Chronic Copper Exposure

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Chronic copper exposure in water

Studies on true chronic copper toxicity, which include exposure at all stages of the life cycle or at sensitive stages of early growth, are relatively rare, whereas studies on chronic toxicity in juvenile fish or adults exposed to copper for a period of 4-6 weeks are more abundant.

Based on a comprehensive assessment of copper exposure early in life as a true chronicity test model (McKim, 1977), the US Environmental Protection Agency (US EPA) accepts 30-day tests of copper exposure in early stages of the life cycle as a chronic toxicity model, in the absence of data in the full life cycle exposure. These 4-6 week models are often considered as chronic toxicity, however, these should be considered as "prolonged exposures" (true chronic toxicity, as well as prolonged exposure, will be discussed below).

Acclimation

In reference literature on toxicology, acclimation usually denotes a higher tolerance (i.e. over LT50 or LC50) at high concentrations that would normally be lethal in a prolonged prior exposure to sub-lethal concentrations of the toxicant (McDonald and Wood, 1993).

Acclimation was first described in 1981 (Dixon and Sprague, 1981) and has been repeatedly confirmed in freshwater fish (Buckley *et al*., 1982, McCarter and Roch, 1984). Acclimation to copper has been described using the term "true acclimation" (Prosser, 1973), referring to the return of normal physiological conditions of the fish despite continuing its copper exposure. Examples include the recovery of appetite and growth (Lett *et al*., 1976; Buckley *et al*., 1982), and restoration in Na+ homeostasis (Lauren and McDonald, 1987a, b). Restoration of Na+ homeostasis in rainbow trout acclimated in fresh water to continuous exposure involves an increase in the synthesis of Na^+/K^+ATPase to compensate for the inhibition of this enzyme caused by copper (Lauren and McDonald, 1987a, McGeer *et al*., 2000b). In addition to the increase in Na^+/K^+ATPase and the restoration of Na+ absorption parameters, freshwater rainbow trout reduces the diffusive loss of Na^+, presumably through the gills (Lauren and McDonald, 1987b).

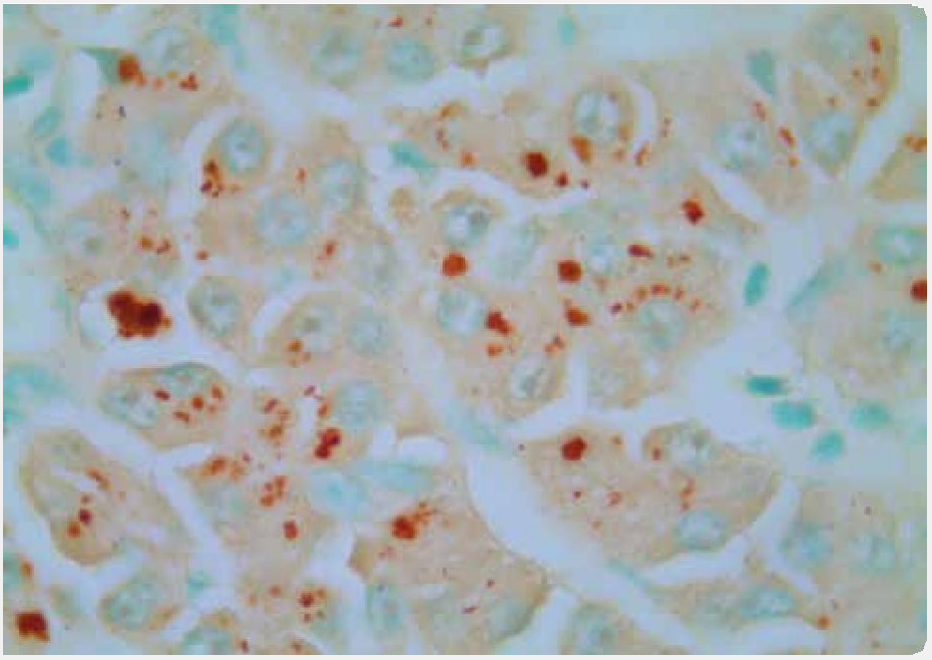
In addition to the gill modifications during copper exposure, the kidney plays a role in restoring the Na^+ balance.

