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INTERACTIONS BETWEEN COPPER AND CADMIUM MODIFY METAL ORGAN DISTRIBUTION IN MATURE TILAPIA, *Oreochromis mossambicus*

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Abstract

Sexually mature female tilapia were exposed to sublethal concentrations of waterborne Cu and/or Cd over 6 days, and subsequent body concentrations of these metals were determined in several organs. The results show that the distribution of Cu and Cd was metal and organ specific. This is demonstrated, for example, by the observation that in tilapia, Cu exposure did not result in Cu accumulation in the liver, whereas in the intestinal wall, notably high concentrations of Cu and Cd were measured in metal exposed fish.

In addition to single metal exposed fish, we also determined Cu and Cd body distribution in Cu–Cd co-exposed fish. The observed interactions in metal accumulation were most pronounced in the organs of fish exposed to low, environmentally realistic, metal concentrations.

Keywords: Copper, cadmium, metal accumulation, Cu–Cd interaction, organ distribution.

INTRODUCTION

Heavy metals such as copper (Cu) and cadmium (Cd) are frequently present at elevated concentrations in freshwaters, generally as a result of industrial pollution. As a consequence, aquatic organisms, including fish, are exposed to elevated levels of these metals. Cu, as an essential metal, plays an important role in cellular metabolism (Cousins, 1985) and its concentration is well regulated. However, exposure of fish to increased Cu concentrations results in Cu accumulation (Brungs *et al.*, 1973; Buckley *et al.*, 1982). As a result, various blood parameters (McKim *et al.*, 1970; Christensen *et al.*, 1972), enzyme activities in blood (Christensen and Tucker, 1976) and reproduction (Horning and Nieheisel, 1979) are affected. In contrast to Cu, a biological function for Cd is unknown, and the metal is toxic to organisms at very low concentrations (Chmielnicka and Cherian, 1986). For example, exposure to Cd resulted in reduced growth, reproduction and survival in flagfish *Jordanella floridae* (Spehar, 1976). Also, Cd produces a variety of pathological effects in various organs in fish

after acute exposure (Hawkins *et al.*, 1980; Karlsson-Norrgrén *et al.*, 1985).

No clear picture exists concerning the accumulation of Cu and Cd in fish tissues, partly because of differences in species, analytical techniques and experimental designs of the published studies (McCracken, 1987; Douben, 1989). More importantly however, each of the metals, Cu and Cd, has been studied separately, not taking into account a possible concomitant influence of other metals. Few investigations have been made concerning the effect of one heavy metal on the accumulation of another metal in fish (Gill *et al.*, 1992; Pelgrom *et al.*, 1994a,b). Nevertheless, many of the toxic effects of Cd have been suggested to be the result of induced secondary deficiencies of essential trace elements, such as Zn and Cu, since the uptake of Cd both modulates, and is modulated by, the uptake of these metals (Bremner, 1974). Since heavy metals often occur together in polluted areas, it is of importance to study metal–metal interactions in fish at environmentally relevant concentrations.

In a previous paper (Pelgrom *et al.*, 1994a), interactions between Cu and Cd on whole body metal accumulation in juvenile tilapia during waterborne metal exposure were demonstrated. The present study examines Cu–Cd interactions in mature tilapia, paying particular attention to organs with diverse biological functions, because it is anticipated that differences in metal accumulation between organs will be related to their functions. Organ metal concentration or metal–metal interaction at this level may be a link to toxicity (Foulkes, 1990; Landrum *et al.*, 1992). In keeping with this, it has recently been suggested that metal concentrations in the organs of fish, rather than the metal concentrations in the ambient water, could be used as a biomonitor for water pollution in natural freshwaters (Handy, 1992).

We have investigated the effects of two concentrations of Cu and Cd, single as well as in combination, over 6 days on the Cu and Cd concentration of organs associated with osmoregulation (gills), metal detoxification (liver, kidney), digestion (intestine), neuro-endocrine regulation (brain, head kidney), locomotion (muscle)

and reproduction (gonads). At one intermediate concentration of Cu and Cd, gills and liver were compared after 6 and 11 days of exposure, to compare effects of a more prolonged exposure on metal accumulation and interaction.

