potentially confounding factor was conducted by members of the Marine Science Research Center at the State University of New York, Stony Brook. The mussel *Mytilus golloprovincialis* was used to compare the accumulation and retention of metals from food and water under both field and laboratory

conditions (Fisher et al. 1996). The results from this study indicate that AE estimates derived from exposures conducted in a laboratory setting are applicable to field conditions (Fisher et al. 1996).

Kinetic modeling can be used to predict trace element concentrations in situ where environmental data exist for comparison. Wang, Fisher and Luoma (1996) have used such a model, which utilizes values obtained from laboratory experiments employing radio tracer methodology, to determine metal AEs from ingested seston in the mussel *Mytilus edulis*. Such models can assist in identifying pathways and the most important processes that are responsible for bioaccumulation (Wang, Fisher and Luoma 1996). These models are also critical in predicting the flow of contaminants along trophic pathways and can also aid in the prediction of the fate of contaminants in the marine environment. Although this type of modeling is not the goal of this study, it is an example of how the data obtained from this research can be expanded upon to predict levels of bioaccumulation in aquatic organisms, and ultimately be used in establishing regulatory criteria.

III. MATERIALS AND METHODS

A. Overview

Models can provide a conceptual overview of important processes. It is possible to employ a bioaccumulation model to describe the kinetic uptake of trace elements to bivalves and other aquatic biota (Wang, Fisher and Luoma 1996; Luoma et al. 1992; Fisher et al. 1996; Lee and Luoma 1997a). Kinetic bioaccumulation modeling can assist in the determination of contaminant doses where multiple pathways of exposure exist. Because trace metals are available to bivalves through both dissolved and particulate phases, it is important to assess both potential pathways of accumulation.

The model assumes that uptake from each source is proportional to its influx and is described by the following general equation:

$$dC/dt = \alpha_w \times FR \times C_w + \sum_{j=1}^n (AE \times IR \times C_p) - (k_e + g) \times C \quad (1)$$

where C is the trace element concentration in bivalve soft tissue ($\mu\text{g g}^{-1}$), t is the time of exposure (d), and α_w is the trace element absorption efficiency from the dissolved phase, FR is the bivalve filtration rate ($\text{L g}^{-1} \text{d}^{-1}$), C_w is the dissolved trace element concentration ($\mu\text{g L}^{-1}$), AE is the trace element assimilation efficiency (%) from ingested particles, IR is the ingestion rate of the bivalve ($\text{mg g}^{-1} \text{d}^{-1}$), C_p is the trace element concentration in ingested particles ($\mu\text{g mg}^{-1}$), j is the food compartment, k_e is the efflux rate constant (d^{-1}), assuming a first order growth (Wang, Fisher and Luoma 1996).

This study attempted to determine two critical values in the model, absorption

efficiency (AE) and dissolved trace metal influx (μ metal /gram tissue / unit time (t), to quantify the effect of the presence of dithiocarbamates on cadmium and zinc, and their combined effects on the bivalve *Potamocorbula amurensis*. The dissolved influx rate describes the rate at which the dissolved metal species is accumulated by the organism. Absorption Efficiency (AE) describes that portion (%) of the element which is accumulated through the particulate phase (seston, food particles, etc.) and retained after the gut has been cleared (approximately 24 hours after feeding).

Experimental trace metal exposures to determine influx from the dissolved phase were calculated by exposing two groups of animals consisting of twelve replicates (three clams per replicate). One group was exposed to free (or inorganically complexed) Cd(II) and Zn(II) in solution and the other to Cd(II) and Zn(II) complexed with diethyldithiocarbamate (DDC) in solution. In all exposures, stable metal forms are equilibrated with ^{109}Cd and ^{65}Zn used as radio tracers. DDC (purchased from Fluka Chemicals) was chosen because it has a similar structure to the dithiocarbamate fungicides described earlier.

Assimilation efficiency (absorption efficiency) was determined by feeding clams phytoplankton cells that had been exposed to free (inorganically complexed) Cd(II) and Zn(II) and compares this group to clams fed phytoplankton exposed to Cd(DDC)₂ and Zn(DDC)₂ complexes. Sample groups for Assimilation Efficiency (AE) both consisted of nine replicates (containing three clams per replicate).

Radioactivity analysis for dissolved influx and AE was conducted by using a Wizard® 1480 gamma counter equipped with a 3" well type NaI crystal detector. Photon

emission of ^{109}Cd and ^{65}Zn is determined from their characteristic 88 keV emission energies.

All exposures occurred in chemically defined Estuquail medium, synthetically formulated estuary water solution. It was necessary to perform the exposures in a Estuquail for two major reasons. First, it was essential to limit the additions of organic matter in the exposure medium. Near-shore seawater, even when filtered, contains large concentrations of dissolved organic compounds, such as humic substances, that could potentially bind with dissolved trace metal addition and confound the results for speciation calculations (Piana 1996). Second, in order to accurately model the equilibrium concentrations of the components within the exposure medium, it must be chemically defined. The procedure for Estuquail production is outlined in section C below.

B. Glass and Plasticware Washing Procedure

To limit trace metal contamination that may be introduced from glass and plasticware used during the experiment and to eliminate the potential contamination by organic ligands, all vessels that might come into contact with the exposure medium during the experiment underwent the rigorous washing procedure outlined below. The use of intensive washing procedures has been employed in similar studies (Phinney and Bruland 1994; Kuwabara 1980; Price et al. 1989):

2. Preliminary rinse in Milli-Q water inside and outside of container or item
3. Rinsing in Micro solution (Baxter Scientific)

4. Soaking in Micro solution for 1 day
5. Rinsing in Milli-Q water (at least 5 times)
6. Rinsing in HPLC grade methanol (to remove residual organics)
7. Rinsing with Milli-Q water (at least 3 times)
8. Soak item in 3N HNO₃ for at least 1 week
9. Rinse item with Milli-Q water (at least 5 times)
10. Allow item to dry under laminar air flow
11. Place lids on all containers and store in clean room or place item under laminar air flow until use

Steps 8-10 occurred in a class 1000 clean room located in the McKelvey building of the Water Resources Division of the U.S. Geological Survey in Menlo Park California. The lab is equipped with extremely efficient air make-up and filtration units that limit airborne particles to a maximum of 1000 per cubic meter.

C. Estuquail Production

Because Estuquail is a chemically defined medium it is important to limit any potential contamination during its production. The medium was prepared in a class 1000 Clean Room in the McKelvey Building at the Water Resources Division of the U.S. Geological Survey in Menlo Park, California. The column used was composed of Bor-silicate glass and all fittings, tubing and filter membranes made of Teflon. Silicon stoppers are used instead of rubber to further limit contamination.

The Synthetic Ocean Water (SOW) culture medium, Aquil, has been used in

previous studies where it is important to accurately predict the chemical speciation of the medium being used. It was primarily designed for studying trace metal physiology and nutrient requirements in algae (Price et al. 1989; Kuwabara 1980; Morel et al. 1975). The recipe for this medium has evolved and been improved over the course of the past few decades (Price et. al 1989). The Estuquil medium utilized in this experiment was specifically designed for this use, and was formulated to imitate the natural inorganic composition of estuary water where it exists along a natural (theoretical) estuarine mixing gradient at 20 parts per thousand dissolved solids. The name “Estuquil” was given to this medium by the author of this thesis because of its similarities to Aquil in its production and use.

Aquil, and its artificial freshwater counterpart, Fraquil, were used as endpoints in a theoretic mixing curve to determine the inorganic salt composition of the Estuquil medium. Figure 4 demonstrates how this curve is used to determine the concentrations of a salt such as Sodium Bicarbonate (NaCO_3) in Estuquil. Because natural estuarine water is composed of riverine inputs and a seawater sink, it is necessary to replicate the freshwater input in terms of its dissolved inorganic content as opposed to simply diluting SOW with de-ionized water, which may result in unrealistic levels of alkalinity and hardness.

Like its counterparts Aquil and Fraquil, Estuquil medium was prepared in a manner that replicated the inorganic components of natural estuarine water while eliminating potential organic contaminants. Its production was completed in five stages: (1) preparation of the salt and nutrient mixtures; (2) resin preparation; (3) purification of

To determine mass of NaHCO₃ at 20ppt.

$$10\text{g} - .63\text{g} = 9.37\text{g}$$

$$9.37\text{g} \times (20/34)\text{ppt} = 5.51\text{g}$$

$$5.51\text{g} + .63\text{g} = 6.14\text{g NaHCO}_3 \text{ at } 20\text{ppt.}$$

Figure 4. Estuquil Mixing Curve. The determination of concentrations of NaHCO₃ for Estuquil at 20 ppt. Synthetic riverine water (Fraquil) and synthetic seawater (Aquil) were used as endpoints.

the medium by column ion-exchange; (4) pH adjustment; and (5) final manipulation. All high density polyethylene (HDPE) and glass containers and apparatuses used in preparation of the medium had undergone the exhaustive glass and plastic washing procedure outlined in section B.

Chelex resin purchased from Bio-Rad Laboratories in Richmond, California was used for purification of the salt mixture and nutrient mixtures by passing the solutions through a column containing the resin. The resin is available in various mesh sizes with a mesh size of 100 recommended for purification of artificial culturing medium like artificial seawater (Price et al., 1989; Kuwabara, 1980; Morel et al., 1975). The selectivity of Chelex 100 for metals corresponds to those of iminodiacetic acid (Bio-Rad, 1978). Table 2 below lists the selectivity factors of several divalent cations. The selectivity factor is a quantitative measurement of the affinity that resin binding sites have for a particular ion with respect to a reference cation, Zn(II) in this case.

Table 2. Selectivity Factors of Divalent Cations for Chelex Resin (Source: Bio-Rad).

Ion	Selectivity Factor	Ion	Selectivity Factor
Hg ⁺²	1060	Fe ⁺²	0.130
Cu ⁺²	126	Mn ⁺²	0.024
UO ⁺²	5.70	Ba ⁺²	0.016
Ni ⁺²	4.40	Ca ⁺²	0.013
Pb ⁺²	3.88	Sr ⁺²	0.013
Zn ⁺²	1.000	Mg ⁺²	0.009
Co ⁺²	0.615	Na ⁺²	0.0000001

Ion	Selectivity Factor	Ion	Selectivity Factor
Hg ⁺²	1060	Fe ⁺²	0.130
Cd ⁺²	0.390		

(Table 2 continued)

The resin has an unusually high preference for the heavy metals such as Cu and Fe over cations such as Ca and Na, making it particularly useful for purifying solutions such as Estuquail. The resin is shipped in the sodium form, and its chemical structure consists of a cross-linked polystyrene matrix with iminodiacetic acid, $R-N(CH_2 COOH)_2$, as the functional group (Heitner-Wirguin and Markovits, 1963).

C 1. Salt and Nutrient Mixture Preparation.

The salt portion of Estuquail was prepared at double strength concentration (40 ppt) which aided in the ease of production and manipulation. The salt mixture was prepared in a 25 L High Density Polyethylene (HDPE) carboy equipped with spigot. First the carboy was filled to the 5 L mark with Milli-Q water and placed on a large magnetic spin plate. A large 5 cm Teflon coated magnetic spin bar was used to mix the solution. Reagent grade salts were weighed and added individually. Additions were made slowly, ensuring that the previous solute was fully dissolved before making any subsequent additions. Table 3 lists the major salts and gives the masses necessary to make a double strength solution (40 ppt). The last column gives final molar concentrations for Estuquail at 20 ppt (after dilution of the double strength salt solution).

The nutrient solution as previously mentioned was prepared separately. The

Table 3. Major Salt Composition in Estuquil.

Salt	Mass (grams) for 40 ppt. Solution	Molecular weight of salt	Total Salt Added (in Moles)	Final Estuquil Molarity
NaCl	721.4706	58.4427	12.3449	0.2469
MgCl ₂ ·2H ₂ O	326.4706	203.2998	1.6059	0.0321
Na ₂ SO ₄	120.2941	142.0355	0.8469	0.0169
CaCl ₂ ·2H ₂ O	46.0508	147.0156	0.3132	0.0063
KCl	20.5882	74.5510	0.2762	0.0055
NaHCO ₃	6.1418	84.0057	0.07311	0.0015
KBr	2.9412	119.0020	0.0247	0.0005
H ₃ BO ₃	0.8824	61.8307	0.0143	0.0003
SrCl ₂ ·6H ₂ O	0.0500	266.6148	0.0019	3.75E-05
NaF	0.08824	41.9882	0.0021	4.20E-05
MgSO ₄ ·7H ₂ O	0.7612	246.4800	0.0031	6.18E-05

nutrient stock solutions were prepared from reagent grade salts dissolved in Milli-Q water. Seventy-five grams of NaNO₃ was dissolved in 1 L Milli-Q water and 5.0 g of NaH₂PO₄·H₂O was dissolved in 1 L Milli-Q water. 500 mL of each of the stock solutions were then added together at an equal ratio (1:1) to make 1 L of the final stock solution mixture, called Nutrient Stock 2 (if a diatom species is to be grown in the medium, it is also necessary to add an available form of silica to the nutrient mixture). By adding 1 mL of Nutrient Stock 2 to 500 mL of Estuquil, the final desired Estuquil nutrient concentrations were achieved. The concentrations of the stock solutions as well as final Estuquil nutrient concentrations are in Table 4. The final concentrations are equal to

Guillard's f/2 culturing medium concentrations. Both salt and nutrient stock solutions should be stored in the dark under refrigeration until they are purified by column ion-exchange.

Table 4. Major Nutrient Concentrations in Estuquill.

Nutrient	Mass of Salt / L Stock 1	Concentration of Stock Solution (2)	Final Estuquill Concentration
NaNO ₃	75.0	0.4415 M	883 μ M
NaH ₂ PO ₄ ·H ₂ O	5.0	0.1815 M	36.3 μ M

C 2. Chelex Resin Preparation

It has been suggested that the use of Chelex 100 resin may leach organic substances during column use and collect in elutant solutions, therefore Price et al. have outlined a Chelex preparation procedure that removes residual organics from the resin prior to its use (Price et. al, 1989). The procedure for Chelex purification is outlined in Table 5 below.

Table 5. Chelex 100 Resin Purification Procedure.

(a)	Soak in HPLC-grade methanol for 3-4 hrs. At room temperature (w/v; 40 g : 200 mL); rinse with 750 mL Milli-Q water (Q-H ₂ O). In all steps it is suggested that a scintered glass filter is used.
(b)	Soak in 1 M HCl overnight at room temperature; rinse with Q-H ₂ O (1 L).
(c)	Soak in 3 M NH ₄ for 1 week at room temperature; rinse with Q-H ₂ O (1 L).
(d)	Soak in 0.1 M HCl for 10 min; rinse with Q-H ₂ O (2 L).
(e)	Rinse and suspend in solution to be purified, if nitrogen nutrition is being studied NH ₄ ⁺ concentrations should be measured.

(f)	Titrate slowly to desired pH with (1 M NaOH).
(g)	Rinse again with Estuquill and slurry-pack column (maintain 3 in. fluid head).
(h)	Discard the first 500 mL of elutant.

(Table 5 continued) Source: Price et al., (1989).

One additional step that has been suggested to those list above is to measure the pH of the solution entering the column and to monitor the pH of the elutant until pH in is equal to pH out (Morel et al. 1975). In this study, salinity was also used as an indication that the solution entering the column had purged any fluid occupying the resin pore space. Salinity was measured by refractometer and Estuquill was run through the column until salinity (inflow) was equal to salinity (outflow).

C 3. Solution Purification by Ion Exchange

When the column had been purified, slurry packed into the column, and both the major salt and nutrient solutions had been produced, the solution was then purified. The column used to purify the salt solution was a 1500 mL capacity Boro-silicate glass column (a large fritted column is recommended for this step). The column was placed in-line between the carboy containing the salt solution and a filtered Teflon receiving vessel that contained a washed 1 L Teflon bottle. A .45 μ M hydrophilic polycarbonate filter was also placed directly after the column to filter the solution. Figure 5 shows the complete column, filtering and receiving vessel. The receiving vessel was equipped with a vacuum fitting that allowed a slight negative pressure to be applied to the column. It is extremely important that the pressure not exceed 10 p.s.i. unless the column has been rated for a

Figure 5. Chelex 100 Purification Apparatus; Column and Vacuum Pressurized Teflon Receiving Vessel.

higher level. Excess pressure could cause the column to implode, resulting in a major set back and potentially dangerous situation. A fluid head of at least 1 inch should be maintained above the resin in the column, and the column never allowed to run dry.

Once the salt solution had passed through the column, and the elutant collected in the Teflon receiving bottle, it was immediately diluted with Milli-Q water at a ratio of 1:1 to achieve the desired salinity of 20 ppt. After dilution, the Estuquill solution was stored in washed HDPE bottles at 15° in the dark until use. The medium should not be stored over two weeks (Kuwabara, 1980). Because trace metal contamination is potentially a problem whenever performing ultra-trace clean experiments, the final solution was analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analysis revealed no significant contamination for the metals of primary concern, (i.e. Zn and Cd). Zn and Cd levels were below the limits of detection. Some Al contamination in the 26 part per million (ppm) range was detected, most likely a result Al leached from the glass column.

The nutrient solution was purified in the same manner, but it was prepared at full strength and not diluted after it passed through the column. The resin used to purify the nutrient solution was a separate batch from that used for the salt solution. In addition, it is not necessary to use a column of the volume used to purify the salt solution. In this experiment, a 500 mL fritted glass column was used to purify the nutrient stock solution.

C 4. pH Adjustment of Estuquill Medium

Depending on the type of application that the Estuquill medium will be used for, it

may require pH adjustment. In this study, experiments were conducted at a pH of 8.1, similar to the pH of San Francisco Bay surface water samples taken near the clam collection site. To adjust Estuquail pH, micro-liter additions of ultra-pure grade 0.5 M NaCO_3 were made to a 500 mL test aliquot of Estuquail medium, to predetermine the additions that would be necessary to reach the desired pH of 8.1. The unadjusted medium had a pH of approximately 7.1. Because the subsequent trace metal additions as well as the radio-tracer spikes that were made to the medium contained free hydrogen (H^+), this was also considered in adjusting pH. Therefore prior to adjusting the pH of the medium, all additions of trace metals and equivalent volumes of acid for the radio-tracer spikes were made to the 500 mL test aliquot of Estuquail. Measurements to determine pH were conducted using an Orion Research EA 920 expandable ion analyzer. Constant magnetic stirring took place during all pH measurements. Separate pH adjustments were conducted for the dissolved influx and assimilation media. Table 6 and 7 summarize additions made to the pH test media in each treatment, and the amount of 0.5 M NaCO_3 required to achieve a pH of 8.1. All pH adjustments were made as a final step just prior to the experimental exposures.