During continuous exposure, the increase in renal reabsorption of Na^+ between days 3 and 30 of exposure to Cu was demonstrated, contributing, in this way, to the recovery of plasma Na^+ levels at day 30 of the exposure (Grossel *et al*., 1998b). Evidence of an acclimation response in seawater has been reported in the European flounder (Stagg and Shuttleworth, 1982b), although specimens such as Gulf toadfish do not appear to show recovery in the excretion of Na+ (and Cl^-) after 30 days of continuous exposure to Cu (Grossel *et al*., 2011). Although acclimation to Cu by marine teleosts has not been conclusively demonstrated, there is evidence of a present compensatory response. After an initial reduction, a compensatory increase in Gulf toadfish consumption rates is observed, from 8 to 30 days of copper exposure, when disturbances in osmoregulation and in the excretion of gill Na^+ and Cl^- are evident (Grossel *et al*., 2004a). In addition, the ionic composition of intestinal fluids strongly suggests an improvement in the absorption of Na^+ and water from part of the distal portions of the intestine during the 30-day exposure to Cu, which in turn would act to compensate osmoregulatory distress, caused initially by Cu exposure (Grossel *et al*., 2004b).

Although acclimation involves the restoration of the osmoregulatory balance and adjustments of the homeostatic control of copper, the results of chronic exposure to this metal involve late effects, which will be discussed below.

Impact on reproductive performance

Among the many studies of true chronicity, two stand out to identify reproductive performance as a highly sensitive endpoint and, obviously, from an ecological point of view. In early toxicity studies in Brook trout, it was revealed that the reproductive performance has been damaged at concentrations of 17 µg Cu L^{-1} (McKim and Benoit, 1971), while in subsequent studies conducted in Bluntnose minnow, a decrease in reproductive performance was detected at 18 µg Cu L^{-1} (Horning and Neiheisel, 1979) in waters of mild and intermediate hardness, respectively.



S. salar. Liver. Rhodanine Brown copper granules are observed in the hepatic cytoplasm, this being a positive reaction to copper (image: Carlos Sandoval).

In subsequent studies, at a concentration of 9.4 µg, no consequences were found on the reproductive performance of Brook trout, suggesting that the threshold for the effects on reproductive performance in Brook trout is between 9.4 and 17 µg Cu L-1 (McKim and Benoit, 1974).

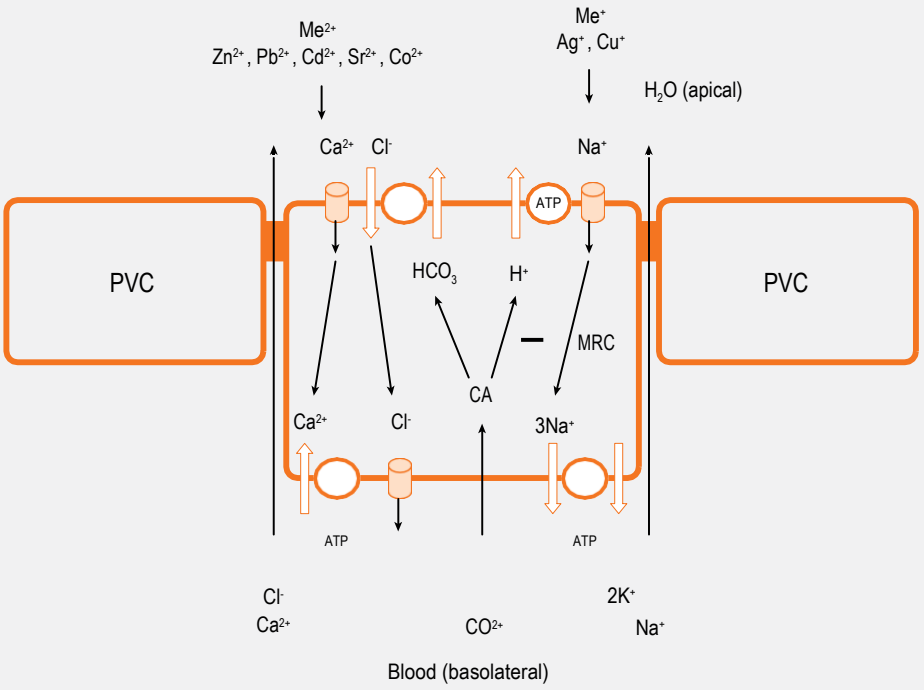
Reproductive damage induced by copper exposure occurs at concentrations well below those needed to induce reduction in growth and mortality (Horning and Neihesel, 1979).