MATERIALS AND METHODS

Fish and control water conditions

Tilapia (*Oreochromis mossambicus*) were obtained from our own laboratory stock. Fish were kept, from 9 days after hatching, in artificial freshwater with undetectable Cu and Cd concentrations (detection levels below 0.1 and 0.01 $\mu\text{g litre}^{-1}$, respectively). The artificial freshwater consisted of demineralized water supplemented with 1.3 mM NaHCO_3 , 0.5 mM CaCl_2 , 0.06 mM KCl and 0.2 mM MgCl_2 , at pH 7.8. The composition and preparation of the water was based on the EEC instructions for artificial water for use in toxicity studies in fish (EEC Directives 84/449/EEC Annex 5 method c1: Acute toxicity for fish). Water was continuously aerated, filtered and subjected to flow-through, resulting in water of constant quality and with a stable pH (pH = 7.6). The light/dark regime was 12/12 h and the water temperature 26°C. Fish were fed commercial tropical fishfood Tetramin™, 2% dw/ww per day. The food was eaten within 1 min. The Cu and Cd contents of the food were: $9.86 \pm 0.16 \mu\text{g Cu g}^{-1}$ dry food and $0.22 \pm 0.01 \mu\text{g Cd g}^{-1}$ dry food (means \pm SE; $n = 10$).

Experimental design

Six weeks before the start of the experiments, sexually mature female fish (mean weight 20 g) were divided into four groups of 14 fish each, and kept in 80-litre aquaria with continuously filtered and refreshed artificial freshwater by means of flow-through. The experiment started with the connection (by means of a 16 channel peristaltic pump; Watson Marlow) of each of the aquaria to its own reservoir filled with artificial freshwater with or without (controls) a well-defined metal concentration (added as nitrate; Spectrosol, BDH, England). During the first 6 h the flow rate was 4.5 l h^{-1} , followed by a flow rate of 1.5 l h^{-1} . In this way, the metal concentrations in the aquaria were gradually raised, reaching a plateau after 18 h. The measured concentrations deviated maximally 5% from the nominal concentrations (Pelgrom *et al.*, 1994a). Two experiments were performed, with LOW and HIGH Cu and Cd concentrations. The experiment with the HIGH metal concentrations was performed first, followed by experiments with LOW concentrations. The experimental period lasted 6 days. In an additional experiment with intermediate metal concentrations ($50 \mu\text{g Cu litre}^{-1}$ and $20 \mu\text{g Cd litre}^{-1}$), fish were exposed over 6 and 11 days (Table 1).

Feeding was ended the day prior to sacrifice. Cu and Cd concentrations in both stock solutions and aquaria were monitored every hour during the first 6 h, and at least once a day during the rest of the exposure period. Water samples were acidified with nitric acid in a final

Table 1. Cu and Cd concentrations in the aquaria of the metal exposed fish

	[Cu]	[Cd]	[Cu] + [Cd] ($\mu\text{g litre}^{-1}$)
6 Days			
LOW	20	5	20 + 5
HIGH	100	35	100 + 35
6 and 11 days			
	50	20	50 + 20

concentration of 0.2% (v/v). Water Cu and Cd concentrations were determined with a flameless Atomic Absorption Spectrometer (AAS, Philips PU9200) connected with an electrothermal atomiser (Philips PU9390X). After exposure, the fish were killed by spinal dissection. Gills, head kidney, brain, liver, intestine (after removal of contents), kidneys, gonads (LOW experiment only) and white muscle were dissected carefully. The tissues were weighed, lyophilized and, after determination of the dry weights, digested with nitric acid (65% HNO_3 , ultrapur, Merck). Finally, the samples were dissolved in 0.2% HNO_3 , and stored at 4°C until metal analysis by means of AAS. Cu and Cd determinations in the tissues were performed under standard matrix conditions, with the exception of Cd determination in the gonads and muscle. The Cd concentrations in the latter tissues were determined in the presence of a matrix modifier (AAS matrix modifier, Merck). Interference between Cu and Cd during measurements can be excluded, as has been demonstrated previously (Pelgrom *et al.*, 1994a). From the (wet) weights of the the gonads and the total body weight, the Gonad Somatic Index (GSI) was determined, with the gonad weight expressed as a percentage of the total body weight.

Statistics

Data are presented as means \pm SE. For statistical evaluation the Student's *t*-test was applied, and significant differences between control and metal exposed groups are indicated by asterisks, whereas significant differences between single metal and Cu–Cd co-exposed groups are indicated by circles: * or \circ : $P < 0.05$; ** or $\circ\circ$: $P < 0.02$; *** or $\circ\circ\circ$: $P < 0.01$; and **** or $\circ\circ\circ\circ$: $P < 0.001$.