Table 6. Dissolved Influx Media pH Adjustments.

Solution	Addition to 500 mL Estuquail
trace metal stock solution (TM stock)	500 μL (actual TM stock)
H^+ equivalent for ^{109}Cd spike	30 μL of 0.5N HCl
H^+ equivalent for ^{65}Zn spike	200 μL of 0.1N HCl
vol. NaCO_3 required to achieve pH=8.1	225 μL of 0.5M NaCO_3

Table 7. Assimilation Efficiency Media pH Adjustments.

Solution	Addition to 500 mL Estuquil
trace metal stock solution (TM stock)	500 μ L (actual TM stock)
H ⁺ equivalent for ¹⁰⁹ Cd spike	30 μ L of 0.5N HCl
H ⁺ equivalent for ⁶⁵ Zn spike	200 μ L of 0.1N HCl
nutrient solution	1 mL (actual solution)
vitamin solution	500 μ L (actual solution)
Fe-CIT solution	50 μ L (actual solution)
vol. NaCO ₃ required to achieve pH=8.1	260 μ L of 0.5M NaCO ₃

C 5. Trace Metal Addition and Final Manipulation of Estuquil Medium.

Trace metal additions to Estuquil solution were based on two factors. First, it was important to realistically represent trace metal concentrations that are present in the San Francisco Bay. Second, it was important to have the necessary trace metals in the culturing medium to allow for maximum growth of algal cultures. Table 8 lists the final Estuquil trace metal concentrations present in Estuquil medium compared to the trace metal concentrations that are found in the artificial seawater culturing medium Aquil.

The trace metal stock solution (TM) was prepared with individual SPEX-ICP standard solutions of trace metal in 2% nitric acid solution. SPEX standards were added to the TM stock solution in concentrations that would allow a 1 mL addition of the TM stock solution to every 500 mL of Estuquil to yield the final concentrations in Table 8. Fe was added separately as a Fe-Citrate complex in the AE experiment only. The TM stock solution was made as a 1 L volume of the SPEX standard solutions diluted to volume

Table 8. Final Trace Metal Concentrations Estuqu coastal versus Aquil.

Trace Metal	Concentrations in Aquil	Concentrations in Estuqu coastal
Fe (AE only)	2.5E-05 M	2.0E-08 M
Zn	2.6E-07 M	9.2E-08 M
Mn	1.3E-06 M	1.8E-08 M
Co	1.5E-07 M	3.4E-09 M
Cu	6.3E-08 M	1.6E-09 M
Mo	9.6E-06 M	1.0E-07 M
Cd	NA	8.9E-09 M
Se	4.5E-07 M	1.1E-08 M

Source: (Aquil concentrations) Price et al. (1989).

with Milli-Q water. The TM stock was prepared in a 1 L volumetric flask and immediately transferred to a 1 L Teflon bottle.

Iron (Fe) presented a unique problem in the Assimilation Efficiency (AE) experiment. The standard Aquil recipe calls for Fe to be added as a Fe-EDTA complex to prevent the precipitation of $\text{Fe}(\text{OH})_3$ in solution (Price et al. 1989). However, the stability constant of the ligand to be studied (DDC) would favor a Fe-DDC complex to a Fe-EDTA complex, potentially rendering Fe unavailable to algae and limiting its growth (Morel and Hering 1993; Lubuda et al. 1984). In addition, the presence of EDTA could confound trace metal speciation calculations by out competing trace metal-DDC complexes. To address these potentially confounding factors, Fe was added to solution as an Iron-Citrate complex (Phinney and Bruland, 1994). Fe additions were not made in the Dissolved Influx exposures.

D. Chemical Speciation Modeling in Estuquail Medium

HYDRAQL, a program for the computation of chemical equilibria in aqueous batch systems, was used for all equilibrium calculations in Estuquail medium.

HYDRAQL is an enhanced version of the program MINEQL which was designed to incorporate Surface Complexation Models and enhanced error handling routines in the computation of equilibrium speciation in aqueous systems (Papelis, Hayes and Leckie 1988). The program calculates species equilibria in solution based on inputs, by the user, of the free concentrations of the components that define the system being investigated (Papelis et al. 1988). Equilibrium calculations based on the conditional stability constants of the major species in solution estimate the concentrations of the components. A series of several iterations “improves” the solution until the mass balance for all components is satisfied (Papelis, Hayes and Leckie 1988). A full description of the capabilities of HYDRAQL can be found in Technical Report No. 306, September 1988, Department of Civil Engineering at Stanford University by Papelis, Hayes and Leckie.

Because Estuquail is chemically defined, concentrations of its components can be put into the HYDRAQL input file, where the concentrations of the major species are then calculated. In the experimental treatments where DDC was added, at least 98 percent of the metals being studied (Cd and Zn) were bound as Me-DDC complexes and in most cases the percentage was close to 100. DDC was present at a final concentration of 10 μM in Estuquail. Table 9 and 10 summarize the percentage distribution of Cd and Zn species in the control and DDC exposures for the Dissolved Influx experiment as calculated by HYDRAQL. Table 11 and 12 summarize the percentage distribution of Cd

and Zn species in the control and DDC exposures for the Assimilation Efficiency culturing medium. Percent of total refers to the percent of the listed species relative to the total amount of the specific trace metal added. For Cd, the total added was 8.9×10^9 M;

Table 9. Percent Distribution of Cd and Zn in Control Exposure (Dissolved Influx).

Component	Species	% of Total
Cd^{2+}	Cd^{2+}	5.1
	CdCl^+	47.8
	CdCl_2	42.1
	CdCl_3^-	3.5
Zn^{2+}	Zn^{2+}	45.2
	ZnCO_3	9.3
	$\text{Zn}(\text{CO}_3)_2^{2-}$	2.5
	ZnSO_4	8.4
	ZnCl^+	11.4
	ZnCl_2	2.1
Zn^{2+}	$\text{Zn}(\text{OH})^+$	2.4
	$\text{Zn}(\text{OH})_2$	2.6
	$\text{Zn}(\text{OH})\text{Cl}$	12.9
	ZnHCO_3^+	1.4

Table 10. Percent Distribution Cd and Zn in DDC Exposure (Dissolved Influx).

Component	Species	% of Total
Cd^{2+}	$\text{Cd}(\text{DDC})_2$	99.6
Zn^{2+}	$\text{Zn}(\text{DDC})_2$	98.2

Table 11. Percentage Distribution Cd and Zn in Control Exposure (AE Culturing Media).

Component	Species	% of Total
Cd ²⁺ (Cit = Citrate)	Cd ²⁺	4.9
	CdCl ⁺	45.9
	CdCl ₂	40.4
	CdCl ₃ ⁻	3.3
	CdCit ⁻	3.2
Zn ²⁺	Zn ²⁺	9.5
	ZnCO ₃	2.0
	ZnSO ₄	1.8
	ZnCl ⁺	2.4
	FeZnCl(OH) ³⁺	2.7
	ZnCit ⁻	78.1

Table 12. Percent Distribution Cd and Zn in DDC Exposure (AE Culturing Media).

Component	Species	% of Total
Cd ²⁺	Cd(DDC) ₂	99.9
Zn ²⁺	Zn(DDC) ₂	99.2

for Zn the total was 9.2×10^{-8} M. Difference between the sum of all of the species and 100 percent is due to computational error in HYDRAQL iterations. The complete list of percentage distribution of all components in solution are located in Appendices 1 and 2

E. Animal Collection and Acclimation

Bivalve clams of the species *Potamocorbula amurensis* were collected from South

San Francisco Bay on October 19, 1998 at a location just south of the San Mateo Bridge on the southwest edge of the main channel, from the U.S. Geological Survey vessel, The Frontier, a seventeen foot Boston Whaler. Figure 6 shows the approximate site of collection. Clams were collected at an approximate depth of three meters of water in high tide conditions. Salinity measured 23.5 parts per thousand (ppt) at a water temperature of 17.2° C. Animals were retrieved from their benthic habitat where they orient themselves approximately 5 mm in depth into surface sediments so that they are able to feed and respire via their exposed siphons.

To collect the clams, a Van Veen type stainless steel jaw-type grab was lowered into the water, the device is tripped when it hits the bottom. Upon retrieval, the jaws close shut and dig approximately 15 centimeters into the bottom surface sediments which are retrieved with the grab. Once on board, sediments were washed through a 5 mm mesh screen exposing the *P. amurensis* (and other material >5 mm) so that they would be easily gathered. Figure 7 shows the collection procedure, and a sub-sample of the animals collected is shown in figure 8. Two to three hundred animals were initially collected and placed into 5 L of San Francisco Bay water also collected from the site.

Once the clams arrived at the U.S. Geological Survey laboratory, they were cleaned and acclimated. First the animals were rinsed thoroughly with seawater diluted to 20 ppt and loose sediment and organic matter was scrubbed from the shells with the use of a tooth brush. Clams were then placed in an aquarium containing filtered seawater obtained from Long Marine Laboratory in Santa Cruz California that had been diluted to 20 ppt with Milli-Q water. The aquarium was heavily aerated with an air stone, and

Figure 6. Location of Animal Collection From South San Francisco Bay.

Figure 7. *P. amurensis* Benthic Collection Procedure. Benthic sample contained in Van Veen grab being emptied into box sieve with 5 mm screen mesh.

Figure 8. *P. amurensis*.

temperature remained at 20°C within a constant temperature room.

Animals were then fed and acclimated in Estuquil medium. The clams were fed once per day with 100 mL aliquots of *Rhodomonas salinas* algae cultures that had been grown in 20 ppt seawater containing F2 nutrient concentrations under fluorescent lighting for 3 to five days or until culture became a deep reddish hue. Daily feeding occurred until 48 hours before the experimental exposures, allowing time for gut depuration. Forty-eight hours prior to experimental exposures, the animals were again scrubbed gently but thoroughly to remove all remaining surface bound sediment and organic matter. The clams were then placed in clean containers filled with 50% Estuquil medium and 50% 20 ppt filtered seawater mixture. At 24 hours prior to the experimental exposures, the clams were placed in 100% Estuquil medium.

To ensure healthy experimental subjects, the time from collection to experimental exposure should not exceed 5 days. In addition all manipulations of the animals should be done quickly and gently to limit their disruption.

F. Influx and Efflux from the Dissolved Phase

To determine the influx rates of Cd and Zn in *P. amurensis*, clams in the 13-14 millimeter size range were exposed for four hours to 1 µg/L Cd and 6 µg/L Zn. The experimental medium, Estuquil, was prepared per the method outlined in section C above. Thirteen replicates each consisting of three animals were used in both the control and DDC exposures. Exposures took place in 2.5 L Pyrex glass beakers that had undergone the washing procedure outlined in Section B. Two liters of pH adjusted, 20

ppt Estuquail medium, spiked with TM stock solution was placed into two beakers for the exposures. Both vessels were spiked with 10 μCi of carrier free ^{109}Cd and 15 μCi of carrier free ^{65}Zn to be used as tracers for stable metals. Radioisotopes were added to the exposure medium 12 hours before the experiment to allow stable metals to equilibrate with the isotopes. The DDC exposure received 100 μL per L of 0.1 M DDC stock solution to yield a final concentration of 10 μM DDC in the exposure media. The DDC stock solution was prepared by dissolving 2.25 g of sodium-dimethyldithiocarbamate (Fluka Chemicals) in 5 mL of HPLC grade methanol and topping off the remaining 95 mL of a 100 mL volumetric flask with Milli-Q water. All media manipulations and experimental exposures took place under a laminar air flow hood in a constant temperature room at 20°C. Experimental exposure chambers (beakers) were very gently aerated during the exposures to continually mix the media.

After 3.5 hours of exposure, 3 clams were removed from each treatment, and assayed for whole body and tissue activity. This preliminary assay was conducted to determine if 4 hours of exposure would result in enough activity to conduct the subsequent 21 day efflux that was to follow. Whole body activity counts ranged from approximately 35,000 disintegrations (dpm) to 316,000 for ^{109}Cd and 271,000 to 678,000 dpm for ^{65}Zn . At least half of this activity was in the tissues, determined by dissection and subsequent analysis. From these levels, it was determined that enough activity existed for the subsequent efflux experiment, which would only require approximately 3500 dpm (whole body) activity to allow detectable levels of radiation after three weeks of efflux considering average efflux rates (Lee, Wallace and Luoma 1998).

The remaining twelve replicates in each exposure were used for influx and efflux determinations. At the end of four hours of exposure time, the clams were immediately placed in 2 L of unlabeled Estuquill for 20 minutes to rinse loosely bound metals from the shell and gill surfaces. Immediately after the 20 minute short depuration period, all groups were assayed for whole body activity and four replicates from each exposure were immediately sacrificed to obtain shell and tissue counts at time zero ($t=0$). The remaining animals were placed in a single 20 gallon efflux chamber equipped with continuous activated charcoal filtration. The efflux chamber was filled with filtered seawater diluted to 20 ppt with de-ionized water. Clams were fed cultures of *Rhodomonas salinas* daily over the course of the next 21 days. Whole body activity measurements were made at approximately 4, 20, 70, 100, 120, 150, 250, 300, 400, and 500 hours after the initial exposure. Of the 8 remaining replicates from each exposure, 3 were sacrificed at day 6 and the remaining replicates at day 21 for efflux calculations.

F 1. Dissolved Influx Calculations

Dissolved influx calculations were based on raw tissue activities after the four hour exposure. Tissue activities in disintegrations per minute (dpm) were multiplied by 1/6 day, then multiplied by their respective wet weights yielding dpm/d/g for ^{109}Cd and ^{65}Zn where d is one day, and g is the wet tissue weight in grams. Next, dpm for ^{109}Cd and ^{65}Zn were converted to mass of stable Cd and Zn. Three 5 mL samples of exposure media were taken from each exposure vessel before, and immediately after the exposure, the results of the activities was averaged, and converted to total dpm/L. Because the amount

of stable metal added was known (1 $\mu\text{g/L}$ Cd and 6 $\mu\text{g/L}$ Zn), the mass of each metal per dpm could be calculated resulting in $\mu\text{g metal/dpm}$. The final influx rate was determined by the following equation:

$$\mu\text{g metal/g/d} = (\text{dpm metal/g/d}) \times (\mu\text{g metal/dpm})$$

The final trace metal influx rate was described as $\mu\text{g metal/g/d}$ and was calculated for both Cd and Zn.

F 2. Efflux Rate Calculations

The efflux rates characterize the loss of assimilated metal from the tissues. In the case of this experiment, efflux calculations were carried out on those animals that had been exposed to metal in the dissolved phase. Efflux rates were determined on clams from both the control and DDC exposures and compared. To obtain efflux rates, whole body activity counts carried out over the 21 day period were corrected for decay to time zero ($t=0$; immediately after exposure). Clams were dissected at $t=0$, $t=6$, and $t=21$ days to determine the proportion of radioactivity partitioned in the tissues. Prior to the dissections, whole body assays were taken. Tissue and shell measurements taken after the dissections were used to calculate tissue/whole ratios. By regressing these ratios against time (in days), estimates in the temporal changes of radioactivity in the soft tissues were made. Linear regressions were then made between the natural log transformed fraction of retained metal and depuration time. The physiological loss of metals from the tissues

follows a first order exponential function:

$$C_{(t)} = ae^{kt}$$

where $C_{(t)}$ is the natural log (ln) of the fraction of metal retained in the tissues at time (t), a is a constant, k is the rate constant of loss, and t is time (in days) (Reinfelder et al. 1997). Regressions were performed for both Cd and Zn effluxes from clams in each treatment.

G. Assimilation Efficiency (AE)

Assimilation Efficiency (AE) can be defined as the percent of an ingested element that crosses an animal's gut lining (Reinfelder et al., 1997). AE is important because it is believed to be one of the key parameters needed to quantify trophic transfer (Reinfelder et al., 1997). In this experiment, the filter feeding bivalve, *P. amurensis*, was fed radio labeled cultures of *Rhodomonas* (*sp. unknown*) for 1 hour, then allowed to depurate for 48 hours at which point the metal retained within the soft tissues was considered assimilated. Algae were grown in the chemically defined medium Estuquill.

G 1. Algae Culturing

Algal cultures preparation began 6 days prior to the AE experimental exposures. *Rhodomonas salinas* cultures were grown in 20 ppt seawater with f/2 concentrations of trace metals and nutrients for three days under fluorescent lighting. One hundred mL of

the culture was filtered onto a 0.45 μm polycarbonate filter under 10 psi vacuum pressure. The filter was then placed into a 250 mL HDPE centrifuge vessel containing 200 mL of Estuquail medium (no TM stock added) and shaken vigorously to suspend the cells. Cells were centrifuged at 3000 x gravity (g) for 10 minutes, the supernate decanted, then re-suspended in Estuquail and spun a second time at 3000 x g in a Sorvall RC 5C Plus Centrifuge. These cells were used as the seed culture for the AE experiment.

When the seed culture was ready, the Estuquail algal culturing medium was prepared. The culturing medium was prepared in a 1 L batch with TM stock additions identical to the dissolved influx exposure with the addition of Fe added as a Fe-Citrate complex. Final Fe and Citrate concentrations in the medium were 20 nM and 300 nM respectively. The seed culture was added to the medium by placing 100 mL of Estuquail medium into the centrifuge vessel and shaking vigorously until the cells were fully suspended, then mixing the cells with the remaining 900 mL of Estuquail. The inoculated medium was then divided into 2 separate 500 mL aliquots. Both treatments received 3 μCi of ^{109}Cd and 6 μCi ^{65}Zn to be used as radiotracers for stable metals. The DDC treatment was spiked with 50 μL DDC stock solution, the final DDC concentration was 10 μM . Cells were placed under fluorescent lighting and allowed to divide for 3 days (6 generations) to ensure uniform labeling.

Periodic assays of 5 mL aliquots of culturing medium were taken to verify that cells were no longer taking up radio tracer. The aliquots were taken and the cells filtered onto a 0.33 μm polycarbonate filter. Filtered cells were then washed in with 5 mL of a 0.1 M sodium-EDTA solution followed by 10 mL of Estuquail to remove loosely bound

metal from the cell surfaces.

Labeled cells were then prepared for the feeding experiment. Two hundred mL from each culture were centrifuged in two stages both at 1912 x *g*. Cells were washed and resuspended in Estuquil each time, and the supernate was decanted after each spin. After the second rinse and spin, cells were resuspended in 200 mL of fresh Estuquil solution. The cells were then ready to be fed to the clams.