The exact mechanism by which copper induces reproductive damage is unknown, but it could be the simple result of reducing the availability of resources that arises when the energy must be destined to treat the morphological changes induced by copper.

Impact on sensory systems

Olfactory damage occurs almost immediately after copper exposure and its effects are highly persistent. In rainbow trout brood and one-year-old fish exposed to Cu (20-40 µg L-1) for 40 weeks, no total or partial recovery of the olfactory capacity was recorded based on observations on the behaviour of the fish; even so, several weeks are required for recovery after the end of

exposure to Cu (Saucier and Astic, 1995). These observations agree with similar studies of Fathead minnow embryonic specimens, which showed persistent damage to the chemical-sensory function, which continues even after the exposure term. There is evidence of partial recovery of the olfactory capacity despite continuous exposure to the toxic, at least, at lower concentrations of exposure (Saucier and Astic, 1995). However, the extensive recovery period of development by the fish, after the exposure term (Saucier *et al*., 1991, Carreau and Pyle, 2005), suggests that these early stages of life are more vulnerable to olfactory deterioration induced by long-term exposure. Intuitively, it seems clear that the deterioration of the sensory function (smell, as well as mechanoreception) can translate into a lower capacity to detect and avoid predators, to locate prey and suitable spawning sites, it also affects the orientation, control of the speed and swimming direction.



General model of how metal ions enter the gills through ionic similarity and thereby compete with nutrient ions for consumption and in sufficient concentration eventually blocks the consumption of nutrient ions by inhibition of basolateral ATP-dependent enzymes that normally drive these processes.

However, at present, no links have been reported between altered sensory function, as evidenced by laboratory studies on sensory neurons, and fish behaviour or effects such as predator damage or impact of reproductive success (Wood *et al*., 2012).

Impact on immune function

Copper is a fungicide frequently used in aquaculture, but copper exposure has also reported an increase in susceptibility to viral and bacterial diseases. Even copper exposure in the short term and at low concentrations (9% of the LC50 of 96 h or 3-4 µg L^{-1}) induces a higher mortality due to infections with *Vibrio anguillarum* in Chinook salmon and rainbow trout, when exposed experimentally to the pathogen in water (Baker *et al*., 1983).

A study using wild European eel carrying *V. anguillarum* revealed that control fish not exposed to copper remained healthy over a period of 12 months, whereas fish exposed to 30-40 µg L-1 showed mortality and symptoms consistent with *V. anguillarum* infections within 50-120 days of exposure.

In fact, the blood collected immediately post mortem from the infected individuals exposed to copper indicated the presence of the bacteria, while the unexposed individuals did not contain bacteria (Rodsaether *et al*., 1977). Similar results were found in fish exposed to copper and infection with pathogens, either by inoculation or by brief exposures to high concentrations of the pathogen in water (Hetrick *et al*., 1979, Mushiake *et al*., 1984).

