



## METALS IN THE AQUATIC FOOD WEB: BIOAVAILABILITY AND TOXICITY TO FISH

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### *Dietborne Metals—Bioavailability and Toxicity to Fish*

Early research on dietborne metals and fish was driven by the aquaculture industry's interest in factors that affect the provision of essential metals such as Cu and Zn to cultured fish. These studies examined the concentrations of essential elements required by different species in practical fish food.

#### *Terminology*

**Dietborne metals:** Fish (like other vertebrates) require low concentrations of some metals (e.g. Cu, Zn) in their diet to remain healthy. The term "dietborne" is used in toxicology to describe metal exposures above required nutritional limits and to differentiate this route from water-to-gill exposures.

**Purified vs. practical diets:** Purified diets, consisting of purified forms of protein (usually casein or egg white) and other essential ingredients, are used in dietary research to simplify the ingredients being fed to a test organism. Purified diets are usually expensive and difficult to feed to fish because they are less palatable than regular diets and dissolve readily in water. Practical diets are the familiar form of food fed as pellets to farmed fish. Practical diets usually contain a protein source such as fish, beef, blood or soybean meal that may contain many other constituents, such as ash, that would complicate the interpretation of a nutritional study.

A few examined the metal concentrations required to cause toxicity, defined here as negative effects on growth, survival or reproduction (Table 1). This research shows that dietborne metal toxicity depends on many factors such as the fish species, life stage and diet composition. Therefore, toxicity thresholds are difficult to define.

The research on dietborne copper (Cu) toxicity is a good example. One study showed that only 16 mg Cu kg<sup>-1</sup> dry diet was toxic to channel catfish, while a later study showed no toxic effects to catfish fed 40 mg Cu kg<sup>-1</sup> dry diet. In yet another example, the threshold for copper toxicity to rainbow trout may lie between 500-664 mg Cu kg<sup>-1</sup> dry diet, depending on the life stage (e.g. fry, fingerling or adult) and daily dose. There is contradictory evidence for the threshold of copper toxicity for Atlantic salmon; it may be less than 34 mg Cu kg<sup>-1</sup> dry diet, or between 100-467 mg Cu kg<sup>-1</sup> dry diet. The data on gurnard indicate this species may tolerate 2000 mg Cu kg<sup>-1</sup> dry diet.

### *Dietborne Metals in Polluted Environments*

Recently, there has been interest in dietborne metal bioavailability and toxicity in the natural environment. It had long been assumed that metal-contaminated diets prepared in the laboratory were comparable to natural diets contaminated with metals in polluted environments. However, in the mid 1990s, a series of studies where metal-contaminated invertebrates from the Clark Fork River in Montana, USA were fed to juvenile brown and rainbow trout demonstrated toxicity at metal concentrations far below those

**Table 1**

The effect of different concentrations of dietborne Cu on different fish species, at different life stages. Toxicity is indicated by negative (x) effects on growth (G) or survival (S). None of the studies examined potential effects of metals on reproduction, and daily dose is a more useful parameter than dietborne metal concentration for predicting toxicity to fish.

0 indicates no effect of diet on parameter, - indicates the parameter was not examined or reported upon, + indicates positive effect of diet on parameter. Data summarized from Clearwater et al. (*in prep*).