RESULTS

During all experiments, no mortality occurred, and no differences in the feeding behaviour and body weights between the experimental and control groups were observed. The water in the aquaria was continuously refreshed by means of flow-through, resulting in water of constant quality, as demonstrated by the constant pH, and no changes in ammonia concentrations (data not shown). The results of the 6-day metal exposures on organ metal concentrations are presented in Fig. 1, Fig. 2, Fig. 3 and Fig. 4(A), with LOW: $5 \mu\text{g Cd litre}^{-1}$ and/or

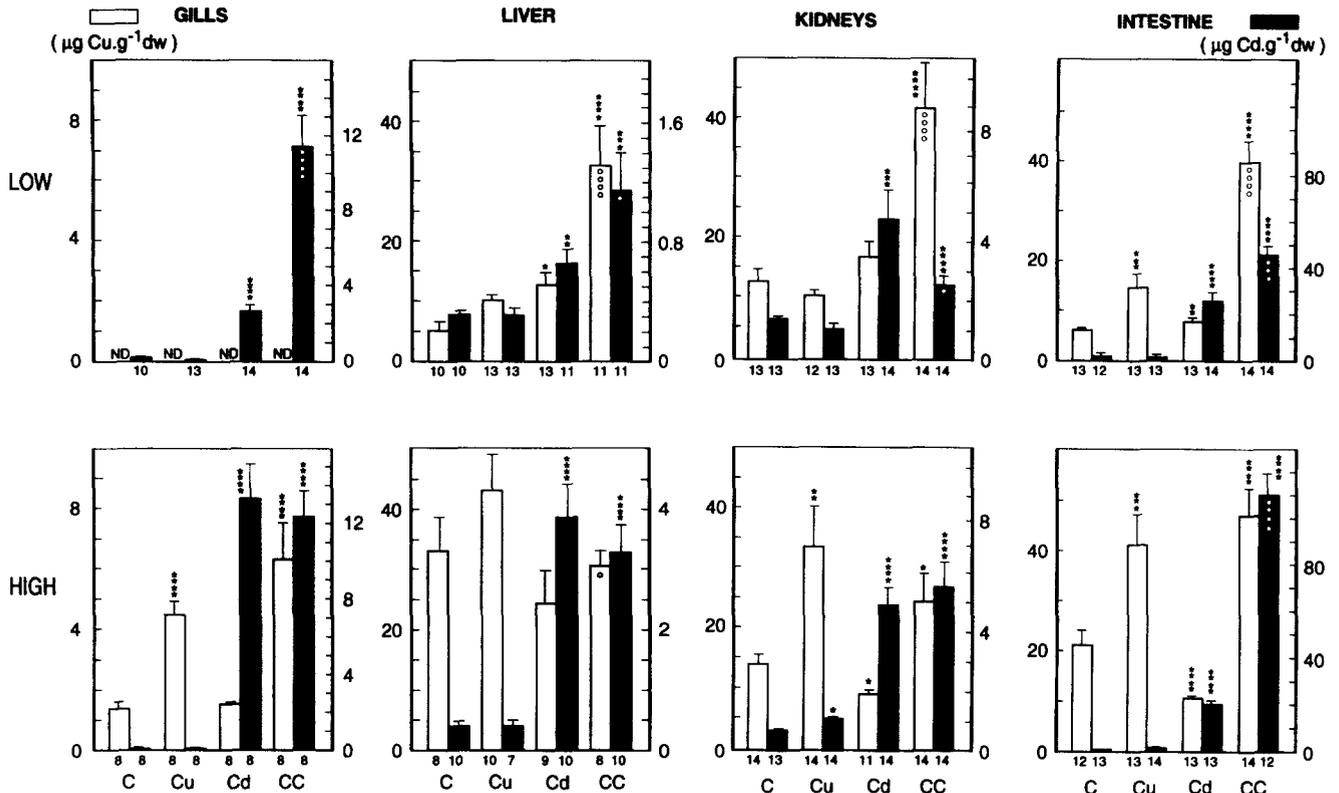


Fig. 1. Concentrations of Cu (open bars) and Cd (closed bars) in the gills, liver, kidneys and intestine of fish exposed over 6 days to LOW ($5 \mu\text{g Cd litre}^{-1}$ and/or $20 \mu\text{g Cu litre}^{-1}$; upper panels) or HIGH ($35 \mu\text{g Cd litre}^{-1}$ and/or $100 \mu\text{g Cu litre}^{-1}$; lower panels) metal concentrations. The Cu concentration is expressed on the left axis and the Cd concentration on the right axis. The Cu and Cd concentrations in the organs are given of the controls (C), Cu exposed (Cu), Cd exposed (Cd) and Cu + Cd co-exposed (CC) fish successively. Asterisks indicate significant differences between control and experimental fish, circles indicate significant differences between single metal exposed fish and Cu + Cd co-exposed fish. The number of fish per group is indicated under the bar. In the LOW group, Cu was not determined (ND) in the gills.