The phagocytic function of macrophages is affected by copper exposure (Mushiake *et al*., 1985, Khangarot and Tripathi, 1991, Rougierycol, 1994),

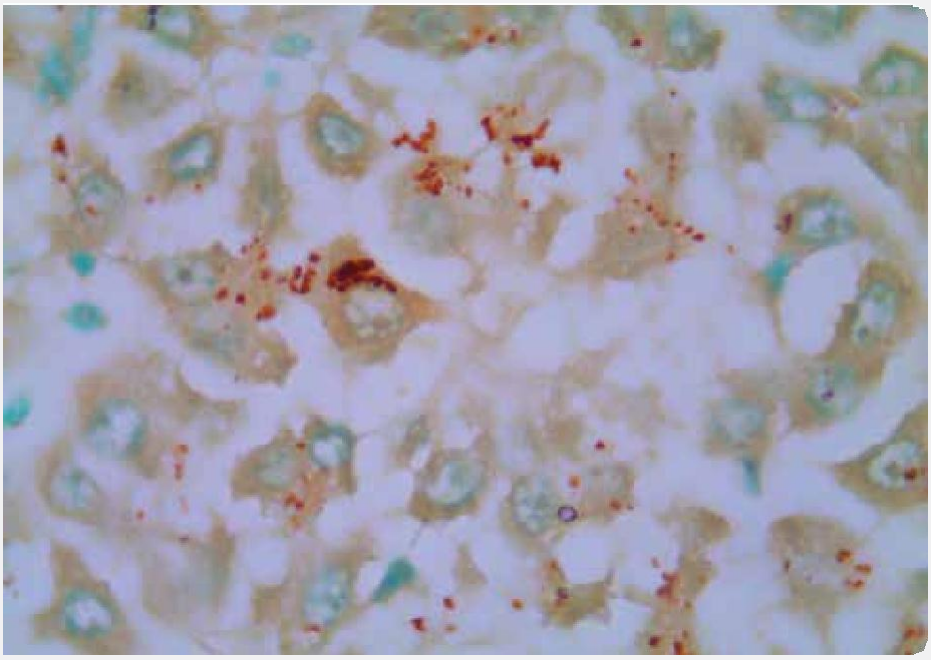
as well as blastogenesis and responses in the production of antibodies (O ' Neill, 1981; Anderson *et al*., 1989), decreasing the magnitude of some specific immune responses such as humoral and cellular (Dethloff *et al*., 1998). Consistently with this range of effects induced by copper in the immune response present in fish, alterations in the transcription of genes related to the immune system are observed, either by water-based exposure (Geist *et al*., 2007) or by copper injections (Osona-Jiménez *et al*., 1989; Prieto-Alamo *et al*., 2009). In contrast to most previous studies that focus on freshwater, gene expression studies were conducted in marine species and suggest that copper affects the immune response of both marine and freshwater species.

The exact mechanism by which copper interacts with the immune system is unknown, but one study points out that corticosteroid injection results in increased susceptibility to pathogens in a manner similar to copper exposure in fish immune responses, which could be secondary to a general stress response (Mushiake *et al*., 1984) which is often reported for fish exposed to copper.

Impact on the stress response

The hypothalamic-pituitary-interrenal (HPI) axis in freshwater fish is activated at sub-lethal copper concentrations, even in short periods of time, resulting in high levels of plasma cortisol (Dethloff *et al*., 1999; DeBoek *et al*., 2001; Tales *et al*., 2005) and, in some cases, hyperglycaemia (Pelgrom *et al*., 1995). However, the plasma cortisol increase is transient in nature, with levels returning to control values after a few weeks, despite continuous exposure (Dethloff *et al*., 1999; DeBoek *et al*., 2001).

The ability of adrenal cortex cells isolated from fish exposed to copper to release cortisol in response to adrenocorticotropic hormone (ACTH) increased instead of decreasing, compared to cells isolated from control fish, demonstrating a reduction in the stress response in vivo (Gagnon *et al*., 2006).