G 2. Feeding

Two groups 9 of replicates (each replicate containing 3 animals) were used in the AE experiment. The clams were placed in 200 mL of unlabeled 20 ppt Estuquil solution in a 2.5 L glass beaker under a laminar air flow at 18°C. The 200 mL of Estuquil containing the labeled cells was then poured into the respective beakers, one containing cells labeled in the DDC solution and the other, the control. Clams were allowed to feed for 1 hour.

Immediately after feeding, 4 replicates were immediately dissected to obtain tissue and shell activities. The remaining 5 replicates were placed into a depuration chamber within 150 mL beakers for feces collection for the next 48 hours.

To correct for any radioactivity that may have been obtained from the dissolved phase in the feeding solution, a separate exposure was conducted. Two hundred milliliter aliquots taken from the remaining initial culturing medium were used. The cells underwent centrifugation and washing identical to the cells used for feeding. However, one last centrifugation was conducted (3 total) on the Estuquil containing the washed

cells. In this case, the supernate from each treatment was mixed with 200 mL of unlabeled Estuquill containing 3 replicates of clams in two clean beakers. The total exposure time was 1 hour. After the 1 hour dissolved exposure, the clams were immediately dissected and assayed for shell and tissue activity.

G 3. Depuration Period

Depuration of clams exposed to radioactive cells occurred for 48 hours, 24 hours longer than the gut residence time of *P. amurensis* which has been placed at approximately 24 hours (Decho and Luoma 1991). During this time clams were removed from the chamber periodically (at 1, 2, 3, 5, 8, 12, 18, 24, and 48 hours), and assayed for whole body radioactivity. Each time clams were assayed for whole body radioactivity, feces that had been expelled up until that time, were also assayed. Feces were collected by pouring the contents of the 150 mL beakers in which the clams were contained, through a plastic tube equipped with removable 20 μ m mesh. The mesh was then removed, and folded carefully so as not to lose any particles, and placed into a scintillation vial that was analyzed for radioactivity. Figures 9 shows the depuration chamber and figure 10 is a photograph of the feces collection vessels.

G 4. Assimilation Efficiency Calculations

Absorption Efficiency (AE) was calculated using the following two methods. Method 1 employs a mass-balance approach to determine the initial ingested activity, as follows:

Figure 9. Depuration chamber used for 21 day efflux. Chamber is equipped with an activated charcoal filtration system to absorb metals lost during efflux.

Figure 10. Feces collection chamber and individual feces collection beakers. Clams are place into individual beakers during depuration. Feces are removed periodically by pouring beaker contents through 20 μ M mesh which is then radio-assayed.

$$\%AE_I = \frac{(C_{24} - C_{diss})}{[(\text{sum } C_{feces}) + (C_{24} - C_{diss})]} \times 100 \quad (1)$$

where C_{24} is dpm in *P. amurensis* soft tissue after 24 hours of depuration, C_{diss} is dpm in soft tissue after 1 hour of uptake from the dissolved exposure (Cd alone and complexed with DDC) and sum_{feces} is the sum of all activity in feces collected during the 24 hour depuration period (Lee and Luoma 1997b).

The second method for calculating assimilation efficiency (AE) estimates the initial activities from the whole body analysis at $t=0$ corrected for the activity associated with the shell. The shell correction is made by multiplying the ratios of tissue/tissue + shell activities obtained from dissected clams at $t=0$. In this equation, AE is defined as:

$$\% AE_{II} = \frac{(C_{24} - C_{diss})}{(W_{t=0}^* - C_{diss})} \times 100 \quad (2)$$

Where $(W_{t=0}^*)$ is the whole body activity corrected for shell activity, and the remaining variables are the same as those defined for equation 1.

H. Subcellular Metal Distribution

Subcellular distributions of Cd and Zn were analyzed from clams used in both the DI and AE experiments. For the clams from the DI experiment, 4 replicates each containing 2 clams were analyzed from both the control and DDC exposures at $t = 0$ (no depuration). A second group containing 3 replicates (2 clams each) was taken from both

the control and DDC exposures (DI experiment) that had undergone $t = 6.06$ days of depuration. For the AE clams, 4 replicates each containing 2 clams were analyzed from both the control and DDC treatments taken immediately after feeding ($t = 0$ days) and 4 replicates each containing 2 clams were analyzed from both the control and DDC treatments taken after 48 of depuration ($t = 2$ days).

Subcellular Cd and Zn distributions were obtained for all replicates in the same manner. Clams from the DI and AE experiments had been frozen at -70°C at the end of the experiments, therefore prior to obtaining fractions, clam tissues were slowly thawed from their deep frozen state by being placed on ice. Once thawed, the clams were homogenized in 0.5 mL of Tris-HCl buffer solution (preadjusted to a pH of 7.6) by a high speed electric homogenizer. Homogenate from each replicate was then fractionated according to the procedure outlined in Figure 11. At each step in the procedure activity measurements were taken to determine the dpm from Cd and Zn isotopes. Homogenized clams were fractionated by centrifugation at $300 \times$ gravity (g) for 15 minutes at 4°C . The pellet obtained from the first centrifugation contained tissue fragments and cellular debris [(membranes, setae, and metal rich granules (MRG))] (Wallace, Lopez and Levinton 1998). MRG were isolated from the tissue fragments by suspending the pellet (P1) in 0.5 mL 1 N NaOH and heating at 100°C for 1 hour to dissolve the tissue (Wallace Lopez and Levinton 1998). MRG were collected by a second centrifugation at $5000 \times g$ for 10 minutes at 20°C . The supernate from this second centrifugation (S3) contains the tissue fraction.

The supernate from the initial $300 \times g$ (S1) centrifugation were further

Figure 11. Procedure for Obtaining Subcellular Fractions.

fractionated to obtain the intracellular, heat stable protein, and non heat-stable protein fractions. This was accomplished by centrifuging S1 at 100,000 x g for 1 hour at 4°C which produces an intracellular pellet (P2) containing nuclear, mitochondrial, and microsomal fractions, the supernate (S2) contained the cytosol and incorporated proteins (Wallace, Lopez and Levinton 1998). The cytosol fraction (S2) was then fractionated into heat stable (metallothionein-like) proteins and heat sensitive or non heat-stable proteins. The cytosol S2 was heat denatured by being heated at 80°C for 10 minutes, then subsequently chilled on ice for 1 hour (Wallace, Lopez and Levinton). Proteins were then separated by centrifugation at 50,000 x g for 10 minutes. Final activities of all fractions were summed to determine percent recoveries of intact homogenate assayed prior to obtaining fractions.

Concentrations of metal were obtained by measuring gamma emission of ^{109}Cd ^{65}Zn and converting dpm to nanograms (ng) of metal, and normalizing metal mass per wet tissue weights of the clams.

Obtaining subcellular metal distribution was important for two major reasons. The first, was to test the hypothesis that the addition of DDC would increase the amount of metal that was found in the debris fraction that contains the cellular membrane. In addition, metallothionein-like proteins and metal-rich granules have been found to play a role in the detoxification of metal from several aquatic organisms, therefore the second reason for assessing subcellular metal distributions was to determine if DDC had an effect on the compartmentalization of metal in these fractions (Simkiss 1981; Viarengo et al. 1987; Roesijadi 1992; Wallace and Lopez 1997).

I. Statistical Analysis

Where it was necessary to conduct a statistical analysis on values obtained experimentally, a statistical t -test was employed. The t -test was conducted to establish significance (or insignificance) between a treatment parameter (μ). The parameter analyzed was generally the experimentally determined mean value obtained for replicate groups within a given treatment. Determination of the difference between means were described by an equation for the two-tailed hypothesis to be tested: $H_0: \mu_1 - \mu_2 = 0$ and $H_2: \mu_1 - \mu_2 \neq 0$. In all statistical analysis, determination of significance was made at a 95% confidence interval.

IV RESULTS

Dissolved Influx

Initial analysis of clams exposed to metals from the dissolved phase revealed significant differences between the control and DDC treatments. Based on average whole body activity, clams exposed to the DDC treatment had 10 times the activity of the control group for Cd and had accumulated approximately 3 times the Zn. However, whole body activity measurements can be confounded by the “shielding effect” of the shell and vary greatly depending upon the energy of the decaying particle of a particular isotope. Therefore, it was critical to examine the activity that resided within the tissue component of the animals, and use these actual (or estimates of) measurements as points of comparison. The average tissue activities for the clams exposed to the DDC treatment were approximately 25 times higher than those in the control treatment for Cd and approximately 10 times greater in the DDC treatment for Zn. Table 13 summarizes the dpm (corrected for background activity) in the control and DDC exposures for the dissolved influx experiment for both ^{109}Cd and ^{65}Zn . The values in Table 13 are the whole clam activities as well as the activities obtained from the dissected components (shell and tissue) for four replicate groups. The designations C1-C12 and D1-D12 stand for the replicate samples in each exposure (12 replicates in each). Each replicate, consists of three animals. Only 4 replicates (C9-C12 and D9-D12) from each exposure were dissected immediately after influx; the remaining groups were placed into the depuration chamber.

Table 13. Total Radioactivity in clams (in dpm) Immediately After Dissolved Exposure

CONTROL	Cd			Zn		
	whole clam	shell	tissue	whole clam	shell	tissue
C1	35094.4	x	x	273505.1	x	x
C2	34995.8	x	x	275705.1	x	x
C3	28161.4	x	x	238145.8	x	x
C4	29560.0	x	x	251549.2	x	x
C5	28997.5	x	x	221805.1	x	x
C6	30192.6	x	x	214067.8	x	x
C7	33236.5	x	x	242698.3	x	x
C8	39856.1	x	x	257093.2	x	x
C9	30168.4	16504.9	26880.4	231347.5	134166.1	57745.8
C10	32497.5	23293.7	27155.1	244211.9	158715.3	56232.2
C11	28943.5	13114.7	31796.5	214364.4	115062.7	64696.6
C12	37974.0	24038.9	30544.2	240440.7	157932.2	49284.7
<i>avg</i>	32473.2	19238.1	29094.0	242077.8	141469.1	56989.8
<i>std</i>	3659.6	4595.2	2125.2	19441.0	18159.7	5475.1
DDC TX	Cd			Zn		
	whole clam	shell	tissue	whole clam	shell	tissue
D1	213470.2	x	x	501593.2	x	x
D2	270014.7	x	x	743333.9	x	x
D3	276293.0	x	x	642533.9	x	x
D4	223840.7	x	x	599254.2	x	x
D5	336106.0	x	x	797432.2	x	x
D6	308997.5	x	x	781106.8	x	x
D7	217794.7	x	x	692676.3	x	x
D8	354220.4	x	x	834706.8	x	x
D9	361576.5	24741.4	841639.6	1012869.5	143315.3	659408.5
D10	372371.2	20103.5	898009.8	955076.3	115383.1	670940.7
D11	382133.7	20336.1	806885.6	806778.0	110213.6	551264.4
D12	217508.1	20943.5	453119.3	516854.2	116874.6	331725.4
<i>avg</i>	294527.2	21531.1	749913.6	740351.3	121446.6	553334.7
<i>std</i>	63279.6	1878.6	174412.5	152020.4	12865.5	136196.3

The large differences in the tissue activities relative to those obtained for the whole body measurements can be seen in the ratios obtained from the 4 groups of animals immediately dissected after the exposure. Tissue activities were divided by the sum of the shell plus the tissue activities ((tissue/(shell+tissue))) to yield tissue to whole body ratios. In the control treatment, 60.6% of the total activity resided in the tissue for Cd, and 29.0% for Zn. In the DDC exposure, 97.0% of the total activity was in the tissues for Cd, and 81.2% for Zn.

Dissolved influx rate calculations where metal uptake rates were normalized for actual wet tissue weights and expressed as micrograms of metal per gram wet weight per day ($\mu\text{g me/g/d}$) were consistent with the initial activities obtained from tissues. Figure 12 summarizes the influx rates from the dissolved phase for Cd and Zn for both the control and DDC treatments. Metal Influx rates for the DDC exposure were approximately 25 and 10 times greater than those seen for the inorganic (control) exposure for Cd and Zn respectively.

The activities of 5 mL aliquots of the exposure media taken before and after the dissolved exposure, indicated that no substantial loss of activity occurred (< 5%) from the media during the experiment. Table 14 summarizes the activities of media samples taken just before and just after clam exposures. A significant reduction in metal concentrations in solution could have confounded influx rate calculations, which were obtained for fixed concentrations.

Figure 12. Dissolved Influx of Cd and Zn from the Dissolved Phase - Inorganic Versus DDC Metal Complex. Rates are expressed as the mass of metal (μg) entering the tissues per day. DDC complexed Cd and Zn are taken up 25 and 10 times faster than the control.

Table 14. Radioactivity (in dpm) of 5 mL Aliquots of Exposure Media from Dissolved Influx Treatment Before and After Exposure

time = 0	Control - Cd	Control - Zn	DDC -Cd	DDC -Zn
avg. (N=3)	1.65e+04	1.80e+04	1.58e+04	1.68e+04
std.	1.28e+02	7.86e+02	2.12e+02	6.89e+02
time = final				
avg. (N=3)	1.65e+04	1.70e+04	1.52e+04	1.62e+04
std	3.88e+02	1.19e+02	1.67e+02	1.90e+02

Efflux Rate of Metals Taken Up from the Dissolved Phase

The efflux of metals from the dissolved exposure was carried out over a 21 day period. Declining condition and mortalities experienced after day 7 made it difficult to calculate efflux rates over the entire 21 day period, therefore several methods were used to calculate and compare efflux rates between the control and experimental treatments. Figures 13 and 14 show the general loss patterns exhibited by whole clams during efflux of Cd and Zn. The largest difference between the two treatments occurred in the first three days, where the rapid losses seen from the dissolved metal treatment exceeded losses in the DDC treatment on a percentage basis. The pattern of an accelerated initial loss over the first three days occurred for both Cd and Zn. This is visible in figures 13 and 14.

In general, the loss of radioisotopes during efflux followed the two-compartment exponential model described in previous studies (Fisher et al. 1996). An initial rapid release was seen in the first three days, followed by a slower release that continued

Figure 13. Efflux of Cd from Whole Clams. Actual whole body activities of ^{109}Cd were measured during a 21 day loss period. Points represent percentage of disintegrations per minute (dpm) that have been retained from the end of the initial influx period.

Figure 14. Efflux of Zinc from Whole Clams. Actual whole body activities of ^{65}Zn were measured during a 21 day loss period. Points represent the percentage of disintegrations per minute (dpm) that have been retained from the end of the initial influx period.

throughout the depuration period. The initial rapid release occurs primarily from fecal pellets during gut clearance if the uptake occurred from food, or desorption of radioisotopes from the shell surface if uptake had occurred from the dissolved phase (Fisher et al. 1996). Efflux calculations are generally based on this second, more slowly exchanging pool (Fisher et al. 1996; Reinfelder et al. 1997). The rapid initial loss is clearly seen in figures 13 and 14. In these figures, the initial loss (on a percentage basis), of the metal from the control group exceeded that found in the DDC treatment. In the case of Cd, approximately 75% of the total activity in the shell fraction was lost in the first 6 days of efflux for the control treatment, where in the DDC treatment, no loss of activity was seen from the shell fraction during the same time period. In addition, the ^{109}Cd activity associated with the shell fraction was approximately 58% of the total clam activity in the control treatment while the shell activity only accounted for approximately 6% of the total clam activity in the DDC treatment. The differences in shell to total activity seen amongst the two treatments, as well as the larger decline in activity seen from the shell fraction in the control treatment, account for the major differences seen in the whole clam efflux plots (Figures 13 and 14).

Figures 15 and 16 show the least squares regression fits for whole body efflux of Cd and Zn for the slowly exchanging pools (2.85-21 days) obtained from whole clam activities for this study. Whole body efflux rates for Cd were between 2.6-2.9% per day with no significant differences between the control and DDC treatments. Whole body efflux rates for Zn were approximately 4% per day for both treatments. Whole body efflux rates are generally not as accurate as tissue efflux rates, however, these rates were

Figure 15. Whole Clam Efflux Rates of ^{109}Cd for Slowly Exchanging Pools of Isotopes.

Figure 16. Whole Clam Efflux Rates of ^{65}Zn for Slowly Exchanging Pools of Isotopes.

used here to demonstrate the similarity in the rates found for the two treatments.

The physiological loss rates of metals from soft tissues are of the most importance, because these rates represent the loss of metal that could potentially disrupt the health of the organism. To estimate the tissue activities based on the whole body activities, it was necessary to periodically dissect organisms during efflux to obtain the ratios of tissue to whole body activity. Clams were dissected at 3 stages: immediately after influx, after 6 days of depuration, and after 21 days of depuration. Ratios of tissue to whole body activity were then applied to the remaining intact clams to estimate tissue activities. Tables 15 and 16 show the change in the ratio of tissue/whole clam activity during the course of efflux for Cd and Zn respectively.

For Cd, the average ratio of tissue/total activity was 48.2% immediately after influx, 59.9% after approximately 6 days of efflux and 78.6% after 21 days of efflux (for the control exposure). With respect to Cd, the DDC exposure had 118.6% of the total activity in the tissue immediately after influx, 75.3% after 6 days of efflux and 89.2% after 21 days of efflux. It is possible for the tissues to have percentages exceeding 100% for cadmium, because the gamma ray emitted from the decaying isotope can be “shielded” by the shell of the organism when the animal is intact. For Zn, the activity distributions for the control group were more consistent. The control group maintained approximately 25% of the total activity within the tissue component during the course of efflux, and the DDC group started at 63.5% activity in the tissues after influx then declined to 52.6% at day 6 , and 49.5% at day 21.