Species	Life Stage	Diet	[Cu] (mg Cu kg <sup>-1</sup> dry diet)	Daily Dose (mg Cu g <sup>-1</sup> body weight d <sup>-1</sup> )	Toxicity	
					G	S
channel catfish <sup>1</sup>	fingerlings	purified (casein)	16	0.4	x	-
channel catfish <sup>1</sup>	fingerlings	semi-purified (egg white)	40	1	0	0
rainbow trout <sup>2</sup>	fingerlings	purified (casein)	150	3	0	0
rainbow trout <sup>2</sup>	fingerlings	purified (casein)	500	10	0	0
rainbow trout <sup>2</sup>	juvenile	purified (casein)	990	20	x	0
rainbow trout <sup>2</sup>	fry	practical (fish-soy meal)	664	27	x	0
rainbow trout <sup>2</sup>	fry	practical (fish-soy meal)	730	44	x	x
rainbow trout <sup>2</sup>	fry	practical (fish-soy meal)	800	54	x	0
Atlantic salmon <sup>3</sup>	parr	practical (fish-wheat meal)	34	1	x	-
Atlantic salmon <sup>3</sup>	parr	practical (fish-corn meal)	100	2	0	-
Atlantic salmon <sup>3</sup>	fry	practical (fish-wheat meal)	≥467	16	x	0
Atlantic salmon <sup>3</sup>	parr	practical (fish-wheat meal)	691	17	x	-
dab <sup>4</sup>	adult	purified (casein)	200	2	0	0
gurnard <sup>5</sup>	juvenile/adult	polychaete worms	2000	20-40	0	-
grey mullet <sup>6</sup>	juvenile	practical (fish meal)	2400	60	x	0

<sup>1</sup>*Ictalurus punctatus*, <sup>2</sup>*Oncorhynchus mykiss*, <sup>3</sup>*Salmo salar*, <sup>4</sup>*Limanda limanda*, <sup>5</sup>*Trigla lucerna*, <sup>6</sup>*Chelon labrosus*

required to cause toxicity in laboratory prepared diets (Table 2). In addition, the natural diets caused a toxic response, even though waterborne metal concentrations were not above US EPA Water Quality Criteria. Previously, it had been assumed that as long as waterborne metals were kept at non-toxic levels, dietborne metals would be unlikely to cause toxicity since it generally takes 1000- to 100 000-fold higher concentrations of metal in food than in water to have toxic effects on fish. Thus, the Clark Fork River studies have shown that some metals may concentrate in dietary items to levels that cause toxicity and that natural exposures differ in some manner from laboratory-prepared diets.

**Table 2**

Dietborne metal concentrations (mg kg<sup>-1</sup> dry diet) toxic to rainbow trout in either laboratory-prepared diets or in invertebrates taken from the Clark Fork River. Because a mixture of metals was present in the Clark Fork River invertebrates, toxicity cannot be attributed to one metal alone, whereas only one metal was present in the laboratory diets. Despite this, the difference in toxic concentrations is surprising.

Metal	Laboratory-prepared diets	Clark Fork River invertebrates
Cadmium	2265	<1
Copper	664	174
Zinc	>1010	648

Why do laboratory studies of dietborne metals sometimes fail to mimic the effects of dietborne metals on fish in natural environments?

There are two main theories that explain why the naturally contaminated invertebrates appear to be more toxic than laboratory-prepared diets:

**Theory 1. Biological Incorporation of Metals**—Metals in natural diets are “biologically incorporated” into the prey organisms (e.g. in proteins) in ways that make them more “bioavailable” or easily absorbed by the fish. Usually (but not always), if the fish can absorb more of the metal, it will be more toxic to the fish. In contrast, the metals added to laboratory diets are usually added as metal salts (e.g.  $\text{CuSO}_4$ ,  $\text{PbNO}_3$ ), which might not be as easily absorbed by fish or are less “bioactive” once they are inside the fish. These differences between natural and laboratory diets might make laboratory diets less toxic than natural diets.

Sometimes, however, biological incorporation of metals in natural diets will actually decrease the toxicity of metals to fish. For example, some polychaete worms can store high concentrations of metals in insoluble storage granules. These insoluble granules protect the worm from the toxic effects of the metals and may be indigestible and non-toxic to fish that eat the worms (e.g. gurnard, Table 1).