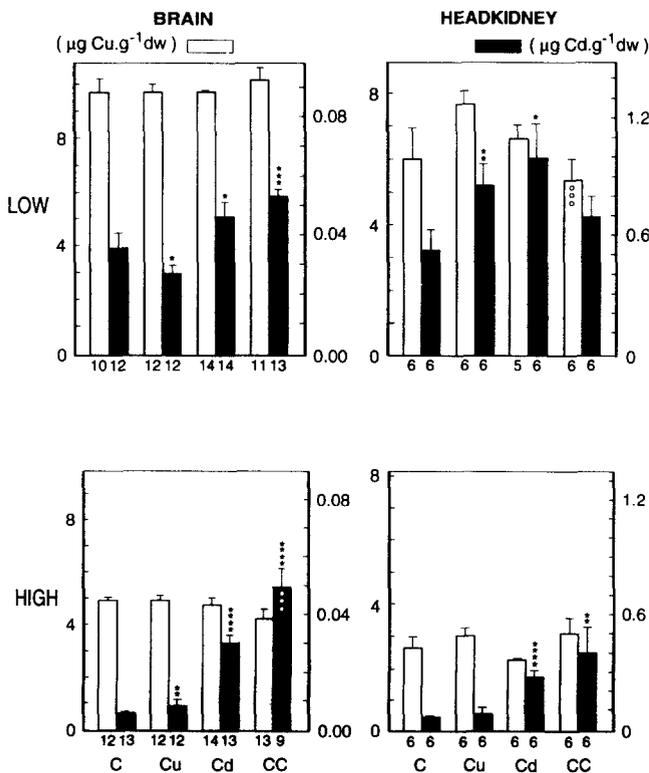


Fig. 2. Concentrations of Cu (open bars) and Cd (closed bars) in brain and head kidney of fish exposed during 6 days to LOW or HIGH Cu and Cd concentrations. Symbols are used in the the same way as described in Fig. 1.

$20 \mu\text{g Cu litre}^{-1}$, and HIGH: $35 \mu\text{g Cd litre}^{-1}$ and/or $100 \mu\text{g Cu litre}^{-1}$. Throughout the organs and concentrations, only incidental differences in the per cent water content were observed (data not shown). Therefore, Cu and Cd concentrations in the organs are expressed as dry weights.

Tissue Cu concentration

LOW

Effects of Cu. Cu-exposure resulted in increased Cu concentrations in the intestine, muscle and gonads.

Effects of Cd. Compared to control fish, an increased Cu concentration in the liver and intestine was observed in fish exposed to Cd singly.

Compared to fish exposed to Cu singly, significantly more Cu accumulation was observed in the liver, kidneys and intestine of Cu-Cd co-exposed fish. In contrast, the Cu concentrations in the head kidney and muscle of Cu-Cd co-exposed fish were lower than those observed in Cu exposed fish. As a result, the Cu concentration in the head kidney of fish co-exposed to Cu and Cd was not statistically different from the controls.

HIGH

Effects of Cu. Exposure to $100 \mu\text{g Cu litre}^{-1}$ resulted in accumulation of Cu in the gills, kidneys, intestine and muscle.

Effects of Cd. The Cu concentration in the kidneys and intestine were significantly lower in Cd-exposed fish than in control fish.

Combined Cu–Cd exposure resulted in a lower Cu concentration in the liver when compared to the liver Cu concentration of fish exposed to Cu singly.

Tissue Cd concentration

LOW

Effects of Cd. Exposure to $5 \mu\text{g Cd litre}^{-1}$ resulted in increased Cd concentrations in all tissues examined.

Effects of Cu. In Cu-exposed fish, the Cd concentration in the brain and gonads were significantly lower, whereas in the head kidney we observed an increase in the Cd concentration compared to control fish. Combined Cu–Cd exposure resulted in an increased Cd accumulation in the gills, liver, intestine and gonads when compared to fish exposed to Cd singly. The Cd concentration in the kidneys of Cu–Cd co-exposed fish was lower than in Cd-exposed fish.

HIGH

Effects of Cd. The Cd concentration was increased in all tissues examined of Cd-exposed fish.

Effects of Cu. In Cu-only exposed fish, the Cd concentration was higher in the kidneys and brain compared to controls. Combined Cu–Cd exposure resulted in an increased accumulation of Cd in the intestine, brain and muscle when compared to fish exposed to Cd singly.