The condition of the animals was also measured during the course of the 21 day

Table 15. Distribution of Activity in Clams (in dpm) During Efflux of Cd

Control							
Rep #	Total Activity	Tissue Activity	Shell Activity	% tissue/total	% recovery	avg % tiss/total	Days of Efflux
9	56837.3	26880.4	31095.3	47.3	102.0		0
10	61225.4	27155.1	43885.3	44.4	116.0		0
11	54529.6	31796.5	24708.2	58.3	103.6		0
12	71543.1	30544.2	45289.4	42.7	106.0	48.2	0
5	24397.5	14451.9	4883.2	59.2	79.3		6
7	23138.8	14803.6	4773.5	64.0	84.6		6
8	19877.9	11236.6	5358.5	56.5	83.5	59.9	6
1	12374.2	10631.0	2528.5	85.9	106.3		21
6	7797.8	5564.1	1693.0	71.4	93.1	78.6	21
DDC							
Rep #	Total Activity	Tissue Activity	Shell Activity	% tissue/total	% recovery	% avg tiss/tot	Days of Efflux
9	681210.1	841639.6	46612.8	123.6	130.4		0
10	701547.4	898009.8	37875.0	128.0	133.4		0
11	719939.9	806885.6	38313.3	112.1	117.4		0
12	409785.2	453119.3	39457.6	110.6	120.2	118.6	0
6	457340.7	318255.9	57130.8	69.6	82.1		6
7	281776.3	224322.3	50382.8	79.6	97.5		6
8	484254.8	370952.3	60704.5	76.6	89.1	75.3	6
2	216215.1	210198.9	39871.4	97.2	115.7		21
3	169888.5	137800.4	23371.5	81.1	94.9	89.2	21

Table 16. Distribution of Activity in Clams (dpm) During Efflux of Zn

Control							
Rep #	Total Activity	Tissue Activity	Shell Activity	% tissue/total	% recovery	avg % tiss/total	Days of Efflux
9	243840.2	57745.8	64150.7	23.7	50.0		0
10	257399.3	56232.2	62469.3	21.8	46.1		0
11	225940.1	64696.6	71872.5	28.6	60.4		0
12	253424.5	49284.7	54751.2	19.4	41.1	23.4	0
5	55013.4	15567.0	17293.7	28.3	59.7		6
7	65903.6	16887.2	18760.3	25.6	54.1		6
8	65362.3	14956.1	16615.0	22.9	48.3	25.6	6
1	28461.6	8021.1	8910.8	28.2	59.5		21
6	17423.2	4591.2	5100.4	26.4	55.6	27.3	21
DDC							
Rep #	Total Activity	Tissue Activity	Shell Activity	% tissue/total	% recovery	% avg tiss/tot	Days of Efflux
9	1067564.4	659408.5	151054.3	61.8	75.9		0
10	1006650.4	670940.7	121613.7	66.7	78.7		0
11	850344.0	551264.4	116165.1	64.8	78.5		0
12	544764.4	331725.4	123185.8	60.9	83.5	63.5	0
6	312298.4	172427.3	52791.1	55.2	72.1		7
7	228251.7	104167.4	54863.4	45.6	69.7		7
8	309175.7	175825.1	49698.8	56.9	72.9	52.6	7
2	116758.2	59729.6	29871.1	51.2	75.4		21
3	127530.4	60989.1	27125.3	47.8	69.1	49.5	21

efflux period. Condition was assessed by measuring the wet tissue weight per unit of shell length, in this case 1 mm. Condition measurements were limited to the three composite samples taken from each treatment at 0, 6, and 21 days of efflux. Figure 17 shows the changes in condition that occurred during efflux. The animals gained weight from the 0 to 6 days, probably as a result of the daily feeding of high quality food, but then deteriorated from 7 to 21 days, perhaps as a result of stress from laboratory conditions. The lack of additional data points during efflux made it difficult to accurately characterize the changes in condition throughout the 21 day period.

The increases in activity that were observed (per gram of wet tissue) over time could be partially attributed to the deteriorating condition that occurred from 7 to 21 days, although it would be difficult to quantify this effect. Mortalities coincided with the deteriorating condition. Figures 18 and 19 plot the dpm/gram wet tissue weight during efflux at the three points that dissections were made (0, 6.06 and 21 days of efflux).

Because significant decline in condition and mortalities did occur after day 7 of efflux, tissue estimates that relied on the tissue/whole body ratios were only computed for the first 6.06 days of loss. Figures 20 and 21 are estimated tissue activities (in dpm) normalized per gram weight tissue weight during the loss of activity that occurred from 0 to 6.06 days. Estimates were made by multiplying ratios of tissue/whole clam activities by intact whole clam activities. Ratios were obtained at 0 and 6.06 days after exposure and the slope of the curve of these two points was used to estimate ratios along the line. Points on the figures represent natural log (ln) conversions of the fraction of activity retained from immediately after exposure over 6.06 days for both rapid and slowly

Figure 17. The Change in Clam Condition During Efflux. Clam condition is defined as the wet tissue wet per unit shell length (i.e. 1 mm).

Figure 18. The Change in Activity During the Loss of Cd. Dissections occurred at three points, immediately after exposure ($t=0$), after 6 days of efflux ($t=6$), and after 21 days of efflux ($t=21$). Points represent the fraction of ^{109}Cd retained in tissues since the initial dissection after exposure. Measurements are actual tissue activities, corrected for decay, and normalized on a per gram wet tissue basis.

Figure 19. The Change in Activity During Efflux. Dissections occurred at three points, immediately after exposure ($t=0$), after 6 days of efflux ($t=6$), and after 21 days of efflux ($t=21$). Points represent the fraction of ^{65}Zn retained in tissues since the initial dissection after exposure. Measurements are actual tissue activities, corrected for decay, and normalized on a per gram wet tissue basis.

Figure 20. Estimated ^{109}Cd Tissue Efflux from t=0 to t=6.06 Days.

Figure 21. Estimated ^{65}Zn Tissue Efflux from t=0 to t=6.06 Days.

exchanging pools. An attempt to characterize the efflux rates obtained from tissue estimates during the period of 2.85 to 6.06 days was made. However, a curve based on these measurements, resulted in dramatically different rates (approximately 5 times greater for the DDC exposure). This large variation can be attributed to calculating curves that rely on points taken close to the inflection point of the efflux curves.

Therefore these estimates were omitted because it is believed that they are misleading, overestimate the efflux rates, and exaggerate the difference between the treatments.

Because the methods used to estimate tissue efflux rates may have the effect of compounding errors introduced at each stage of the calculation, the final method used to compare efflux rates simply involved comparing the actual losses of activity from 0 to 6.06 days of efflux. Days 0 and 6.06 days were used because these were the only points where actual tissue activity measurements were made without estimation, where decline in condition had little to no influence. Figures 22 and 23 show the actual decline in tissue activities from 0 to 6.06 days of efflux. Table 17 summarizes the loss constants calculated from all of the above methods.

Table 17. Depuration Rate Constants (k) (in Units of Day⁻¹).

Loss From:	Whole Clams	Tissues (Estimated)	Tissues (Actual)
Time Frame:	2.85-21 days (slow)	0-6.06 days (rapid & slow)	0-6.06 days (rapid & slow)
Cd (control)	-0.0293	-0.0859	-0.1095

Figure 22. Actual ^{109}Cd Activity Loss From Tissues From 0 to 6.06 Days. Points are natural log (ln) conversions of the fraction of activity retained from immediately after exposure ($t=0$). Actual activities (dpm) have been normalized to dpm per gram wet tissue weight.

Figure 23. Actual ^{65}Zn Activity Loss From Tissues From 0 to 6.06 Days. Points are natural log (ln) conversions of the fraction of activity retained from immediately after exposure ($t=0$). Actual activities (dcpm) have been normalized to dpm per gram wet tissue weight.

Loss From:	Whole Clams	Tissues (Estimated)	Tissues (Actual)
Time Frame:	2.85-21 days (slow)	0-6.06 days (rapid & slow)	0-6.06 days (rapid & slow)
Cd(DDC) ₂	-0.0265	-0.0961	-0.1392
Zn (control)	-0.0420	-0.1515	-0.1677
Zn(DDC) ₂	-0.0413	-0.1535	-0.1816

(Table 17 continued)

Assimilation Efficiencies

Assimilation efficiencies were calculated using the two equations presented in the methods section, the mass balance approach (equation 1) and the shell correction method (equation 2). Assimilation efficiency (AE) calculations obtained from the two methods had a large degree of variability. Table 18 summarizes the AE calculation obtained for both treatments from the two equations.

Table 18. Assimilation Efficiency Calculations (Percentage of Activity Retained from Food); A Summary of Two Approaches.

Method	Cd ²⁺	Cd(DDC) ₂	Zn ²⁺	Zn(DDC) ₂
Equation 1 (Mass Balance)	90 ± 2	58 ± 13	73 ± 5	73 ± 6
Equation 2 (Shell Correction)	68 ± 3	46 ± 7	40 ± 3	47 ± 5

The values in table 18 represent mean AE (in percent) ± standard deviations from the mean for 5 replicates each containing 3 clams. The large discrepancies between the

AEs determined by the two different methods are probably related to the loss of fecal matter and the desorption of metal during handling. This loss, or the failure to accurately measure all fecal matter activity has the result of overestimating AE by underestimating the activity lost through defecation. Similar problems associated with AE by the mass balance approach have been reported in previous studies (Reinfelder et al. 1997). For this reason, AEs obtained by equation 2, are believed to be more accurate, and will be referred to in this discussion.

The gut residence time for *P. amurensis* is approximately 24 hours, consistent with the results of this experiment (Lee, Wallace and Luoma 1998). The total depuration period of 48 hours following feeding was chosen based on the gut residence time of this species. Although it was believed that the total activity measured from the feces lost during depuration was underestimated, as mentioned above, measurement of defecation rates was important in confirming the gut passage of radioactive particles. The defecation rate, obtained by dividing the dpm of feces by the amount of time that elapsed between feces collections demonstrated that the gut passage of ingested particles was complete by the end of the 48 depuration period and most likely complete at the end of 24 hours for both Cd and Zn (Lee, Wallace and Luoma 1998; Reinfelder et al. 1997). Figures 24 and 25 depict the decline in defecation rates for Cd and Zn respectively, during the course of the 48 hour depuration period.

Following the 1 hour 30 minute labeled algae feeding, clams followed an immediate rapid and variable loss for both metals for approximately the first 24 hours of depuration, then loss slowed throughout the remaining 24 hours. The loss of radioactivity

Figure 24. Defecation Rate (dpm in feces/hour) During the Assimilation of Cd.

Figure 25. Defecation Rate (dpm in feces/hour) During the Assimilation of Zn.

from the clams that were fed food particles labeled with the Cd-DDC complex was greater during the entire period, relative to the control group. Cd from the control treatment was assimilated more efficiently than the Cd obtained by the DDC exposure where the slope of the retention curve remained steep throughout depuration. Figure 26 plots the percentage of activity from ^{109}Cd retained during the course of depuration of labeled food particles.

The loss of Zn from food was much more similar between treatments than the loss of Cd during the gut clearance stage. Both treatments underwent very rapid loss of activity from ^{65}Zn during the first 12 hours of depuration then loss slowed, but remained consistent until the end of the depuration period. Differences in assimilation of Zn did not vary significantly between the two treatments (47 ± 5 for the control treatment and 40 ± 3 for the DDC treatment) when mean values underwent an independent statistical *t*-test. Figure 27 plots the percentage of activity retained from ^{65}Zn during the 48 hour depuration period.

Table 19 summarizes the statistical variables obtained by subjecting the individual AE values used to obtain the mean AEs to a statistical *t*-test for independent variables. The 95% confidence interval was the determination of significance for the two-tailed hypothesis, $H_0: \mu_1 - \mu_2 = 0$ and $H_2: \mu_1 - \mu_2 \neq 0$. The analysis shows that for Cd, with mean AE values of 68% and 46% for the control and DDC exposures, respectively, a significant difference does exist between the mean AE values ($p = .0016$). However, for the Zn treatments, the mean AE values of 40% and 47% for the control and DDC exposures, respectively, no significant difference can be inferred from the mean values at

Figure 26. Percentage of ^{109}Cd Activity (in dpm) Obtained From Feeding During 48 Hour Depuration Period. Final AE values were obtained by equation 2.

Figure 27. Percentage of ^{65}Zn Activity (in dpm) Obtained From Feeding During 48 Hour Depuration Period. Final AE values were obtained by equation 2.

a 95% confidence interval ($p = .0559$).

Table 19. Statistical variables obtained from T-test analysis for mean AE values.

Treatment	N	Mean	Std. Dev.	Degrees of Freedom	t - value	p - value	F - ratio variance
Cd ²⁺	4	.6750	.0379	7	-2.289	.0016	4.3256
Cd(DDC) ₂	5	.4600	.0787	7			
Zn ²⁺	4	.3975	.0287	7	-4.971	.0559	3.7242
Zn(DDC) ₂	5	.4680	.0554	7			

Subcellular Distribution of Cd and Zn From DI Experiment

Subcellular fractions were obtained from 4 groups of animals from the initial DI experiment: control clams at $t=0$ days (4 replicates), control clams at $t=6$ days (3 replicates), DDC clams at $t=0$ days (4 replicates), and DDC clams at $t=6$ days (3 replicates). Each replicate contained 2 clams.

The analysis of fractions obtained for both treatments (control and DDC) revealed that the addition of DDC had no significant effect (95% confidence $t = \text{test}$) on the relative subcellular distribution of either Cd or Zn in clams at both $t=0$ and $t=6$. Figure 28 shows pie charts representing the relative subcellular distribution of Cd and Zn from each treatment at $t=0$ (immediately after dissolved influx). Percentages were calculated by summing mean metal concentrations for each fraction for all replicates in that groups, and expressing this as a percent of the mean total body burden.

Figure 28. Subcellular compartmentalization of Cd and Zn after dissolved uptake ($t=0$). Percentages are based on mean concentration values obtained from all replicates for each compartment.

Figure 29 compares the subcellular metal distributions after uptake ($t=0$) with that after loss ($t=6$). The DDC treated clams had a significant loss of Cd from the MT fraction as compared with the controls, which showed no significant loss from the MT fraction. Approximately 79 percent of the Cd found in the MT fraction of the DDC clams at $t=0$ was lost by $t=6$. For both treatments, the non heat-stable protein fraction showed the largest total losses of Cd. Although significant differences in Cd loss from the MT fraction were apparent between the two treatments on a concentration basis, proportional analysis revealed that no relative changes of Cd distribution took place in either treatment.

Also seen in Figure 29, is that the relative partitioning of Zn was similar between the control and DDC treatments both at $t=0$ and $t=6$. Statistical analysis showed that there were no significant differences in the relative partitioning of Zn at $t=0$ or $t=6$. Appendices Five and Six contain the raw activity data, the calculated masses of Cd and Zn, and relative percentage distributions for fractionated clams from the DI experiment.

Subcellular Partitioning of Cd and Zn From AE Experiment

Subcellular fractions were obtained from 4 groups of animals from the initial AE experiment: control clams at $t=0$ days (4 replicates), control clams at $t=2$ days (4 replicates), DDC clams at $t=0$ days (4 replicates), and DDC clams at $t=2$ days (4 replicates). Each replicate contained 2 clams.

Subcellular partitioning of clams immediately after feeding ($t=0$), revealed a treatment effect in the partitioning of Cd. It should be noted however, that fractions

Figure 29. Subcellular distribution (concentration) of metals after dissolved uptake ($t=0$ dark bars) vs. after 6 days of efflux ($t=6$ light bars).

obtained at $t=0$ include cellular material from both the clams and the algae that were consumed and still within the gut. Figure 30 shows pie charts representing the proportional subcellular partitioning of Cd and Zn at $t=0$. Significant differences of Cd were found between the metal-rich granule (MRG), metallothionein (MT), non heat-stable protein (NHS), and tissue debris (Debris) fractions of the control and DDC treatments. Of these differences, the debris fraction was the largest with respect to Cd concentrations at $t=0$. Approximately 32 percent of the total body burden of Cd was found in the debris fraction of the control group versus 48 percent in the DDC group at $t=0$. No significant differences in relative Zn distributions were found between the control and DDC treatments at $t=0$.

Following 2 days (48 hours) of depuration, no significant differences in relative distributions of Cd or Zn existed between the two treatments as seen in proportional distributions in Figure 31. The largest differences between the control and DDC treatments found at $t=0$, were in the debris fraction (relative to total body burden), however these difference were not present at the end of the 48 hour depuration period. Approximately 57 percent of the Cd in the debris fraction of the DDC treated clams was lost during this time versus a 19 percent loss in the control. Metal loss from all other fractions was similar between treatments. Figure 32 shows the concentrations of Cd and Zn for each treatment at $t=0$ and $t=48$ hours. Appendices Seven and Eight contain the raw activity data, the calculated masses of Cd and Zn, and relative percentage distributions for fractionated clams from the AE experiment.

Figure 30. Compartmentalization of Cd and Zn after AE feeding (t=0). Percentages are mean values of obtained from all replicates for each compartment.

Figure 31. Suncellular compartmentalization of Cd and Zn 48 hours after feeding (t=48) from AE experiment. Percentages are mean values (% of total body burden) obtained from all replicates for each compartment.

Figure 32. Subcellular distribution (concentration) of metals from AE experiment after feeding (t=0 dark bars) vs. after depuration (t=2 light bars).

V. DISCUSSION

The results obtained from the dissolved influx experiment supported the hypothesis that the addition of DDC to the exposure media would increase the dissolved influx rates for both Cd and Zn. The accelerated rates were a result of the increases in both the bioavailable fraction of metal as well as an acceleration in the cellular uptake mechanism. Comparison of the increases in the estimated bioavailable fraction of the metals indicated that this alone, could not have accounted for the overall increased rates in uptake. Table 20 summarizes the differences in the bioavailable fraction of metal in the dissolved influx exposure. The percentages shown are those that were obtained from the speciation modeling calculations that were performed for the Estuquill media using the HYDRAQL program. In the control group, it is the free ion component that is considered to be the bioavailable fraction of metal (Blust, Bailieu and Decleir 1995; Luoma 1983; Lee, Wallace and Luoma 1998). This is consistent with previous studies that showed significant increases in Cd influx rates with decline in salinity (Lee, Wallace and Luoma 1998). In the DDC exposure, because nearly 100 percent of the metal available is present as a DDC-metal complex, it is known that this complex is the bioavailable form.

Table 20. Percentages of Bioavailable Metal; A Comparison Between Experimental Treatments.

Metal	Control Exposure	DDC Exposure
Cd (total = 1 μg / L)	5.1%	99.6%
Zn (total = 6 μg / L)	45.2%	98.2%

The addition of DDC had the effect of increasing the amount of bioavailable Cd by almost 20 times (19.53) and the fraction of bioavailable Zn is more than doubled (2.17 times). However, the increase in uptake rate that occurs is over 25 times faster for Cd and 10 times faster for Zn. Therefore, the dominant mechanism that is responsible for uptake of the metal-DDC complex, which is assumed to be diffusion across the plasma membrane, occurs 1.3 times faster for Cd and 5 times faster for Zn. When assessing the effect of carbamate pesticides on metal bioavailability, both the relative increase of the bioavailable form of metal, as well as the effect of an increased influx rate, should be considered.