**Theory 2. Indirect Effects of Metals**—Metals in natural diets are not directly responsible for the decreased growth and survival of fish; instead, differences in the species composition of the invertebrate diets, or perhaps decreased health of the invertebrates due to the metals, made them an inferior diet (e.g. decreased fat and protein content). In other words, the decreased fish growth and survival were due to differences in diet quality rather than direct metal toxicity.

**Theories 1 and 2 combined**—In fact, biological incorporation of metals and decreased diet quality might act together to negatively affect fish that consume metal-contaminated diets from natural environments. Also, other factors might increase the toxicity of the natural diets. For example, fish are most often exposed to a mixture of metals in natural diets, not just a single metal in high concentrations.

**Follow-up studies to the Clark Fork River research:** Theory 2 (indirect effects of metals) has

been tested in laboratory studies using live prey that were artificially contaminated with metals to the same concentration as the organisms collected from the Clark Fork River. In theory, the organisms used in the preparation of metal-contaminated and control diets in the laboratory should be very similar, thus avoiding the variability encountered when using field-collected diets (e.g. invertebrate species, size, health). Some of these lab studies using live prey have shown low toxicity of dietborne metals, which supports the hypothesis that the type of invertebrates making up the field-collected diets was the main cause for decreased growth and fish survival rather than direct metal toxicity. However, these lab studies have been criticized because it is difficult to create metal-contaminated live prey in the laboratory in a biologically realistic manner. Most metal-contaminated invertebrates are exposed in the laboratory by being “dipped” into water containing a very high concentration of metals. Compositional analysis of “dipped” lab invertebrates shows that their metals distribution can differ from that found in wild invertebrates. The wild invertebrates probably have taken up metals from their diet as well as from the water (Munger and Hare 1997), and have been exposed over their entire life span rather than via a 24-to-72-hour bath in waterborne metals.

### *Ingestion of Sediments*

Fish can be exposed to metals in the sediment as well as to metals in food. Incidental ingestion of sediments increases dietborne exposure of fish to metals. Many fish tend not only to consume prey items such as worms and shellfish, but also to deliberately ingest sediments as a feeding strategy and/or to incidentally ingest significant quantities of sediment that are in the digestive tract of the invertebrates, attached to the surface of the invertebrates or in the water when the invertebrate is sucked into the fish’s mouth.

Since sediments tend to accumulate high concentrations of metals, the incidental ingestion of sediment might constitute a significant dietborne metal exposure for some species. It is not known whether metals bound to sediment particles are bioavailable to fish, and most laboratory studies of fish and dietborne metals fail to consider this potential source of metal exposure.

### *Evidence for Factors that Alter Dietborne Metal Bioavailability and Toxicity*

The mechanisms that control dietborne metal bioavailability and toxicity are not well understood. The following are some examples of important influences on dietborne metal bioavailability and toxicity.

- There is evidence that metal partitioning within a prey organism can strongly influence its bioavailability to juvenile fish. Reinfelder and Fisher (1994) fed marine crustaceans containing radioactive metals to fish and showed that dietborne metal bioavailability correlated most strongly with the metal concentrations in the soft tissues of the crustaceans, even though the majority of the dietborne metal was associated with the exoskeleton of the contaminated prey.
- Chelation of metals with absorbable compounds, for example amino acids (e.g. Cu-methionine), may improve bioavailability for intestinal uptake. Paripatananont and Lovell (1997) compared the relative bioavailabilities of chelated and inorganic forms of five minerals in purified or practical diets fed to channel catfish (*Ictalurus punctatus*). Chelation of Cu, Cd, Zn or Fe to amino acids improved the absorption of the mineral from purified (egg white-based) diets on average by 38%, and even more so from practical (soybean-based) diets (on average by 95%). Chelation of the metals to absorbable compounds probably prevents non-absorbable compounds such as phytate from binding to the metals, thereby preventing intestinal absorption. Such a mechanism would explain why chelation enhanced absorption from the practical feed (with more ingredients) to a greater extent than from the purified feed.
- Bioavailability may decrease as metal concentration increases. Many different studies have shown that as Cu and Zn concentrations increase in the diet, a smaller percentage of the metal is retained, which indicates that fish are capable of regulating Cu and Zn uptake across the intestine. Beyond a critical dietary concentration, however, the proportion of assimilated metal increases with increasing dietary concentrations, indicating that regulatory mechanisms within the intestine are compromised. This has been shown for Cu and Zn, which are both essential elements; it is not known if the same relationship exists for nonessential elements such as Cd.