Table 2 shows the Cu and Cd concentrations in the gills and liver of fish exposed to $20 \mu\text{g Cd litre}^{-1}$ and/or $50 \mu\text{g Cu litre}^{-1}$ after 6 or 11 days of exposure. No differences were observed between the Cu concentrations of both gills and liver of fish exposed to Cu over 6 or 11 days. In the liver, 11 days of Cd exposure resulted in a significantly decreased Cu concentration. Compared to

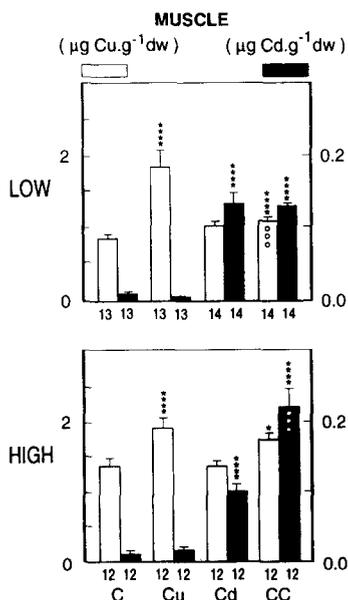


Fig. 3. Concentrations of Cu (open bars) and Cd (closed bars) in muscle of fish exposed over 6 days to LOW or HIGH Cu and Cd concentrations. Symbols are used in the same way as described in Fig. 1.

the Cu concentration in the liver of fish exposed to Cd over 6 days, the Cu concentration was lower in the liver of fish exposed to Cd over 11 days ($P < 0.05$). The Cd concentration in the gills of Cd exposed fish doubled between days 6 and 11 of exposure ($P < 0.05$). In the liver of Cu exposed fish, a significantly decreased Cd concentration was observed after both 6 and 11 days of exposure. Prolonged Cu–Cd exposure resulted in a significant increase in both Cu and Cd concentrations in the gills compared to single Cu or Cd exposure. In Cu–Cd co-exposed fish, significant differences between 6 and 11 days of exposure were observed in the Cu and Cd concentrations of the gills ($P < 0.001$ and $P < 0.05$, respectively) and the Cd concentration in the liver ($P < 0.05$).

The relation between GSI and the Cd concentration in the gonads of control, Cd and Cu–Cd co-exposed fish is best described by non-linear functions (Fig. 4(B)). For the Cu-exposed fish, however, no significant relation exists between GSI and the Cd concentration in the gonads. The observed higher Cd concentrations in the gonads of Cd- and Cu–Cd-exposed fish compared to the non-Cd-exposed fish (Fig. 4(A)) appeared consistent throughout the GSI range (Fig. 4(B); compare left and right panels).

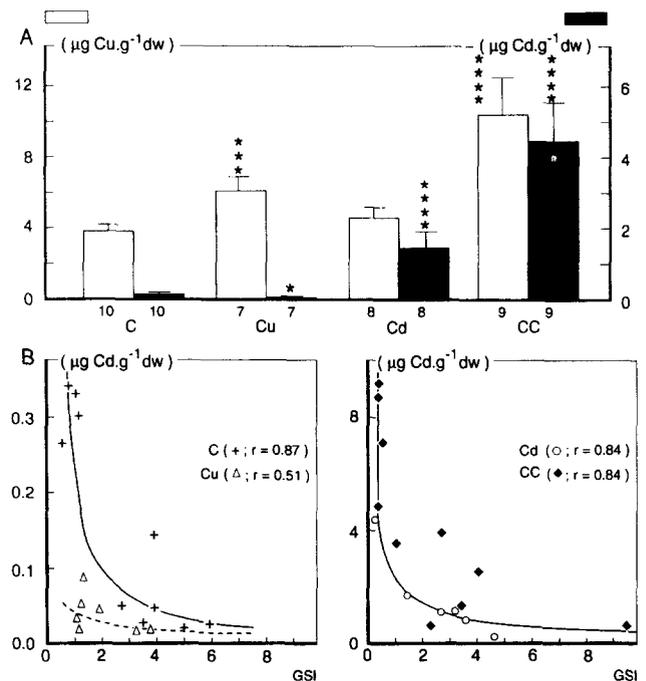


Fig. 4. (A) Cu (open bars) and Cd (closed bars) concentrations in gonads of fish exposed for 6 days to $5 \mu\text{g Cd litre}^{-1}$ (Cd), $20 \mu\text{g Cu litre}^{-1}$ (Cu) or co-exposed to $5 \mu\text{g Cd litre}^{-1}$ + $20 \mu\text{g Cu litre}^{-1}$ (CC). Symbols are used in the same way as described in Fig. 1. (B) Relationship between the Cd concentration in the gonads and the GSI of the exposed fish described in (A). In control and Cu exposed fish, the relation between the GSI and the Cd concentration is best described by $y = 0.23x^{-1.17}$ (****) and $y = 0.04x^{-0.51}$ (ns), respectively. In the Cd and Cu–Cd co-exposed fish, the GSI and the Cd concentration in the gonads are related in a comparable way, and can be best described by the function $y = 2.21x^{-0.73}$ (****), with y representing the Cd concentration in the gonads and x representing the GSI.