The determination of efflux rates of Cd and Zn that were accumulated during the dissolved influx experiment was complicated by mortalities and a decline in the condition of the animals. However, comparisons of efflux rates obtained by using three separate methods, show that metals taken up by way of dissolved metal-DDC complexes, exhibit similar loss patterns to metals taken up in ionic form (inorganically complexed). The efflux rates seen for this experiment generally fall within the ranges of those seen for various species in other experiments. Table 21 summarizes the loss rate constants as determined in similar studies using *P. amurensis*. Physiological loss rates determined in a previous study, indicate that rates may not be particularly variable among different bivalve species (Reinfelder et al. 1997). However, when comparing the efflux rate constant of the control exposure for Cd and Zn, to the rates seen in previous studies using *P. amurensis*, the rates observed in this study are substantially faster (Lee, Wallace and Luoma 1998). This difference was most likely a function of the methods used to calculate

the rate constants. In determining efflux rates *P. amurensis*, Lee, Wallace and Luoma (1998) used a depuration period of 21 days as opposed to the 6 days used in this experiment. A rate constant based on a shorter duration will generally be faster due to the bias of the increased efflux found closer to $t = 0$. In the absence of the mortalities and reduction in condition that were seen in this study after day 7, it is hypothesized that the rates seen for *P. amurensis* in similar studies would have been observed, had rate calculations been extended to 21 days. Because no previous studies have investigated the effect of carbamate compounds on bivalve uptake and loss rates, no data exists for this comparison.

Table 21. Comparison of Physiological Loss Constants (k , d^{-1}) of Present and Previous Studies.

Species	Element		Source
	Cd	Zn	
<i>P. amurensis</i>	-0.029	-0.042	Present Study (control)*
<i>P. amurensis</i>	-0.027	-0.041	Present Study (DDC)*
<i>P. amurensis</i>	-0.011	-0.027	Lee, Wallace and Luoma 1998
<i>M. mercenaria</i>	-0.01	-0.01	Reinfelder et al. 1997
<i>C. virginica</i>	-0.01	-0.01	Reinfelder et al. 1997
<i>M. balthica</i>	-0.01	-0.04	Reinfelder et al. 1997
<i>M. edulis</i>	-0.0232	-1.06	Fisher et al. 1996

* The loss rate constant used was calculated based on whole clam activities for the slowly exchanging pools (2.85 - 21 days).

The loss of metals from the shell fraction of the control group followed a similar pattern to those seen in previous studies. Shell activities from the DDC exposure however, followed a much different pattern. The shell activities from the DDC treatment remained generally unchanged during the entire course of efflux. This is apparent when reviewing the shell activities as seen in tables 15 and 16. The retention seen in the DDC exposure is probably the result of the association of the metal-DDC complex with the organic film that may exist on the shell surfaces. Unlike the tissues, loss of metal from the shell component is not regulated by physiological processes but rather chemical processes.

Assimilation efficiencies determined from the two separate methods described earlier (equation 1 and equation 2) did not support the hypothesis that AE would be increased with the addition of DDC to the algae culturing media. In the case of Cd, the addition of DDC to the culturing media actual had the effect of decreasing AE as seen in Figure 26. Assimilation of Cd from food occurred more quickly and more thoroughly in the control treatment indicating the Cd taken up by algae as a Cd-DDC complex was less available to the predator (clams). By 10 hours after depuration began, retention of Cd from the control treatment began to level off while substantial loss continued for the DDC treatment.

The reasons for the reduction in Cd assimilation in the DDC treatment are not fully understood, but the results of the subcellular distribution analysis revealed that Cd associated with the tissue fraction was lost much more quickly than for the control treatment. The amount of Cd associated with that fraction was significantly greater at $t=0$

(48% versus 32% in the control). The excess Cd in the tissue fraction of the DDC exposure was most likely associated with the ingested algae cells that were still present in the gut at the $t=0$. This was surmised because the DI experiment showed that there was not an increase in the clam tissue fraction of Cd accumulated from $\text{Cd}(\text{DDC})_2$ complexes.

Previous studies have shown that bivalves undergo a biphasic digestion process, intestinal and glandular (Decho and Luoma 1991). During the more rapid intestinal digestion process, food is ingested, enters the stomach, is passed into the intestine where it undergoes extracellular digestion and is ultimately released as intestinal feces (Decho and Luoma 1991). It is possible that if more of the Cd associated with the algae cells was present in the membrane or other lipid dominated cell fractions, larger amounts of Cd might pass through the animal within the undigested membrane, ultimately exiting as feces. Further research will be necessary to identify the processes responsible for lower assimilation of the Cd associated with the DDC complex, but these results indicate that the affinity of $\text{Cd}(\text{DDC})_2$ to the debris fraction of the algae cells was the cause.

Zn assimilation was not significantly different between the two treatments with final AEs calculated at 47% and 40% for the DDC and control treatments respectively. In addition, the subcellular distribution of Zn was essentially identical in the control and DDC treatment.

AE may be affected by several factors including food concentrations, food quality and type and the species being studied (Fisher et al. 1996; Reinfelder et al. 1997; Wang, Fisher and Luoma 1995; Lee and Luoma 1997a). Table 22 compares the AEs obtained for various species for Cd and Zn from several previous studies. AE for Cd (in the

control treatment) determined during this study is consistent with AEs determined for *C. virginica* and *M. balthica* fed *Isochrysis galbana* but Zn AEs are lower than those found for those species, but consistent with those seen in *M. edulis* (Reinfelder et al. 1997).

Table 22. Comparison of Assimilation Efficiencies (AE) Obtained From This and Earlier Studies.

Species	Element		Source
	Cd	Zn	
<i>P. amurensis</i>	68 ± 3	40 ± 3	Present Study (control)*
<i>P. amurensis</i>	46 ± 7	47 ± 5	Present Study (DDC)
<i>C. virginica</i>	69 ± 8.6	73 ± 8.2	Reinfelder et al. 1997
<i>M. balthica</i>	69 ± 2.3	64 ± 4.8	Reinfelder et al. 1997
<i>M. edulis</i>	96	43	Reinfelder et al. 1997
<i>M. edulis</i>	34	44	Wang and Fisher 1996

The hypothesis that DDC would increase the amount of Cd and Zn that would be found in the subcellular (debris) fraction that contained cell membranes was not supported. The DI experiment revealed that cellular partitioning of metal was not significantly different from the control. In addition, both Cd and Zn assimilated from food did not show significant differences in their subcellular partitioning at the end of the 2 day depuration period.

Additionally, the fact that metals accumulated from the DDC treatment were sequestered by proteins found in the metallothionein fraction (MT), indicated that the Cd and Zn entering the cell as a metal-DDC complex, were able to undergo exchange reactions with intracellular proteins. The proteins found in this fraction have been found to play a significant role in the regulation and detoxification of metals in aquatic organisms (Simkiss 1981; Viarengo et al. 1987; Roesijadi 1992; Wallace and Lopez 1997).

The increased loss of Cd from the MT fraction of the DDC group from the DI experiment is a finding that should be investigated in greater detail. The increased loss may indicate an increased production of metallothionein-like proteins, with subsequent elimination of sequestered metal through excretion. Or conversely, the toxic effect of DDC may actually inhibit the further production of these proteins. Little is known about the binding rates of metals and the mechanisms responsible for their subsequent release or excretion, and additional research on the effects of DDC on MT production are necessary to test these hypotheses (Roesijadi 1992).

VI. RECOMMENDATIONS

The results presented in this thesis highlight the importance in giving consideration to a multi-contaminant approach when assessing metal bioavailability to aquatic organisms. Mechanisms that enhance metal uptake, such as the one studied here, should be incorporated into models that attempt to establish the relationships between environmental metal contamination and bivalve tissue concentrations.

Further research is needed to qualify and quantify the actual effects of the introduction of carbamates into aquatic systems. The development of methods to analyze environmental concentrations of metallo-carbamates will be a critical element in carrying research in this area to the next level. In addition, much research needs to be done on examining the biochemical and toxicity effects of metal-carbamate exposures to aquatic organisms. Little is known about the biochemical processes that occur on a subcellular level once these complexes pass through the cell membrane.

It is recommended that an additional efflux study be conducted to more accurately characterize the efflux rates of DDC bound metals. The decline in condition, and the mortalities that were experienced during the course of the efflux phase of the experiment, may have confounded the results obtained in this study. A larger number of animals, and more frequent analysis of changes in condition and tissue/whole activities would reduce uncertainties in future efflux experiments of this type.

An additional study that would characterize the partitioning of Cd and Zn in algae cells is necessary to support or refute the conclusions drawn in the assimilation efficiency

experiment conducted for this thesis. Such an experiment would not be complicated, but may have strong implications in its findings.

The next phase in this study would be to vary the concentrations of the contaminant inputs, to include the range of actual environmental concentration of DDC. In addition, different durations of exposure should be considered as well as exposure to additional species. The fact that the majority of carbamate inputs occur near fresh water flows may highlight the need to further study interactions in a freshwater systems.

Regulating agencies such as the Regional Water Quality Control Board and the Environmental Protection Agency must promulgate new pollution control standards that take potential synergistic effects of contaminants into consideration. The current methods of enforcing standards based on individual pollutants without consideration of multiple contaminant interactions may not be effective in limiting the harmful effects that may occur to organisms and their potential predators, including humans.

APPENDIX ONE**PERCENTAGE DISTRIBUTION OF IONIC COMPONENTS IN DISSOLVED
INFLUX EXPOSURE MEDIA (CONTROL)**

DESCRIPTION OF CONTENT OF APPENDICES ONE THROUGH FOUR

This explains how to read the HYDRAQL printouts that follow. Each major ionic component is placed in the left column. The indented rows beneath each major heading list the species distribution the major component. The percent of the total found as each species is followed by number that has been assigned to that species by the HYDRAQL program. What follows is the number of cations and anions in the particular component.

For example:

SO₄

57.2	Pct Bnd in Spcs no. 102	SO4	1.0		
19.0	Pct Bnd in Spcs no. 1380	Mg	1.0	SO4	1.0
22.8	Pct Bnd in Spcs no. 2010	Na	1.0	SO4	1.0

In this case, Sulfate (SO₄) is present in three major forms. 57.2% is as free Sulfate indicated by 1.0 meaning that is the only component in the species. 102 is the number that has been dedicated to that particular species by the HYDRAQL program. 19% of the Sulfate exists as MgSO₄ indicated by 1 Mg cation and 1 SO₄ anion. Finally, 22.8% of the Sulfate in solution is found as Na SO₄.

DDC species are all designated with a 4 digit number beginning with 30, such as 3010, 3020, 3040, etc.

Percentage Distribution of Components
DI NO DDC

SeO4									
100.0	Pct	Bnd	in	Spcs	no.	171	SeO4	1.0	
MoO4									
100.0	Pct	Bnd	in	Spcs	no.	152	MoO4	1.0	
Cu2+									
13.5	Pct	Bnd	in	Spcs	no.	9	Cu2+	1.0	
70.3	Pct	Bnd	in	Spcs	no.	3920	Cu2+	1.0	CO3 1.0
1.4	Pct	Bnd	in	Spcs	no.	3930	Cu2+	1.0	CO3 2.0
2.1	Pct	Bnd	in	Spcs	no.	3940	Cu2+	1.0	SO4 1.0
5.1	Pct	Bnd	in	Spcs	no.	3950	Cu2+	1.0	Cl 1.0
6.7	Pct	Bnd	in	Spcs	no.	4840	Cu2+	1.0	H -1.0
Co2+									
63.2	Pct	Bnd	in	Spcs	no.	16	Co2+	1.0	
15.9	Pct	Bnd	in	Spcs	no.	8560	Co2+	1.0	SO4 1.0
18.8	Pct	Bnd	in	Spcs	no.	8570	Co2+	1.0	Cl 1.0
2.0	Pct	Bnd	in	Spcs	no.	9300	Co2+	1.0	H -1.0
Mn2									
38.6	Pct	Bnd	in	Spcs	no.	8	Mn2	1.0	
6.1	Pct	Bnd	in	Spcs	no.	3300	Mn2	1.0	SO4 1.0
45.7	Pct	Bnd	in	Spcs	no.	3310	Mn2	1.0	Cl 1.0
8.0	Pct	Bnd	in	Spcs	no.	3320	Mn2	1.0	Cl 2.0
SO4									
57.2	Pct	Bnd	in	Spcs	no.	102	SO4	1.0	
19.0	Pct	Bnd	in	Spcs	no.	1380	SO4	1.0	Mg 1.0
22.8	Pct	Bnd	in	Spcs	no.	2010	SO4	1.0	Na 1.0
Na									
98.6	Pct	Bnd	in	Spcs	no.	5	Na	1.0	
1.4	Pct	Bnd	in	Spcs	no.	2010	SO4	1.0	Na 1.0
Mg									
71.0	Pct	Bnd	in	Spcs	no.	2	Mg	1.0	
10.0	Pct	Bnd	in	Spcs	no.	1380	SO4	1.0	Mg 1.0
18.1	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca 1.0 CO3 2.0
K									
94.8	Pct	Bnd	in	Spcs	no.	4	K	1.0	
1.9	Pct	Bnd	in	Spcs	no.	1960	SO4	1.0	K 1.0
3.3	Pct	Bnd	in	Spcs	no.	1962	K	1.0	Cl 1.0
BOH4									
9.0	Pct	Bnd	in	Spcs	no.	12700	BOH4	1.0	H 1.0
90.1	Pct	Bnd	in	Spcs	no.	12702	BOH4	3.0	H 1.0
Cd									
5.1	Pct	Bnd	in	Spcs	no.	11	Cd	1.0	
47.8	Pct	Bnd	in	Spcs	no.	5090	Cd	1.0	Cl 1.0
42.1	Pct	Bnd	in	Spcs	no.	5100	Cd	1.0	Cl 2.0
3.5	Pct	Bnd	in	Spcs	no.	5110	Cd	1.0	Cl 3.0
Zn									
45.2	Pct	Bnd	in	Spcs	no.	12	Zn	1.0	
9.3	Pct	Bnd	in	Spcs	no.	5955	Zn	1.0	CO3 1.0
2.5	Pct	Bnd	in	Spcs	no.	5956	Zn	1.0	CO3 2.0
8.4	Pct	Bnd	in	Spcs	no.	5960	SO4	1.0	Zn 1.0
11.4	Pct	Bnd	in	Spcs	no.	5970	Zn	1.0	Cl 1.0
2.1	Pct	Bnd	in	Spcs	no.	5980	Zn	1.0	Cl 2.0
2.4	Pct	Bnd	in	Spcs	no.	6740	Zn	1.0	H -1.0
2.6	Pct	Bnd	in	Spcs	no.	6750	Zn	1.0	H -2.0
12.9	Pct	Bnd	in	Spcs	no.	6766	Zn	1.0	Cl 1.0 H -1.0

	1.4	Pct Bnd in Spcs no. 6767	Zn	1.0	H	1.0	CO3	1.0	
Cl									
	99.9	Pct Bnd in Spcs no. 103	Cl	1.0					
Br									
	100.0	Pct Bnd in Spcs no. 105	Br	1.0					
Ca									
	6.2	Pct Bnd in Spcs no. 1	Ca	1.0					
	1.0	Pct Bnd in Spcs no. 1020	SO4	1.0	Ca	1.0			
	92.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0	
CO3									
	6.7	Pct Bnd in Spcs no.12530	H	1.0	CO3	1.0			
	91.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0	

APPENDIX TWO**PERCENTAGE DISTRIBUTION OF IONIC COMPONENTS IN DISSOLVED
INFLUX EXPOSURE MEDIA (CONTAINING DDC)**

Percentage Distribution of Components

SeO4	100.0	Pct	Bnd	in	Spcs	no.	171	SeO4	1.0								
MoO4	100.0	Pct	Bnd	in	Spcs	no.	152	MoO4	1.0								
Cu2+	100.0	Pct	Bnd	in	Spcs	no.	3040	Cu2+	1.0			2.0					
Co2+	100.0	Pct	Bnd	in	Spcs	no.	3030	Co2+	1.0			2.0					
Mn2	38.6	Pct	Bnd	in	Spcs	no.	8	Mn2	1.0								
	6.1	Pct	Bnd	in	Spcs	no.	3300	Mn2	1.0	SO4	1.0						
	45.7	Pct	Bnd	in	Spcs	no.	3310	Mn2	1.0	Cl	1.0						
	8.0	Pct	Bnd	in	Spcs	no.	3320	Mn2	1.0	Cl	2.0						
BOH4	9.0	Pct	Bnd	in	Spcs	no.	12700	BOH4	1.0	H	1.0						
	90.1	Pct	Bnd	in	Spcs	no.	12702	BOH4	3.0	H	1.0						
Na	98.6	Pct	Bnd	in	Spcs	no.	5	Na	1.0								
	1.4	Pct	Bnd	in	Spcs	no.	2010	Na	1.0	SO4	1.0						
Mg	71.0	Pct	Bnd	in	Spcs	no.	2	Mg	1.0								
	10.0	Pct	Bnd	in	Spcs	no.	1380	Mg	1.0	SO4	1.0						
	18.1	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca	1.0	CO3	2.0				
K	94.8	Pct	Bnd	in	Spcs	no.	4	K	1.0								
	1.9	Pct	Bnd	in	Spcs	no.	1960	K	1.0	SO4	1.0						
	3.3	Pct	Bnd	in	Spcs	no.	1962	K	1.0	Cl	1.0						
SO4	57.2	Pct	Bnd	in	Spcs	no.	102	SO4	1.0								
	19.0	Pct	Bnd	in	Spcs	no.	1380	Mg	1.0	SO4	1.0						
	22.8	Pct	Bnd	in	Spcs	no.	2010	Na	1.0	SO4	1.0						
Cd	99.6	Pct	Bnd	in	Spcs	no.	3010	Cd	1.0			2.0					
Zn	98.2	Pct	Bnd	in	Spcs	no.	3020	Zn	1.0			2.0					
Cl	99.9	Pct	Bnd	in	Spcs	no.	103	Cl	1.0								
Br	100.0	Pct	Bnd	in	Spcs	no.	105	Br	1.0								
	79.2	Pct	Bnd	in	Spcs	no.	165		1.0								
	1.8	Pct	Bnd	in	Spcs	no.	3010	Cd	1.0			2.0					
	18.0	Pct	Bnd	in	Spcs	no.	3020	Zn	1.0			2.0					
Ca	6.2	Pct	Bnd	in	Spcs	no.	1	Ca	1.0								
	1.0	Pct	Bnd	in	Spcs	no.	1020	SO4	1.0	Ca	1.0						
	92.8	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca	1.0	CO3	2.0				
CO3	6.7	Pct	Bnd	in	Spcs	no.	12530	H	1.0	CO3	1.0						
	91.8	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca	1.0	CO3	2.0				