- Since uptake and excretion occur continuously in fish, the various depuration mechanisms used to excrete metals are important in determining overall retention of metals by fish. One of the primary excretion mechanisms occurs as metal accumulates in the cells of the intestinal tract, which then periodically slough off into the gut lumen in the normal process of cell turnover; this tissue is then excreted in the feces. Mucus secreted by the intestinal tract may be extremely important in protecting the fish from excess dietborne metals. There is evidence that excess dietborne Cu precipitates in intestinal mucus (Handy et al. 2000), thus rendering it non-bioavailable for absorption by the intestinal cells. Other possible excretory paths include urine (i.e. via the kidney), bile (i.e. via the liver and gall bladder), the gills and skin.

### *Can We Apply Laboratory-Derived Effects Concentrations to Protect Fish in Nature?*

Although numerous laboratory studies show threshold concentrations for dietborne metal toxicity to fish, recent field studies have shown that these laboratory studies might significantly underestimate metal toxicity. Current research efforts are directed at understanding factors that control dietborne metal bioavailability and at developing experimental diets that more closely mimic those in the natural environment (Table 3).

***Lab diets created by exposure of live prey organisms to metal:*** Some of the most promising laboratory studies are using metal-contaminated diets created by exposing live fish prey (e.g. worms, insect larvae) to metals in a manner as close as possible to the metal exposure they would experience in a natural environment (i.e. long-term, and via food, sediment and water). These studies should predict toxic dietborne concentrations in natural environments more accurately than previous studies that have used “metal-spiked” fish feed.

***Realistic exposure of invertebrates to sediments:*** Another field of research is exploring how invertebrates relate to their biotic environment in ways that have not been adequately accounted for in laboratory-based research. For example, research has shown that some benthic invertebrates select nutrient-rich sediment particles (Schlekat et al. 2001). Certain particle characteristics that make them nutri-

**Table 3**

Design considerations and recommendations for future dietborne metal studies and interpretation of past studies.

Study Design Parameter	Consideration/Recommendations
Metal dose	Rations should be reported so that daily dose can be calculated as a function of body weight.
Water quality	Can influence dietborne metal uptake, therefore report hardness, pH, alkalinity, dissolved organic carbon, salinity, temperature, flow rates, metal concentrations and major anions and cations.
High metal concentration in diet	Leaching from diet and feces increases waterborne metal concentrations.
Diet composition and preparation	Ingredients, methods and storage should be reported. Need accurate measures of metal concentrations; nominal concentrations are insufficient.
Depurated invertebrates	Including gut contents of invertebrates in the fish diet may significantly increase total metal concentrations and alter diet composition in a way that changes metal bioavailability.
Fish tissue analysis	Analysis of fish tissue with the inclusion of intestine and organs will change the total metal load measurement; intestinal tissue may contain high concentrations of metal that have not been internalized.
Fish reproduction	Effects of chronic dietborne metal exposure on fish reproduction have not been investigated.

tionally beneficial may also tend to make them metal-rich. This means that characterizing invertebrate exposure by bulk-sediment concentrations of metals will underestimate the actual dietborne metal exposure these animals experience.

***Indirect effect of metals on invertebrate health and consequently, fish diet quality:*** Metals are toxic to invertebrates as well as to fish. One result of metal toxicity might be altered content of fat, carbohydrate and protein in the invertebrates; in turn this might alter their nutritional value to fish predators. Therefore, invertebrates from metal-contaminated environments might not be as nutritionally beneficial to fish as would invertebrates from a pristine environment. As mentioned earlier, an important aspect of this research is to untangle the two influences of decreased diet quality and toxicity due to the metals in the invertebrates.