Table 2. Concentrations of Cu and Cd in the gills and the liver of fish exposed for 6 or 11 days to 20 µg Cd litre⁻¹ (Cd), 50 µg Cu litre⁻¹ (Cu) or co-exposed to 20 µg Cd litre⁻¹ + 50 µg Cu litre⁻¹ (CC). Asterisks indicate significant differences between control and metal-exposed fish, while circles indicate significant differences between single metal exposed fish and Cu + Cd co-exposed fish

		Control	Cu	Cd	CC
[Cu] (µg. g ⁻¹ dry wt)					
Gills	6 days	(n = 5) 1.65 ± 0.17	3.19 ± 0.18***	1.64 ± 0.19	2.79 ± 0.17***
	11 days	(n = 9) 1.59 ± 0.19	3.22 ± 0.23****	1.81 ± 0.18	5.69 ± 0.60****●●●
Liver	6 days	(n = 10) 73.1 ± 12.3	100.6 ± 1.26	66.6 ± 9.2	134.1 ± 18.6**
	11 days	(n = 9) 86.2 ± 17.0	119.6 ± 24.2	44.5 ± 6.1*	149.9 ± 26.7
[Cd] (µg.g ⁻¹ dw)					
Gills	6 days	(n = 5) 0.06 ± 0.01	0.07 ± 0.01	2.67 ± 0.54****	7.41 ± 1.52****●●●
	11 days	(n = 9) 0.12 ± 0.04	0.11 ± 0.02	6.13 ± 1.24****	20.98 ± 4.99****●●
Liver	6 days	(n = 10) 0.31 ± 0.04	0.19 ± 0.04*	8.96 ± 1.50****	12.89 ± 2.85****
	11 days	(n = 9) 0.39 ± 0.12	0.20 ± 0.03*	15.59 ± 5.23***	7.07 ± 0.93****

DISCUSSION

The results demonstrate organ specific Cu–Cd interactions after single and co-exposure of tilapia via the water. Four major conclusions can be drawn from the present study. Firstly, compared to single metal exposed fish, Cu–Cd co-exposure resulted in significantly different Cu and/or Cd concentrations in the gills, liver, kidneys, intestine and gonads. Secondly, these metal interactions were most pronounced in the LOW group. Thirdly, in the gills of Cu–Cd co-exposed fish, accumulation and impact of the interactions were time dependent. Finally, Cu and Cd accumulated in notable amounts in the intestinal wall.

Cu–Cd accumulation and interactions were studied after 6 and 11 days of exposure, and therefore conclusions on Cu–Cd accumulation and interactions are restricted to the exposure regimes studied. However, it has been demonstrated that accumulation and toxicity of the metals are mainly critical during an exposure period of days rather than weeks (Gill *et al.*, 1992; Carbonell and Tarazona, 1994).

Water content

Exposure of the fish to Cu and Cd, both singly and combined, did not affect the water content of any of the organs studied, which contrasts with previous results on juvenile tilapia exposed to identical metal regimes (Pelgrom *et al.*, 1994a). This may relate to an increased sensitivity of younger life-stages to osmoregulatory disturbances, i.e. water balance, since the weight-specific surface area of the gills in juvenile fish is nearly two times higher than that of mature fish (Morgan, 1971).

Gills

Cu or Cd exposure resulted in a significant increase of either metal concentration in the gills. This accumulation was concentration- and time-dependent, only for Cd. Increased metal concentrations were also observed in Cu-exposed carp, brown bullhead and roach (Yamamoto *et al.*, 1977; Stagg and Shuttleworth, 1982; Segner, 1987) but not in rainbow trout (Laurén and McDonald, 1987), and in Cd-exposed rainbow trout, pike and American eel (Brown *et al.*, 1986; Norey *et al.*,

1990; Gill *et al.*, 1992). Metal–metal interactions after single metal exposure were shown by Gill *et al.* (1992) who reported an increased Cu concentration in the gills of eels after Cd exposure. Previous experiments with single metal exposed fish have shown effects of sublethal concentrations of Cu or Cd on the number and function of ion transporting cells in the gills (Baker, 1969; Verbost *et al.*, 1987; Reid and McDonald, 1988; Pratap and Wendelaar Bonga, 1993; Pelgrom *et al.*, 1995). Exposure to low Cd concentrations resulted in an unexpectedly high increase in metal concentration in the gills. However, the free available ionic metal concentration is not necessarily equivalent to the total water metal concentration to which the gill is exposed (Playle *et al.*, 1992), since: (i) the pH at the gill surface is lower than the water, due to local release of carbon dioxide, and this facilitates the release of metal ions from complexes (Cusimano *et al.*, 1986), and (ii) the amount of mucus on the gill surface increases during metal exposure (Handy and Eddy, 1991), which may contribute to higher metal concentrations at the gill surface (Reid and McDonald, 1991). Both phenomena might be relatively more important at low-water metal concentrations. In this study, co-exposure to Cu–Cd resulted in an even greater accumulation of Cd than when compared to single Cd exposure. At present it is not clear to what extent these increased metal concentrations affect the function of ion-transporting cells.