APPENDIX THREE

PERCENTAGE DISTRIBUTION OF IONIC COMPONENTS IN ASSIMILATION
EFFICIENCY EXPOSURE CULTURING MEDIA (CONTROL)

SeO4									
100.0	Pct	Bnd	in	Spcs	no.	171	SeO4	1.0	
CIT									
92.9	Pct	Bnd	in	Spcs	no.	117	CIT	1.0	
6.7	Pct	Bnd	in	Spcs	no.	3071	CIT	2.0	Fe3+ 2.0 H -2.0
MoO4									
100.0	Pct	Bnd	in	Spcs	no.	152	MoO4	1.0	
Cu2+									
13.5	Pct	Bnd	in	Spcs	no.	9	Cu2+	1.0	
70.3	Pct	Bnd	in	Spcs	no.	3920	Cu2+	1.0	CO3 1.0
1.4	Pct	Bnd	in	Spcs	no.	3930	Cu2+	1.0	CO3 2.0
2.1	Pct	Bnd	in	Spcs	no.	3940	Cu2+	1.0	SO4 1.0
5.1	Pct	Bnd	in	Spcs	no.	3950	Cu2+	1.0	Cl 1.0
6.7	Pct	Bnd	in	Spcs	no.	4840	Cu2+	1.0	Fe3+ 0.0 H -1.0
Co2+									
63.2	Pct	Bnd	in	Spcs	no.	16	Co2+	1.0	
15.9	Pct	Bnd	in	Spcs	no.	8560	Co2+	1.0	SO4 1.0
18.8	Pct	Bnd	in	Spcs	no.	8570	Co2+	1.0	Cl 1.0
2.0	Pct	Bnd	in	Spcs	no.	9300	Co2+	1.0	Fe3+ 0.0 H -1.0
Mn2									
38.6	Pct	Bnd	in	Spcs	no.	8	Mn2	1.0	
6.1	Pct	Bnd	in	Spcs	no.	3300	Mn2	1.0	SO4 1.0
45.7	Pct	Bnd	in	Spcs	no.	3310	Mn2	1.0	Cl 1.0
8.0	Pct	Bnd	in	Spcs	no.	3320	Mn2	1.0	Cl 2.0
SO4									
57.2	Pct	Bnd	in	Spcs	no.	102	SO4	1.0	
19.0	Pct	Bnd	in	Spcs	no.	1380	SO4	1.0	Mg 1.0
22.8	Pct	Bnd	in	Spcs	no.	2010	SO4	1.0	Na 1.0
Na									
98.6	Pct	Bnd	in	Spcs	no.	5	Na	1.0	
1.4	Pct	Bnd	in	Spcs	no.	2010	SO4	1.0	Na 1.0
BOH4									
9.0	Pct	Bnd	in	Spcs	no.	12700	BOH4	1.0	Fe3+ 0.0 H 1.0
90.1	Pct	Bnd	in	Spcs	no.	12702	BOH4	3.0	Fe3+ 0.0 H 1.0
Mg									
71.0	Pct	Bnd	in	Spcs	no.	2	Mg	1.0	
10.0	Pct	Bnd	in	Spcs	no.	1380	SO4	1.0	Mg 1.0
18.1	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca 1.0 CO3 2.0
K									
94.8	Pct	Bnd	in	Spcs	no.	4	K	1.0	
1.9	Pct	Bnd	in	Spcs	no.	1960	SO4	1.0	K 1.0
3.3	Pct	Bnd	in	Spcs	no.	1962	K	1.0	Cl 1.0
Fe3+									
100.0	Pct	Bnd	in	Spcs	no.	3071	CIT	2.0	Fe3+ 2.0 H -2.0
Cd									
4.9	Pct	Bnd	in	Spcs	no.	11	Cd	1.0	
45.9	Pct	Bnd	in	Spcs	no.	5090	Cd	1.0	Cl 1.0
40.4	Pct	Bnd	in	Spcs	no.	5100	Cd	1.0	Cl 2.0
3.3	Pct	Bnd	in	Spcs	no.	5110	Cd	1.0	Cl 3.0
3.2	Pct	Bnd	in	Spcs	no.	3087	CIT	1.0	Cd 1.0
Zn									
9.5	Pct	Bnd	in	Spcs	no.	12	Zn	1.0	
2.0	Pct	Bnd	in	Spcs	no.	5955	Zn	1.0	CO3 1.0
1.8	Pct	Bnd	in	Spcs	no.	5960	SO4	1.0	Zn 1.0

	2.4	Pct Bnd in Spcs no. 5970	Zn	1.0	Cl	1.0					
	2.7	Pct Bnd in Spcs no. 6766	Fe3+	0.0	Zn	1.0	Cl	1.0	H	-1.0	
	78.1	Pct Bnd in Spcs no. 3083	CIT	1.0	Zn	1.0					
Cl											
	99.9	Pct Bnd in Spcs no. 103	Cl	1.0							
Br											
	100.0	Pct Bnd in Spcs no. 105	Br	1.0							
Ca											
	6.2	Pct Bnd in Spcs no. 1	Ca	1.0							
	1.0	Pct Bnd in Spcs no. 1020	SO4	1.0	Ca	1.0					
	92.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0			
CO3											
	6.7	Pct Bnd in Spcs no.12530	Fe3+	0.0	H	1.0	CO3	1.0			
	91.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0			

APPENDIX FOUR

PERCENTAGE DISTRIBUTION OF IONIC COMPONENTS IN ASSIMILATION
EFFICIENCY EXPOSURE CULTURING MEDIA (CONTAINING DDC)

SeO4	100.0	Pct	Bnd	in	Spcs	no.	171	SeO4	1.0								
CIT	92.9	Pct	Bnd	in	Spcs	no.	117	CIT	1.0								
	6.7	Pct	Bnd	in	Spcs	no.	3071	CIT	2.0	Fe3+	2.0	K	0.0	SO4	0.0		
H	-2.0																
MoO4	100.0	Pct	Bnd	in	Spcs	no.	152	MoO4	1.0								
Cu2+	100.0	Pct	Bnd	in	Spcs	no.	3040	Cu2+	1.0		2.0						
Co2+	100.0	Pct	Bnd	in	Spcs	no.	3030	Co2+	1.0		2.0						
Mn2	35.2	Pct	Bnd	in	Spcs	no.	8	Mn2	1.0								
	5.6	Pct	Bnd	in	Spcs	no.	3300	Mn2	1.0	Fe3+	0.0	SO4	1.0				
	41.6	Pct	Bnd	in	Spcs	no.	3310	Mn2	1.0	Cl	1.0						
	7.3	Pct	Bnd	in	Spcs	no.	3320	Mn2	1.0	Cl	2.0						
	1.0	Pct	Bnd	in	Spcs	no.	3055	Mn2	1.0		2.0						
	7.8	Pct	Bnd	in	Spcs	no.	3056	Mn2	1.0		1.0						
BOH4	9.0	Pct	Bnd	in	Spcs	no.	12700	BOH4	1.0	K	0.0	SO4	0.0	H	1.0		
	90.1	Pct	Bnd	in	Spcs	no.	12702	BOH4	3.0	K	0.0	SO4	0.0	H	1.0		
Na	98.6	Pct	Bnd	in	Spcs	no.	5	Na	1.0								
	1.4	Pct	Bnd	in	Spcs	no.	2010	Na	1.0	Fe3+	0.0	SO4	1.0				
Fe3+	100.0	Pct	Bnd	in	Spcs	no.	3071	CIT	2.0	Fe3+	2.0	K	0.0	SO4	0.0		
H	-2.0																
Mg	71.0	Pct	Bnd	in	Spcs	no.	2	Mg	1.0								
	10.0	Pct	Bnd	in	Spcs	no.	1380	Fe3+	0.0	Mg	1.0	SO4	1.0				
	18.1	Pct	Bnd	in	Spcs	no.	20145	Mg	1.0	Ca	1.0	CO3	2.0				
K	94.8	Pct	Bnd	in	Spcs	no.	4	K	1.0								
	1.9	Pct	Bnd	in	Spcs	no.	1960	Fe3+	0.0	K	1.0	SO4	1.0				
	3.3	Pct	Bnd	in	Spcs	no.	1962	K	1.0	Cl	1.0						
SO4	57.2	Pct	Bnd	in	Spcs	no.	102	Fe3+	0.0	SO4	1.0						
	19.0	Pct	Bnd	in	Spcs	no.	1380	Fe3+	0.0	Mg	1.0	SO4	1.0				
	22.8	Pct	Bnd	in	Spcs	no.	2010	Na	1.0	Fe3+	0.0	SO4	1.0				
Cd	99.9	Pct	Bnd	in	Spcs	no.	3010	Cd	1.0		2.0						
Zn	99.2	Pct	Bnd	in	Spcs	no.	3020	Zn	1.0		2.0						
Cl	99.9	Pct	Bnd	in	Spcs	no.	103	Cl	1.0								
Br	100.0	Pct	Bnd	in	Spcs	no.	105	Br	1.0								
	97.9	Pct	Bnd	in	Spcs	no.	165		1.0								
	1.8	Pct	Bnd	in	Spcs	no.	3020	Zn	1.0		2.0						
Ca	6.2	Pct	Bnd	in	Spcs	no.	1	Ca	1.0								
	1.0	Pct	Bnd	in	Spcs	no.	1020	Fe3+	0.0	SO4	1.0	Ca	1.0				

92.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0		112
CO3									
6.7	Pct Bnd in Spcs no.12530	K	0.0	SO4	0.0	H	1.0	CO3	1.0
91.8	Pct Bnd in Spcs no.20145	Mg	1.0	Ca	1.0	CO3	2.0		

APPENDIX FIVE

INDIVIDUAL REPLICATE CELLULAR DISTRIBUTIONS OF Cd AND Zn IN
CONTROL CLAMS FROM DI EXPERIMENT.
(FOR $t=0$ AND $t=6.06$ DAYS)

Dis. Control (cd only) t=0

pa 1 (c9)	
length	compos.
wet wt.	0.1229
storage t	x
ignore	0
total t	124

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2042	1038	
dpm	7165	17593	
cor. dpm	8630	25031	
dpm/g	70216	203672	
µg/g	0.02129	0.348687	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1742	981	
dpm	6112	16627	
cor. dpm	7362	23657	
dpm/g	59900	192488	
µg/g	0.018162	0.329539	

% cpm recovered before fract	cd	zn
recovery	85.3	94.5

total cpm recovered	cd	zn
total	1578	906

% cpm recovered after fract	cd	zn
recovery	90.6	92.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	46	50	
dpm	161	847	
cor. dpm	194	1206	ng/g
% body	3	5	cd zn
dpm/g	1582	9811	0.479589 16.79609

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	166	86	
dpm	582	1458	
cor. dpm	702	2074	ng/g
% body	10	9	cd zn
dpm/g	5708	16875	1.73069 28.88927

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	711	150	
dpm	2495	2542	
cor. dpm	3005	3617	ng/g
% body	41	15	cd zn
dpm/g	24448	29432	7.412775 50.38826

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	448	304	
dpm	1572	5153	
cor. dpm	1893	7331	ng/g
% body	26	31	cd zn
dpm/g	15405	59650	4.670778 102.1202

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	207	316	
dpm	726	5356	
cor. dpm	875	7620	ng/g
% body	12	32	cd zn
dpm/g	7118	62004	2.15815 106.1513

Dis. Control (cd only) t=0

pa 2 (c10)	
length	compos.
wet wt.	0.1219
storage t	x
ignore	0
total t	124

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2227	1374	
dpm	7814	23288	
cor. dpm	9411	33134	
dpm/g	77206	271812	
µg/g	0.023409	0.465343	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1741	1102	
dpm	6109	18678	
cor. dpm	7358	26575	
dpm/g	60357	218004	
µg/g	0.0183	0.373223	

% cpm recovered before fract	cd	zn
recovery	78.2	80.2

total cpm recovered	cd	zn
total	1610	1025

% cpm recovered after fract	cd	zn
recovery	92.5	93.0

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	37	49	
dpm	130	831	
cor. dpm	156	1182	ng/g
% body	2	4	cd zn
dpm/g	1283	9693	0.388921 16.5952

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	418	347	
dpm	1467	5881	
cor. dpm	1766	8368	ng/g
% body	24	31	cd zn
dpm/g	14491	68645	4.393753 117.5211

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	708	71	
dpm	2484	1203	
cor. dpm	2992	1712	ng/g
% body	41	6	cd zn
dpm/g	24545	14046	7.442051 24.0461

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	305	347	
dpm	1070	5881	
cor. dpm	1289	8368	ng/g
% body	18	31	cd zn
dpm/g	10574	68645	3.205968 117.5211

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	142	211	
dpm	498	3576	
cor. dpm	600	5088	ng/g
% body	8	19	cd zn
dpm/g	4923	41741	1.492615 71.46095

Dis. Control (cd only) t=0

pa 3 (c11)	
length	compos.
wet wt.	0.1415
storage t	x
ignore	0
total t	124

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2177	1424	
dpm	7639	24136	
cor. dpm	9200	34340	
dpm/g	65018	242683	
µg/g	0.019714	0.415474	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1746	1298	
dpm	6126	22000	
cor. dpm	7379	31301	
dpm/g	52146	221210	
µg/g	0.015811	0.378711	

% cpm recovered before fract		
recovery	cd 80.2	zn 91.2

total cpm recovered		
total	cd 1630	zn 1169

% cpm recovered after fract		
recovery	cd 93.4	zn 90.1

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	23	55	
dpm	81	932	
cor. dpm	97	1326	ng/g
% body	1	4	cd zn
dpm/g	687	9373	0.208274 16.04709

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	470	280	
dpm	1649	4746	
cor. dpm	1986	6752	ng/g
% body	27	22	cd zn
dpm/g	14037	47719	4.256029 81.69427

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	719	120	
dpm	2523	2034	
cor. dpm	3039	2894	ng/g
% body	41	9	cd zn
dpm/g	21474	20451	6.510818 35.01183

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	269	334	
dpm	944	5661	
cor. dpm	1137	8054	ng/g
% body	15	26	cd zn
dpm/g	8034	56921	2.435897 97.44959

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	149	380	
dpm	523	6441	
cor. dpm	630	9164	ng/g
% body	9	29	cd zn
dpm/g	4450	64761	1.349252 110.8708

Dis. Control (cd only) t=0

pa 4 (c12)	
length	compos
wet wt.	0.1423
storage t	x
ignore	0
total t	124

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2345	1070	
dpm	8228	18136	
cor. dpm	9910	25803	
dpm/g	69642	181328	
µg/g	0.021115	0.310434	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1801	873	
dpm	6319	14797	
cor. dpm	7611	21052	
dpm/g	53486	147943	
µg/g	0.016217	0.253279	

% cpm recovered before fract		
recovery	cd	zn
	76.8	81.6

total cpm recovered		
total	cd	zn
	1768	869

% cpm recovered after fract		
recovery	cd	zn
	98.2	99.5

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	12	18	
dpm	42	305	
cor. dpm	51	434	ng/g
% body	1	2	cd
dpm/g	356	3050	0.108054 5.222249

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	514	249	
dpm	1804	4220	
cor. dpm	2172	6005	ng/g
% body	29	29	cd
dpm/g	15265	42197	4.628298 72.24112

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	813	90	
dpm	2853	1525	
cor. dpm	3436	2170	ng/g
% body	45	10	cd
dpm/g	24145	15252	7.320635 26.11125

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	269	242	
dpm	944	4102	
cor. dpm	1137	5836	ng/g
% body	15	28	cd
dpm/g	7989	41011	2.422203 70.21024

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	160	270	
dpm	561	4576	
cor. dpm	676	6511	ng/g
% body	9	31	cd
dpm/g	4752	45756	1.440715 78.33374

Dis. Control (cd only) t=6 Days

pa 5 (c5)	
length	compos.
wet wt.	0.1158
storage t	x
ignore	0
total t	1.17

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	782	27.1	
dpm	2744	4593	
cor. dpm	3270	6406	
dpm/g	28240	55323	
µg/g	0.008563	0.094712	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	656	282	
dpm	2302	4780	
cor. dpm	2743	6666	
dpm/g	23690	57568	
µg/g	0.007183	0.098557	

% cpm recovered before fract		
recovery	cd	zn
	83.9	104.1

total cpm recovered		
total	cd	zn
	604	238

% cpm recovered after fract		
recovery	cd	zn
	92.1	84.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3	5	
dpm	11	85	
cor. dpm	13	118	ng/g
% body	0	2	cd zn
dpm/g	108	1021	0.032849 1.747457

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	326	57	
dpm	1144	966	
cor. dpm	1363	1347	ng/g
% body	50	20	cd zn
dpm/g	11773	11636	3.569538 19.92101

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	37	0	
dpm	130	0	
cor. dpm	155	0	ng/g
% body	6	0	cd zn
dpm/g	1336	0	0.405132 0

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	158	101	
dpm	554	1712	
cor. dpm	661	2388	ng/g
% body	24	36	cd zn
dpm/g	5706	20618	1.730022 35.29864

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	80	75	
dpm	281	1271	
cor. dpm	335	1773	ng/g
% body	12	27	cd zn
dpm/g	2889	15311	0.87596 26.21186

Dis. Control (cd only) t=7

pa 6 (c7)	
length	compos.
wet wt.	0.1461
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	904	440	
dpm	3172	7458	
cor. dpm	3780	10401	
dpm/g	25876	71194	
µg/g	0.007846	0.121884	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	809	339	
dpm	2839	5746	
cor. dpm	3383	8014	
dpm/g	23156	54852	
µg/g	0.007021	0.093906	

% cpm recovered before fract	cd	zn
recovery	89.5	77.0

total cpm recovered	cd	zn
total	743	337

% cpm recovered after fract	cd	zn
recovery	91.8	99.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	11	8	
dpm	39	136	
cor. dpm	46	189	ng/g
% body	1	2	cd zn
dpm/g	315	1294	0.095465 2.216077

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	328	66	
dpm	1151	1119	
cor. dpm	1372	1560	ng/g
% body	41	19	cd zn
dpm/g	9389	10679	2.846601 18.28264

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	158	0	
dpm	554	0	
cor. dpm	661	0	ng/g
% body	20	0	cd zn
dpm/g	4523	0	1.371229 0