***Developing models to predict dietborne metal toxicity:*** The better we understand the factors that influence dietborne metal bioavailability and toxicity,

the better we will be able to model dietborne metal uptake and predict metal toxicity as a function of all routes of exposure. Model development is more advanced for invertebrates than for fish. Relevant parameters needed to predict toxicity from dietary pathways include: metal concentrations in the diet, ingestion rates, assimilation efficiencies (a measure of metal bioavailability) and depuration (or excretion) rates.

***Direct effect of metals on fish intestines:*** Most research on dietborne metals has focused on the amount of metal that fish bioaccumulate in their internal organs (e.g. liver, kidneys) before toxicity occurs. But, if dietborne metals are directly toxic to the intestinal tissues, they need not accumulate in internal organs to result in a toxic response. For example, fish eating the metal-contaminated Clark Fork River invertebrate diets were occasionally observed to have “constipation.” This has led to the suggestion that metals are directly toxic to the nerves and muscles that control gut motility. Research is

underway to examine if dietborne metals affect the enteric (or intestinal) nervous system of fish.

### **Implications for Risk Assessment**

Can we use water quality criteria and sediment quality guidelines to protect fish from dietborne metal toxicity?

There is a great deal of debate about this subject. One school of thought says that even if dietborne metals are sometimes a source of toxicity in a polluted environment, safe metal concentrations in the water and/or sediment might successfully protect against dietborne metal toxicity. The idea behind this argument is that metal concentrations achieve an equilibrium between the various compartments of water column, sediment, pore water and food; so a safe concentration in one medium protects against metal toxicity from all other compartments. Therefore, once we understand the relationship of waterborne, sediment and porewater metal concentrations to dietborne metal toxicity, we can use metal concentrations in one medium (e.g. water or porewater) to protect fish from metal exposure via any route.

An opposing school of thought says that not only are dietborne metals sometimes a significant source of metal toxicity, but metal concentrations in the water, sediment and porewater are not predictive of dietborne metal toxicity. This may be because (1) waterborne, sediment or porewater metal concentrations are not in equilibrium with metal concentrations in the diet/food web, and/or (2) the mechanisms of dietborne metal toxicity differ from the mechanisms of waterborne metal toxicity. According to this argument, if effects concentrations are to reflect the natural environment, they will need to take into account both dietborne and waterborne metal exposure.

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### About the Author

**Dr. Susan Clearwater** is a postdoctoral research fellow at the University of Wyoming (USA). Her current research interests include factors that alter the bioavailability of dietborne metals to fish and the effect of dietborne metals on the enteric nervous system of fish. She completed her Ph.D. on the reproductive physiology of fish at the Memorial University of Newfoundland (Canada) in 1996 and was introduced to dietborne metals research during a postdoctoral fellowship at McMaster University (Canada) in 1997. In March 2002, Dr. Clearwater will begin work as an ecotoxicologist at the National Institute of Water and Atmospheric Research Ltd. (NIWA) in New Zealand.

## Fact Sheet on Environmental Risk Assessment

This is the sixth in an occasional series of *Fact Sheets* to be produced by ICMM on metal-specific issues in environmental risk assessment. Authorship selection and editorial review are coordinated by Dr. Anne Fairbrother of Parametrix, Inc. Each *Fact Sheet* is reviewed for technical merit by Dr. Erik Smolders of Katholieke Universiteit (Catholic University) Leuven, Belgium, and by a panel of experts in metal-related regulatory issues. While the *Fact Sheets* reflect the views of the authors, they are intended to provide an objective review of each topic. ICMM hopes these publications provide insights into complex issues in regulatory science, and welcomes questions and comments.

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