Liver and kidneys

Exposure to Cu over 6 as well as 11 days had no effect on the Cu concentration in the liver, whereas the Cu concentration in the kidney increased significantly after exposure to 100 µg Cu litre⁻¹. Opposing effects have been reported in most other fish species. Cu has been shown to accumulate in both the liver and kidneys of catfish (Brungs *et al.*, 1973) and carp (Yamamoto *et al.*, 1977), whereas Stagg and Shuttleworth (1982) registered a decreased Cu concentration in the liver of flounder after Cu exposure in seawater. Starved, but not fed, roach accumulated significant amounts of Cu in the liver (Segner, 1987). Our experiments were performed with fed tilapia, and this may partly account for the absence of Cu accumulation in this study. In contrast to

other species, however, tilapia accumulated extremely high amounts of Cu in the intestinal wall, which may partly explain the absence of Cu accumulation in the liver after Cu exposure. This will be discussed in the following section.

Cd exposure resulted in an increased Cd content of both liver and kidneys, and this agrees with the findings of Gill *et al.* (1992) on eel. The liver and kidneys play a crucial role in detoxification and excretion of toxicants mainly through the induction of metal-binding proteins such as metallothioneins (MTs; Klaverkamp *et al.*, 1984; Cousins, 1985). Relatively few *in-vivo* studies concerning both metal accumulation and MT-induction investigated the effects of Cu–Cd interactions during single and combined exposures. Both in liver and kidneys, the lowest concentrations used in combination, 5 $\mu\text{g litre}^{-1}$ Cd and 20 $\mu\text{g litre}^{-1}$ Cu, resulted in significantly increased Cu concentrations when compared to single Cu exposure. This suggests a disturbance of the Cu metabolism which might involve interactions between Cu and Cd during binding to MT. Changes of the Cu content of the liver and kidney following single Cd exposure are known from studies on mammals (Suzuki *et al.*, 1983; Chmielnicka *et al.*, 1985), and eels (Gill *et al.*, 1992). Our results demonstrate similar interactions in combined Cu–Cd-exposed fish which occur in mixtures at very low, environmentally relevant, metal concentrations.

Intestine

Exposure to waterborne Cu or Cd resulted in a high increase of both metals in the intestinal wall. This is surprising because freshwater fish are known to drink very little (Potts *et al.*, 1967). In Cu–Cd co-exposed fish, the Cd increase was even more pronounced than in Cd exposed fish. After intravenous Cd administration, significantly increased Cd concentrations were found in the intestinal wall of rats and mice (Berlin and Ullberg, 1963; Stonard and Webb, 1976; Barański, 1987). Stonard and Webb (1976) reported that the Cd in the intestinal mucosa could be recovered in the fraction containing MT. Few reports are available on the effects of waterborne metal exposure on the intestinal wall of freshwater fish. Exposure of freshwater fish to high concentrations of Cu (Yamamoto *et al.*, 1977) or Cd (Gill *et al.*, 1992) resulted in a significant increase of these metals in the intestine. In our study, compared to single metal exposed fish, combined Cu–Cd exposure resulted in even higher Cu (LOW) and Cd concentrations. In line with this, Gill *et al.* (1992) observed indications for interaction between Cd and Cu in the intestine of Cd exposed eels. The Cu and Cd content of the food can not account for the amounts of metals found in the intestinal wall of our tilapia, since the contribution of Cd from the food can maximally account for 2.5% of total Cd concentration of the intestine. Also drinking could not explain this phenomenon (Gill *et al.*, 1992), since it would imply an exceptionally high drinking rate (at least 60 ml h⁻¹ in the LOW group, assuming that 100% of the Cu in the water

is taken up). It is therefore more likely that in tilapia the intestine wall serves as a storage organ and possibly excretion route for heavy metals, as has earlier been suggested for rats (Stonard and Webb, 1976). A negative consequence of this mechanism might be, that the high metal concentrations may affect the transport functions of the intestine. The results of *in-vitro* experiments with tilapia intestinal basolateral plasma membrane preparations have shown that Cd²⁺ inhibits the active uptake of calcium (Schoenmakers *et al.*, 1992).