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	158	113	
dpm	554	1915	
cor. dpm	661	2671	ng/g
% body	20	33	cd zn
dpm/g	4523	18284	1.371229 31.30209

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	88	150	
dpm	309	2542	
cor. dpm	368	3546	ng/g
% body	11	44	cd zn
dpm/g	2519	24271	0.763722 41.55145

Dis. Control (cd only) t=6

pa 7 (c8)	
length	compos
wet wt.	0.1426
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	722	360	
dpm	2533	6102	
cor. dpm	3019	8510	
dpm/g	21173	59679	
µg/g	0.00642	0.102171	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	623	328	
dpm	2186	5559	
cor. dpm	2605	7754	
dpm/g	18270	54375	
µg/g	0.00554	0.093089	

% cpm recovered before fract	cd	zn
recovery	86.3	91.1

total cpm recovered	cd	zn
total	566	320

% cpm recovered after fract	cd	zn
recovery	90.9	97.6

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	10	14	
dpm	35	237	
cor. dpm	42	331	ng/g
% body	2	4	cd zn
dpm/g	293	2321	0.088917 3.973321

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	228	69	
dpm	800	1169	
cor. dpm	953	1631	ng/g
% body	37	21	cd zn
dpm/g	6686	11439	2.027301 19.58279

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	52	0	
dpm	182	0	
cor. dpm	217	0	ng/g
% body	8	0	cd zn
dpm/g	1525	0	0.462367 0

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	187	117	
dpm	656	1983	
cor. dpm	782	2766	ng/g
% body	30	36	cd zn
dpm/g	5484	19396	1.662743 33.20561

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	89	120	
dpm	312	2034	
cor. dpm	372	2837	ng/g
% body	14	37	cd zn
dpm/g	2610	19893	0.791359 34.05703

APPENDIX SIX

INDIVIDUAL REPLICATE CELLULAR DISTRIBUTIONS OF Cd AND Zn IN DDC
TREATMENT CLAMS FROM DI EXPERIMENT.
(FOR $t=0$ AND $t=6.06$ DAYS)

Dis. DDC (cd+ddc) t=0

pa 4 (d9)	
length	compos
wet wt.	0.1456
storage t	x
ignore	0
total t	125

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	73303	15682	
dpm	257204	265797	
cor. dpm	310247	379247	
dpm/g	2130815	2604722	
µg/g	0.646063	4.459283	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	60804	13998	
dpm	213347	237254	
cor. dpm	257346	338522	
dpm/g	1767487	2325015	
µg/g	0.535902	3.980426	

% cpm recovered before fract	cd	zn
recovery	82.9	89.3

total cpm recovered	cd	zn
total	57176	13374

% cpm recovered after fract	cd	zn
recovery	94.0	95.5

MRG- NaOH stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	698	570		
dpm	2449	9661		
cor. dpm	2954	13785	ng/g	
% body	1	4	cd	zn
dpm/g	20290	94675	6.151891	162.0834

MT-heat stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	17403	3392		
dpm	61063	57492		
cor. dpm	73656	82031	ng/g	
% body	29	24	cd	zn
dpm/g	505881	563399	153.383	964.5383

non-heat stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	20149	1452		
dpm	70698	24610		
cor. dpm	85278	35115	ng/g	
% body	33	10	cd	zn
dpm/g	585703	241172	177.5852	412.8861

tissue-NaOH dissolve				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	12082	4194		
dpm	42393	71085		
cor. dpm	51136	101426	ng/g	
% body	20	30	cd	zn
dpm/g	351207	696608	106.4859	1192.592

Intra- 100,000xg				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	6844	3766		
dpm	24014	63831		
cor. dpm	28966	91075	ng/g	
% body	11	27	cd	zn
dpm/g	198945	625519	60.32026	1070.888

Dis. DDC (cd+ddc) t=0

pa 5 (d10)	
length	compos.
wet wt.	0.1313
storage t	x
ignore	0
total t	125

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	77636	15234	
dpm	272407	258203	
cor. dpm	328586	368413	
dpm/g	2502556	2805889	
µg/g	0.758775	4.803682	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	62885	14213	
dpm	220649	240898	
cor. dpm	266154	343722	
dpm/g	2027065	2617835	
µg/g	0.614606	4.481733	

% cpm recovered before fract	cd	zn
recovery	81.0	93.3

total cpm recovered	cd	zn
total	58752	13577

% cpm recovered after fract	cd	zn
recovery	93.4	95.5

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	696	879	
dpm	2442	14898	
cor. dpm	2946	21257	ng/g
% body	1	6	cd zn
dpm/g	22435	161899	6.802351 277.1719

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	14083	1774	
dpm	49414	30068	
cor. dpm	59605	42902	ng/g
% body	22	12	cd zn
dpm/g	453958	326746	137.6401 559.3889

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	23445	2414	
dpm	82263	40915	
cor. dpm	99228	58379	ng/g
% body	37	17	cd zn
dpm/g	755737	444625	229.1396 761.1978

tissue- NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	13900	4274	
dpm	48772	72441	
cor. dpm	58830	103361	ng/g
% body	22	30	cd zn
dpm/g	448059	787211	135.8516 1347.705

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	6628	4236	
dpm	23256	71797	
cor. dpm	28052	102442	ng/g
% body	11	30	cd zn
dpm/g	213650	780212	64.77871 1335.722

Dis. DDC (cd+ddc) t=0

pa 6 (d11)	
length	compos.
wet wt.	0.1653
storage t	x
ignore	0
total t	125

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	84065	14796	
dpm	294965	250780	
cor. dpm	355796	357821	
dpm/g	2152423	2164675	
µg/g	0.652615	3.705924	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	69855	13963	
dpm	245105	236661	
cor. dpm	295653	337676	
dpm/g	1788587	2042806	
µg/g	0.542299	3.497284	

% cpm recovered before fract	cd	zn
recovery	83.1	94.4

total cpm recovered	cd	zn
total	66393	13187

% cpm recovered after fract	cd	zn
recovery	95.0	94.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1068	775	
dpm	3747	13136	
cor. dpm	4520	18742	ng/g
% body	2	6	cd zn
dpm/g	27345	113384	8.291115 194.1127

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	14282	2214	
dpm	50112	37525	
cor. dpm	60447	53543	ng/g
% body	20	16	cd zn
dpm/g	365680	323911	110.8743 554.536

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	27663	1695	
dpm	97063	28729	
cor. dpm	117081	40991	ng/g
% body	40	12	cd zn
dpm/g	708291	247981	214.7539 424.5432

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	15794	4182	
dpm	55418	70881	
cor. dpm	66846	101136	ng/g
% body	23	30	cd zn
dpm/g	404394	611832	122.6122 1047.457

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7586	4321	
dpm	26618	73237	
cor. dpm	32107	104497	ng/g
% body	11	31	cd zn
dpm/g	194234	632168	58.89176 1082.272

Dis. DDC (cd+ddc) t=0

pa 7 (d12)	
length	compos.
wet wt.	0.1644
storage t	x
ignore	0
total t	125

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	44082	8394	
dpm	154674	142271	
cor. dpm	186572	202997	
dpm/g	1134867	1234777	
µg/g	0.344092	2.113938	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	36229	7673	
dpm	127119	130051	
cor. dpm	153335	185561	
dpm/g	932696	1128716	
µg/g	0.282793	1.932362	

% cpm recovered before fract	cd	zn
recovery	82.2	91.4

total cpm recovered	cd	zn
total	33411	7436

% cpm recovered after fract	cd	zn
recovery	92.2	96.9

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	237	207	
dpm	832	3508	
cor. dpm	1003	5006	ng/g
% body	1	3	cd zn
dpm/g	6101	30450	1.849955 52.1307

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7590	948	
dpm	26632	16068	
cor. dpm	32124	22926	ng/g
% body	21	12	cd zn
dpm/g	195400	139453	59.24538 238.7435

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	14679	1733	
dpm	51505	29373	
cor. dpm	62127	41910	ng/g
% body	41	23	cd zn
dpm/g	377903	254928	114.5801 436.4372

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7571	2646	
dpm	26565	44847	
cor. dpm	32043	63990	ng/g
% body	21	34	cd zn
dpm/g	194911	389233	59.09707 666.3663

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3334	1902	
dpm	11698	32237	
cor. dpm	14111	45997	ng/g
% body	9	25	cd zn
dpm/g	85832	279789	26.02426 478.998

Dis. DDC (cd+ddc) t=6

pa 1 (d6)	
length	compos.
wet wt.	0.1553
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	15038	2546	
dpm	52765	43153	
cor. dpm	63076	60530	
dpm/g	406158	389761	
µg/g	0.123147	0.667271	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	13155	2431	
dpm	46158	41203	
cor. dpm	55178	57796	
dpm/g	355301	372156	
µg/g	0.107727	0.637131	

% cpm recovered before fract	cd	zn
recovery	87.5	95.5

total cpm recovered	cd	zn
total	12173	2282

% cpm recovered after fract	cd	zn
recovery	92.5	93.9

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	296	99	
dpm	1039	1678	
cor. dpm	1242	2354	ng/g
% body	2	4	cd zn
dpm/g	7995	15156	2.423964 25.9465

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	5132	316	
dpm	18007	5356	
cor. dpm	21526	7513	ng/g
% body	39	13	cd zn
dpm/g	138609	48376	42.02629 82.81914

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1243	17	
dpm	4361	288	
cor. dpm	5214	404	ng/g
% body	9	1	cd zn
dpm/g	33572	2602	10.17901 4.45546

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3656	704	
dpm	12828	11932	
cor. dpm	15335	16737	ng/g
% body	28	29	cd zn
dpm/g	98744	107774	29.93923 184.5085

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1846	1146	
dpm	6477	19424	
cor. dpm	7743	27246	ng/g
% body	14	47	cd zn
dpm/g	49858	175438	15.11702 300.3504

Dis. DDC (cd+ddc) t=6

pa 2 (d7)	
length	compos.
wet wt.	0.128
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	8819	1626	
dpm	30944	27559	
cor. dpm	36991	38657	
dpm/g	288992	302010	
µg/g	0.087622	0.517042	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	6957	1494	
dpm	24411	25322	
cor. dpm	29181	35519	
dpm/g	227976	277493	
µg/g	0.069122	0.475068	

% cpm recovered before fract		
	cd	zn
recovery	78.9	91.9

total cpm recovered		
	cd	zn
total	6532	1474

% cpm recovered after fract		
	cd	zn
recovery	93.9	98.7

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	149	86	
dpm	523	1458	
cor. dpm	625	2045	ng/g
% body	2	6	cd zn
dpm/g	4883	15973	1.480411 27.34661

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	536	92	
dpm	1881	1559	
cor. dpm	2248	2187	ng/g
% body	8	6	cd zn
dpm/g	17564	17088	5.325504 29.25452

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2552	133	
dpm	8954	2254	
cor. dpm	10704	3162	ng/g
% body	37	9	cd zn
dpm/g	83627	24703	25.35576 42.29186

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2371	629	
dpm	8319	10661	
cor. dpm	9945	14954	ng/g
% body	34	42	cd zn
dpm/g	77696	116829	23.55741 200.0119

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	924	534	
dpm	3242	9051	
cor. dpm	3876	12696	ng/g
% body	13	36	cd zn
dpm/g	30279	99184	9.180533 169.8034

Dis. DDC (cd+ddc) t=6

pa 3 (d8)	
length	compos
wet wt.	0.1557
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	21200	3682	
dpm	74386	62407	
cor. dpm	88923	87538	
dpm/g	571116	562220	
µg/g	0.173162	0.962521	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	17079	3366	
dpm	59926	57051	
cor. dpm	71637	80025	
dpm/g	460098	513969	
µg/g	0.139502	0.879915	

% cpm recovered before fract		
recovery	cd	zn
	80.6	91.4

total cpm recovered		
total	cd	zn
	15926	3255

% cpm recovered after fract		
recovery	cd	zn
	93.2	96.7

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	250	205	
dpm	877	3475	
cor. dpm	1049	4874	ng/g
% body	1	6	cd
dpm/g	6735	31302	2.042007 53.58958

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2957	574	
dpm	10375	9729	
cor. dpm	12403	13647	ng/g
% body	17	17	cd
dpm/g	79660	87647	24.15286 150.0508

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	6214	133	
dpm	21804	2254	
cor. dpm	26064	3162	ng/g
% body	36	4	cd
dpm/g	167401	20308	50.75613 34.76787

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	4243	1274	
dpm	14888	21593	
cor. dpm	17797	30289	ng/g
% body	25	38	cd
dpm/g	114304	194532	34.65695 333.0396

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2262	1069	
dpm	7937	18119	
cor. dpm	9488	25415	ng/g
% body	13	32	cd
dpm/g	60937	163230	18.47608 279.45

APPENDIX SEVEN

INDIVIDUAL REPLICATE CELLULAR DISTRIBUTIONS OF Cd AND Zn IN
CONTROL CLAMS FROM AE EXPERIMENT.
(FOR $t=0$ AND $t=2$ DAYS)

AE Control (Cd only) t=0

pa 1 (c6)	
length	compos
wet wt.	0.1321
storage t x	
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3675	2915	
dpm	12895	49407	
cor. dpm	15368	68910	
dpm/g	116340	521647	
µg/g	0.035274	0.89306	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3397	2645	
dpm	11919	44831	
cor. dpm	14206	62527	
dpm/g	107539	473330	
µg/g	0.032606	0.810341	

% cpm recovered before fract	cd	zn
recovery	92.4	90.7

total cpm recovered	cd	zn
total	3105	2603

% cpm recovered after fract	cd	zn
recovery	91.4	98.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	72	231	
dpm	253	3915	
cor. dpm	301	5461	ng/g
% body	2	9	cd zn
dpm/g	2279	41338	0.691087 70.77083

MT-heat stable		
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	371	136
dpm	1302	2305
cor. dpm	1551	3215
% body	11	5
dpm/g	11745	24338
		ng/g
		cd zn
		3.561018 41.66594

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1303	258	
dpm	4572	4373	
cor. dpm	5449	6099	ng/g
% body	38	10	cd zn
dpm/g	41249	46170	12.50675 79.04274

tissue-NaOH dissolved				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	995	744		
dpm	3491	12610		
cor. dpm	4161	17588	ng/g	
% body	29	28	cd	zn
dpm/g	31499	133141	9.550438	227.9372

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	364	1234	
dpm	1277	20915	
cor. dpm	1522	29171	ng/g
% body	11	47	cd zn
dpm/g	11523	220828	3.493829 378.0571

AE Control (Cd only) t=0

pa 2 (c7)	
length	compos
wet wt.	0.136
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	4203	3479	
dpm	14747	58966	
cor. dpm	17577	82242	
dpm/g	129239	604724	
µg/g	0.039185	1.035287	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3955	3198	
dpm	13877	54203	
cor. dpm	16539	75600	
dpm/g	121613	555880	
µg/g	0.036873	0.951666	

% cpm recovered before fract		
	cd	zn
recovery	94.1	91.9

total cpm recovered		
	cd	zn
total	3472	2835

% cpm recovered after fract		
	cd	zn
recovery	87.8	88.6

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	103	188	
dpm	361	3186	
cor. dpm	431	4444	ng/g
% body	3	6	cd zn
dpm/g	3167	32678	0.960288 55.94536

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	326	135	
dpm	1144	2288	
cor. dpm	1363	3191	ng/g
% body	8	4	cd zn
dpm/g	10024	23466	3.039357 40.17353

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1390	197	
dpm	4877	3339	
cor. dpm	5813	4657	ng/g
% body	35	6	cd zn
dpm/g	42741	34243	12.95922 58.62359

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1209	783	
dpm	4242	13271	
cor. dpm	5056	18510	ng/g
% body	31	24	cd zn
dpm/g	37176	136102	11.27173 233.0065

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	444	1532	
dpm	1558	25966	
cor. dpm	1857	36216	ng/g
% body	11	48	cd zn
dpm/g	13653	266294	4.139492 455.8951

AE Control (cd only) t=0

pa 3 (c8)	
length	compos
wet wt.	0.1364
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	8040	6345	
dpm	28211	107542	
cor. dpm	33622	149994	
dpm/g	246499	1099660	
µg/g	0.074739	1.882619	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7527	5737	
dpm	26411	97237	
cor. dpm	31477	135621	
dpm/g	230771	994287	
µg/g	0.06997	1.70222	

% cpm recovered before fract	cd	zn
recovery	93.6	90.4

total cpm recovered	cd	zn
total	6957	5519

% cpm recovered after fract	cd	zn
recovery	92.4	96.2

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	174	471	
dpm	611	7983	
cor. dpm	728	11134	ng/g
% body	2	8	cd zn
dpm/g	5335	81630	1.617476 139.7499

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	602	252	
dpm	2112	4271	
cor. dpm	2518	5957	ng/g
% body	8	4	cd zn
dpm/g	18457	43674	5.596096 74.77067

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2640	546	
dpm	9263	9254	
cor. dpm	11040	12907	ng/g
% body	35	10	cd zn
dpm/g	80940	94628	24.54102 162.0031

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2636	2034	
dpm	9249	34475	
cor. dpm	11023	48083	ng/g
% body	35	35	cd zn
dpm/g	80817	352515	24.50383 603.5061

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	905	2216	
dpm	3175	37559	
cor. dpm	3785	52386	ng/g
% body	12	39	cd zn
dpm/g	27746	384058	8.412735 657.5072

AE Control (cd only) t=0

pa 4 (c9)	
length	compos
wet wt.	0.1407
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	4409	2946	
dpm	15470	49932	
cor. dpm	18438	69642	
dpm/g	131045	494971	
µg/g	0.039733	0.847391	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3637	2666	
dpm	12761	45186	
cor. dpm	15210	63023	
dpm/g	108099	447927	
µg/g	0.032776	0.766851	

% cpm recovered before fract	
cd	zn
recovery	82.5 90.5

total cpm recovered	
cd	zn
total	3434 2658

% cpm recovered after fract	
cd	zn
recovery	94.4 99.7

	MRG- NaOH stable	
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	89	253
dpm	312	4288
cor. dpm	372	5981
% body	2	9
dpm/g	2645	42508
	ng/g	
	cd	zn
	0.802045	72.77321

	MT-heat stable	
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	338	162
dpm	1186	2746
cor. dpm	1413	3830
% body	9	6
dpm/g	10046	27218
	ng/g	
	cd	zn
	3.04597	46.59787

	non-heat stable	
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	1373	245
dpm	4818	4153
cor. dpm	5742	5792
% body	38	9
dpm/g	40808	41164
	ng/g	
	cd	zn
	12.37313	70.47208

	tissue-NaOH dissolve	
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	1174	851
dpm	4119	14424
cor. dpm	4910	20117
% body	32	32
dpm/g	34894	142980
	ng/g	
	cd	zn
	10.57979	244.7826

	Intra- 100,000xg	
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	460	1147
dpm	1614	19441
cor. dpm	1924	27115
% body	13	43
dpm/g	13672	192713
	ng/g	
	cd	zn
	4.145403	329.9244

AE Control (cd only) t=2

pa 1 (c2)	
length	compos
wet wt.	0.102
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3921	1501	
dpm	13758	25441	
cor. dpm	16447	35686	
dpm/g	161240	349858	
µg/g	0.048888	0.598957	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3270	1302	
dpm	11474	22068	
cor. dpm	13716	30954	
dpm/g	134470	303474	
µg/g	0.040771	0.519548	

% cpm recovered before fract		
	cd	zn
recovery	83.4	86.7

total cpm recovered		
	cd	zn
total	3060	1314

% cpm recovered after fract		
	cd	zn
recovery	93.6	100.9

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	151	153	
dpm	530	2593	
cor. dpm	633	3637	ng/g
% body	5	12	cd zn
dpm/g	6209	35662	1.882707 61.05291

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	194	46	
dpm	681	780	
cor. dpm	814	1094	ng/g
% body	6	4	cd zn
dpm/g	7978	10722	2.418842 18.35578

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	652	65	
dpm	2288	1102	
cor. dpm	2735	1545	ng/g
% body	20	5	cd zn
dpm/g	26812	15150	8.129303 25.93751

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1567	588	
dpm	5498	9966	
cor. dpm	6573	13979	ng/g
% body	48	45	cd zn
dpm/g	64439	137053	19.53776 234.6347

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	496	462	
dpm	1740	7831	
cor. dpm	2080	10984	ng/g
% body	15	35	cd zn
dpm/g	20397	107684	6.184255 184.3558

AE Control (cd only) t=2

pa 2 (c3)	
length	compos.
wet wt.	0.1129
storage t	x
ignore	0
total t	1.19

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2244	948	
dpm	7874	16068	
cor. dpm	9412	22538	
dpm/g	83369	199630	
µg/g	0.025278	0.341766	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1873	873	
dpm	6572	14797	
cor. dpm	7856	20755	
dpm/g	69586	183836	
µg/g	0.021098	0.314728	

% cpm recovered before fract	cd	zn
recovery	83.5	92.1

total cpm recovered	cd	zn
total	1735	816

% cpm recovered after fract	cd	zn
recovery	92.6	93.5

MRG- NaOH stable		
	cd	zn
count eff.	0.285	0.059
t 1/2	462	243.7
cpm	81	50
dpm	284	847
cor. dpm	340	1189
% body	4	6
dpm/g	3009	10529
	ng/g	
	cd	zn
	0.912425	18.02566

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	151	32	
dpm	530	542	
cor. dpm	633	761	ng/g
% body	8	4	cd zn
dpm/g	5610	6739	1.70094 11.53642

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	386	45	
dpm	1354	763	
cor. dpm	1619	1070	ng/g
% body	21	5	cd zn
dpm/g	14341	9476	4.348097 16.22309

tissue-NaOH dissolve				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	879	420		
dpm	3084	7119		
cor. dpm	3687	9985	ng/g	
% body	47	48	cd	zn
dpm/g	32657	88444	9.901496	151.4155

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	238	269	
dpm	835	4559	
cor. dpm	998	6395	ng/g
% body	13	31	cd zn
dpm/g	8842	56646	2.680951 96.97804

AE Control (cd only) t=2

pa 3 (c4)	
length	compos.
wet wt.	0.1017
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1337	757	
dpm	4691	12831	
cor. dpm	5608	17997	
dpm/g	55143	176965	
µg/g	0.016719	0.302963	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1151	703	
dpm	4039	11915	
cor. dpm	4828	16713	
dpm/g	47471	164341	
µg/g	0.014393	0.281352	

% cpm recovered before fract	cd	zn
recovery	86.1	92.9

total cpm recovered	cd	zn
total	1078	632

% cpm recovered after fract	cd	zn
recovery	93.7	89.9

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	31	49	
dpm	109	831	
cor. dpm	130	1165	ng/g
% body	3	7	cd zn
dpm/g	1279	11455	0.387656 19.61057

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	116	46	
dpm	407	780	
cor. dpm	487	1094	ng/g
% body	10	7	cd zn
dpm/g	4784	10753	1.450584 18.40992

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	290	32	
dpm	1018	542	
cor. dpm	1216	761	ng/g
% body	25	5	cd zn
dpm/g	11961	7481	3.62646 12.8069

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	486	300	
dpm	1705	5085	
cor. dpm	2039	7132	ng/g
% body	42	43	cd zn
dpm/g	20044	70131	6.077448 120.0647

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	155	205	
dpm	544	3475	
cor. dpm	650	4874	ng/g
% body	13	29	cd zn
dpm/g	6393	47923	1.938281 82.04422

AE Control (cd only) t=2

pa 4 (c5)	
length	compos.
wet wt.	0.1007
storage t	x
ignore	0
total t	1.19

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2101	752	
dpm	7372	12746	
cor. dpm	8813	17878	
dpm/g	87513	177541	
µg/g	0.026534	0.303951	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1737	706	
dpm	6095	11966	
cor. dpm	7286	16785	
dpm/g	72351	166681	
µg/g	0.021937	0.285358	

% cpm recovered before fract	cd	zn
recovery	82.7	93.9

total cpm recovered	cd	zn
total	1593	646

% cpm recovered after fract	cd	zn
recovery	91.7	91.5

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	86	90	
dpm	302	1525	
cor. dpm	361	2140	ng/g
% body	5	13	cd zn
dpm/g	3582	21248	1.086113 36.3771

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	152	29	
dpm	533	492	
cor. dpm	638	689	ng/g
% body	9	4	cd zn
dpm/g	6331	6847	1.919641 11.72151

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	350	18	
dpm	1228	305	
cor. dpm	1468	428	ng/g
% body	20	3	cd zn
dpm/g	14579	4250	4.420226 7.275421

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	775	310	
dpm	2719	5254	
cor. dpm	3251	7370	ng/g
% body	45	44	cd zn
dpm/g	32281	73189	9.787643 125.2989

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	230	199	
dpm	807	3373	
cor. dpm	965	4731	ng/g
% body	13	28	cd zn
dpm/g	9580	46982	2.90472 80.43382

APPENDIX EIGHT

INDIVIDUAL REPLICATE CELLULAR DISTRIBUTIONS OF Cd AND Zn IN DDC
TREATMENT CLAMS FROM AE EXPERIMENT.
(FOR t=0 AND t=2 DAYS)

AE DDC (cd&ddc) t=0

pa 5 (d6)	
length	compos
wet wt.	0.1596
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7868	3664	
dpm	27607	62102	
cor. dpm	32903	86616	
dpm/g	206160	542705	
µg/g	0.062508	0.929111	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7175	3316	
dpm	25175	56203	
cor. dpm	30005	78389	
dpm/g	188002	491160	
µg/g	0.057002	0.840866	

% cpm recovered before fract	cd	zn
recovery	91.2	90.5

total cpm recovered	cd	zn
total	6718	3230

% cpm recovered after fract	cd	zn
recovery	93.6	97.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	458	256	
dpm	1607	4339	
cor. dpm	1915	6052	ng/g
% body	6	8	cd zn
dpm/g	12001	37918	3.638611 64.91606

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	506	236	
dpm	1775	4000	
cor. dpm	2116	5579	ng/g
% body	7	7	cd zn
dpm/g	13258	34956	4.01995 59.8445

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1532	286	
dpm	5375	4847	
cor. dpm	6407	6761	ng/g
% body	21	9	cd zn
dpm/g	40142	42362	12.17107 72.52342

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3549	1027	
dpm	12453	17407	
cor. dpm	14842	24278	ng/g
% body	49	31	cd zn
dpm/g	92992	152117	28.19526 260.425

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	673	1425	
dpm	2361	24153	
cor. dpm	2814	33687	ng/g
% body	9	43	cd zn
dpm/g	17634	211068	5.346692 361.3492

AE DDC (cd&ddc) t=0

pa 7 (d8)	
length	compos
wet wt.	0.1238
storage t	x
ignore	0
total t	117

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3960	1714	
dpm	13895	29051	
cor. dpm	16560	40518	
dpm/g	133767	327289	
µg/g	0.040558	0.560319	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3380	1506	
dpm	11860	25525	
cor. dpm	14135	35601	
dpm/g	114175	287571	
µg/g	0.034618	0.492322	

% cpm recovered before fract		
	cd	zn
recovery	85.4	87.9

total cpm recovered		
	cd	zn
total	3072	1452

% cpm recovered after fract		
	cd	zn
recovery	90.9	96.4

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	195	100	
dpm	684	1695	
cor. dpm	815	2364	ng/g
% body	6	7	cd zn
dpm/g	6587	19095	1.997179 32.69072

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	187	83	
dpm	656	1407	
cor. dpm	782	1962	ng/g
% body	6	6	cd zn
dpm/g	6317	15849	1.915243 27.1333

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	681	129	
dpm	2389	2186	
cor. dpm	2848	3050	ng/g
% body	20	9	cd zn
dpm/g	23004	24633	6.974763 42.17103

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1642	512	
dpm	5761	8678	
cor. dpm	6867	12104	ng/g
% body	49	34	cd zn
dpm/g	55466	97767	16.81727 167.3765

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	367	628	
dpm	1288	10644	
cor. dpm	1535	14846	ng/g
% body	11	42	cd zn
dpm/g	12397	119917	3.758793 205.2977

AE DDC (cd&ddc) t=0

pa 8 (d9)	
length	compos
wet wt.	0.1552
storage t	x
ignore	0
total t	1:17

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	7042	2895	
dpm	24709	49068	
cor. dpm	29449	68437	
dpm/g	189748	440959	
µg/g	0.057532	0.754922	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	5841	2740	
dpm	20495	46441	
cor. dpm	24426	64773	
dpm/g	157387	417350	
µg/g	0.04772	0.714503	

% cpm recovered before fract		
cd	zn	
recovery	82.9	94.6

total cpm recovered		
cd	zn	
total	5436	2590

% cpm recovered after fract		
cd	zn	
recovery	93.1	94.5

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	603	293	
dpm	2116	4966	
cor. dpm	2522	6926	ng/g
% body	10	11	cd zn
dpm/g	16248	44629	4.926388 76.40486

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	434	152	
dpm	1523	2576	
cor. dpm	1815	3593	ng/g
% body	7	6	cd zn
dpm/g	11694	23152	3.545692 39.63665

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	980	177	
dpm	3439	3000	
cor. dpm	4098	4184	ng/g
% body	17	6	cd zn
dpm/g	26406	26960	8.006402 46.15584

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2770	839	
dpm	9719	14220	
cor. dpm	11584	19834	ng/g
% body	47	31	cd zn
dpm/g	74638	127794	22.63034 218.7839

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	649	1129	
dpm	2277	19136	
cor. dpm	2714	26689	ng/g
% body	11	41	cd zn
dpm/g	17487	171966	5.302199 294.4065

AE DDC (metal and ddc) t=2

pa 5 (d1)	
length	compos.
wet wt.	0.1296
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	3295	1583	
dpm	11561	26831	
cor. dpm	13821	37635	
dpm/g	106642	290394	
µg/g	0.032334	0.497154	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2614	1469	
dpm	9172	24898	
cor. dpm	10964	34925	
dpm/g	84601	269481	
µg/g	0.025651	0.461351	

% cpm recovered before fract	cd	zn
recovery	79.3	92.8

total cpm recovered	cd	zn
total	2465	1381

% cpm recovered after fract	cd	zn
recovery	94.3	94.0

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	107	167	
dpm	375	2831	
cor. dpm	449	3970	ng/g
% body	4	11	cd zn
dpm/g	3463	30635	1.049989 52.44771

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	199	54	
dpm	698	915	
cor. dpm	835	1284	ng/g
% body	8	4	cd zn
dpm/g	6441	9906	1.952783 16.95914

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	643	119	
dpm	2256	2017	
cor. dpm	2697	2829	ng/g
% body	25	8	cd zn
dpm/g	20811	21830	6.309746 37.37292

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1175	640	
dpm	4123	10847	
cor. dpm	4928	15216	ng/g
% body	45	44	cd zn
dpm/g	38029	117405	11.53025 200.9972

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	341	401	
dpm	1196	6797	
cor. dpm	1430	9534	ng/g
% body	13	27	cd zn
dpm/g	11036	73562	3.346226 125.9373

AE DDC (metal and ddc) t=2

pa 6 (d2)	
length	compos
wet wt.	0.1206
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	2330	1219	
dpm	8175	20661	
cor. dpm	9773	28981	
dpm/g	81037	240308	
µg/g	0.024571	0.411407	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1985	1178	
dpm	6965	19966	
cor. dpm	8326	28006	
dpm/g	69038	232225	
µg/g	0.020932	0.397569	

% cpm recovered before fract	cd	zn
recovery	85.2	96.6

total cpm recovered	cd	zn
total	1800	1084

% cpm recovered after fract	cd	zn
recovery	90.7	92.0

MRG- NaOH stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	98	177		
dpm	344	3000		
cor. dpm	411	4208	ng/g	
% body	5	15	cd	zn
dpm/g	3408	34893	1.033439	59.73667

MT-heat stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	387	73		
dpm	1358	1237		
cor. dpm	1623	1736	ng/g	
% body	19	6	cd	zn
dpm/g	13460	14391	4.081028	24.63716

non-heat stable				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	156	2		
dpm	547	34		
cor. dpm	654	48	ng/g	
% body	8	0	cd	zn
dpm/g	5426	394	1.645065	0.674991

tissue-NaOH dissolve				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	906	520		
dpm	3179	8814		
cor. dpm	3800	12363	ng/g	
% body	46	44	cd	zn
dpm/g	31511	102510	9.554034	175.4976

Intra- 100,000xg				
	cd	zn		
count eff.	0.285	0.059		
t 1/2	462	243.7		
cpm	253	312		
dpm	888	5288		
cor. dpm	1061	7418	ng/g	
% body	13	26	cd	zn
dpm/g	8799	61506	2.667959	105.2985

AE DDC (metal and ddc) t=2

pa 7 (d3)	
length	compos
wet wt.	0.1099
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1777	689	
dpm	6235	11678	
cor. dpm	7454	16381	
dpm/g	67821	149050	
µg/g	0.020563	0.255174	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1519	615	
dpm	5330	10424	
cor. dpm	6371	14621	
dpm/g	57974	133042	
µg/g	0.017578	0.227768	

% cpm recovered before fract		
	cd	zn
recovery	85.5	89.3

total cpm recovered		
	cd	zn
total	1353	615

% cpm recovered after fract		
	cd	zn
recovery	89.1	100.0

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	64	71	
dpm	225	1203	
cor. dpm	268	1688	ng/g
% body	4	12	cd zn
dpm/g	2443	15359	0.740608 26.29515

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	161	45	
dpm	565	763	
cor. dpm	675	1070	ng/g
% body	11	7	cd zn
dpm/g	6145	9735	1.863091 16.66594

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	374	32	
dpm	1312	542	
cor. dpm	1569	761	ng/g
% body	25	5	cd zn
dpm/g	14274	6923	4.327926 11.85134

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	598	323	
dpm	2098	5475	
cor. dpm	2508	7679	ng/g
% body	39	53	cd zn
dpm/g	22823	69874	6.920053 119.6244

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	156	144	
dpm	547	2441	
cor. dpm	654	3424	ng/g
% body	10	23	cd zn
dpm/g	5954	31151	1.805231 53.33102

AE DDC (metal and ddc) t=2

pa 8 (d4)	
length	compos:
wet wt.	0.0827
storage t	x
ignore	0
total t	119

post dis	cd	pre hom	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1800	721	
dpm	6316	12220	
cor. dpm	7550	17141	
dpm/g	91294	207272	
µg/g	0.02768	0.35485	

post hom	cd	pre fract	zn
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	1429	654	
dpm	5014	11085	
cor. dpm	5994	15549	
dpm/g	72478	188011	
µg/g	0.021975	0.321875	

% cpm recovered before fract	cd	zn
recovery	79.4	90.7

total cpm recovered	cd	zn
total	1324	588

% cpm recovered after fract	cd	zn
recovery	92.7	89.9

MRG- NaOH stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	49	28	
dpm	172	475	
cor. dpm	206	666	ng/g
% body	3	4	cd zn
dpm/g	2485	8049	0.753523 13.78058

MT-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	196	34	
dpm	688	576	
cor. dpm	822	808	ng/g
% body	14	5	cd zn
dpm/g	9941	9774	3.014092 16.73357

non-heat stable			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	137	8	
dpm	481	136	
cor. dpm	575	190	ng/g
% body	10	1	cd zn
dpm/g	6949	2300	2.106789 3.937309

tissue-NaOH dissolve			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	713	297	
dpm	2502	5034	
cor. dpm	2991	7061	ng/g
% body	50	45	cd zn
dpm/g	36163	85381	10.96453 146.1726

Intra- 100,000xg			
	cd	zn	
count eff.	0.285	0.059	
t 1/2	462	243.7	
cpm	229	221	
dpm	804	3746	
cor. dpm	961	5254	ng/g
% body	16	34	cd zn
dpm/g	11615	63533	3.521566 108.7682

Subject:
Re: Uptake of Lipophilic Organics
Date:
Wed, 10 Mar 1999 14:27:49 -0800
From:
Ken Bruland <bruland@cats.ucsc.edu>
To:
"Christopher Wellise, Physical Scientist, Menlo Park, CA" <wellise>

Christopher,

Yes, by all means go ahead. You have my permission. I'd enjoy seeing your thesis some day. I'm not sure what the rules are with respect to ES&T. I never have paid much attention to those details. But, for whatever it is worth, you have my permission. Ken Bruland.

>Dear Mr. Bruland:

>

>I am writing you because I would like to request your permission to
>highlight the concepts outlined in your figure (7) from your ES&T
>publication (vol. 28, No. 11, 1996 Phinney and Bruland).

>

>I conducted my masters thesis research on the effects of DDC on bivalve
>metal accumulation, and feel that your figure would add a strong
>conceptual element to my work.
>I, of course, would cite the source figure accordingly.
>Please let me know if you approve my use of this figure.

>Sincerely,
>>Christopher Wellise.

Professor Ken Bruland
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IMAGE EVALUATION TEST TARGET (QA-3)

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