The metal accumulation in the intestinal wall might be a specific mechanism for tilapia, because in general in fish about 95% of the whole body Cu accumulation was allocated into the liver (Stagg and Shuttleworth, 1982; Laurén and McDonald, 1987). Perhaps an efficient accumulation and excretion route via the intestine contributes to the metal tolerance of tilapia, which is higher than in other species studied.

Brain and head kidney

Because Cd, but not Cu, accumulated significantly in both the brain and head kidney after Cd exposure, the blood–brain barrier might function better for Cu than for Cd. Generally, brain and head kidneys are not considered to be a storage place for metals during exposure (Pelgrom *et al.*, 1994b). In stone loach and carp, waterborne Cu exposure respectively increased (Solbé and Cooper, 1976) and decreased (Yamamoto *et al.*, 1977) Cu concentrations in the brain. Although increased Cd concentrations in fish brains were observed previously, this effect does not appear to be related to the ambient Cd concentration (Gill *et al.*, 1992). Effects of Cd exposure on the Cu content of the brain was observed in studies with rats (Chmielnicka *et al.*, 1985) and eel (Gill *et al.*, 1992). Copper ions may interfere with the control of GnRH (gonadotrophin-releasing hormone) release, whereas Cu deficiency leads to infertility in rats and guinea-pigs (Burrows and Barnea, 1982; Barnea *et al.*, 1986).

To our knowledge, there are no other studies concerning Cu, Cd or Cu–Cd co-exposure on the metal content of the head kidney of fish. In mammals, the adrenal is the endocrine organ most sensitive to chemical induced lesions (Ribelin, 1984). In fish, the cortisol producing interrenal tissue, located in the head kidney, plays a key role in the regulation of ion-homeostasis and in the stress response, via the release of cortisol (Donaldson, 1981). The amount of metal accumulated in the head kidney tissue is remarkably lower than in the kidney. This is in keeping with observations in tilapia that renal and interrenal tissue, despite their common origin, are two separate compartments with different functions (Nandi and Bern, 1960).

Muscle

In contrast to our data, other researchers found no detectable metal concentrations in fish muscle after waterborne Cu (Yamamoto *et al.*, 1977) or Cd exposure (Hawkins *et al.*, 1980). This probably relates to the difficulty of detecting metals in muscle tissue, because its

high protein content influences metal measurements. Such problems can be solved by applying a matrix-modifier during metal detection (Dabeka and Ihnat, 1987), as used presently. Other authors reporting detectable Cu or Cd concentrations found no differences in metal content between control and metal exposed fish. It is important to note that we have reared fish under metal-free water conditions, which is reflected in low metal levels in the control fish. In none of the experimental groups did metal concentrations in the muscle exceed those in the directives of the Food and Drug Act for edibility of fish. From these directives, the norm of safe levels of toxic metals in freshwaters have been deduced (WVC, 1992). Results from the present study and from the study of Gill *et al.* (1992) demonstrate that it is difficult to relate tissue Cu and Cd concentrations to water metal concentration or exposure time, which in particular applies to Cu–Cd co-exposed fish.

Gonads

It is remarkable that, in this tissue, at the low water concentrations used, significantly higher concentrations of Cd were found after co-exposure than after single metal exposure. In a concomitant study on male tilapia, Cu–Cd co-exposure resulted in an even more pronounced difference in the concentrations in the gonads, a three- and eight-fold additional increase of Cu and Cd, respectively, compared to single metal exposure (our unpublished observations). Although no significant effects on the average GSI were noticeable after 6 days of exposure, in all groups of fish there was a negative relationship between the Cd concentration and the GSI. It is of interest, that the gonadal Cd concentration of the Cd and Cu–Cd co-exposed fish was higher than in controls over the entire GSI range, but particularly so at the lower GSI. This indicates that Cd accumulation is predominantly associated with the connective and endocrine tissues. The observed higher Cd concentrations at high GSI indicates, that in the mature ovaria, Cd also accumulated in the eggs. Increased metal concentrations in the gonads likely implicate a direct burden for reproduction and/or for young fish in addition to indirect effects on regulatory systems at other sites. Studies of Eaton (1973) and McFarlane and Franzin (1978) showed that exposure to mixtures of trace metals decreased the reproductive success of fathead minnows and white suckers.

Previously, we demonstrated Cu–Cd interactions on whole body metal accumulation during waterborne metal exposure of juvenile tilapia (Pelgrom *et al.*, 1994a). Data in the present study show that interactions observed are organ specific and therefore data for most organs are not representative for the whole organism. Furthermore, Cu–Cd interactions already occur at low, environmentally relevant metal concentrations in the water. The data in the present study substantiate the observations of Handy (1992) that metal concentrations in the organs of fish, rather than the metal concentrations in the water, are suitable for environmental

monitoring, especially when trying to relate the toxicity of metals to the biological function of specific organs.

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