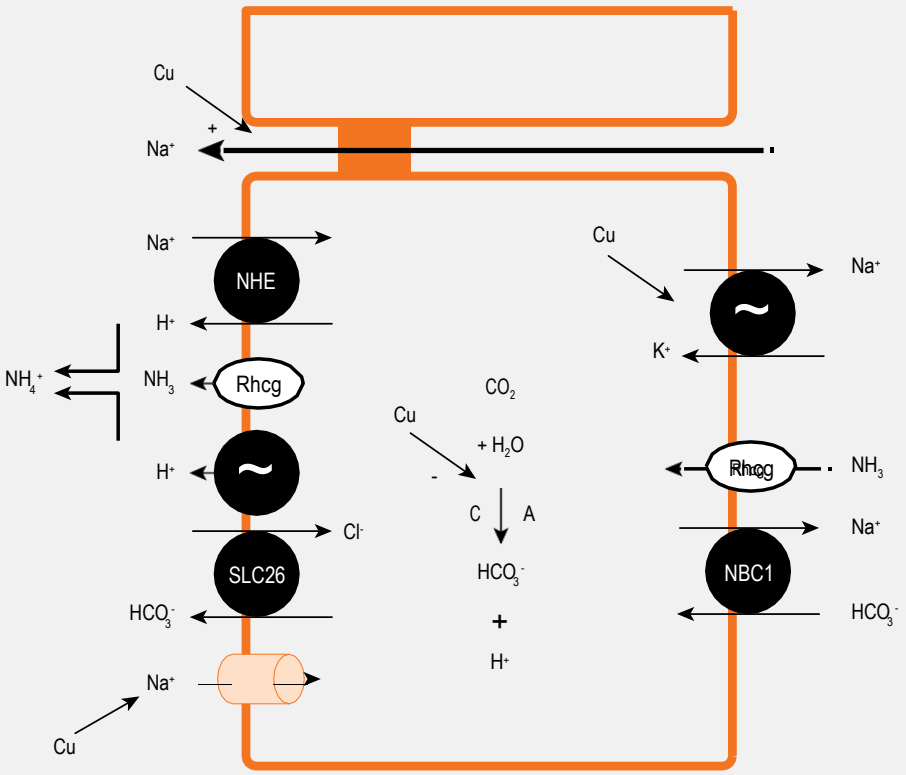
S. salar. Liver. Rhodanine. A positive reaction to copper is observed, consistent with brown granules in the hepatic cytoplasm (image: Carlos Sandoval).

These observations suggest a reduction in the production and release of pituitary adrenocorticotropic hormone (ACTH), and/or alteration of ACTH receptor function in cells of the adrenal cortex in fish exposed to copper (Gravel *et al*., 2005) .

It is clear that copper potentially affects the ability of fish in freshwater to mobilize a complete stress response, in presence of additional stress factors, and that this loss of ability is not directly related to a reduction in steroidogenic capacity. It is unknown whether copper affects the HPI axis in marine fish during an exposure, although it seems very likely.

Impact on development, growth and survival

Growth inhibition seems to occur at relatively low concentrations or below the mortality threshold (Buckley *et al*., 1982; Hansen *et al*., 2002; Kamunde *et al*., 2005; Niyogi *et al*., 2005; Besser *et al*., 2007). However, in some cases, growth inhibition occurs at concentrations less than 20% at 96 h LC50 (Hansen *et al*., 2002), although other studies do not report growth inhibition even at high concentrations



Schematic and simplified representation of the sensitivity to copper in gills of freshwater fish, relevant in the transport process of salt balance, acid base balance and ammonium excretion (Evans et al., 2005). The inhibition of Na+/K+-ATPase induced by copper (~) has been well documented (Grossel et al., 2002). It has also been reported that the efflux of Na+ by the paracellular pathways increases during exposure to high copper concentrations (Lauren and McDonald, 1985, 1986). Although there are no studies demonstrating the inhibition of gill carbonic anhydrase (CA) activity during or after copper exposure, there are numerous reports indicating disturbances in the acid-base balance, where ammonium excretion is the consistently altered parameter for copper.

such as 80 % to 96 hr. of LC50 (McGeer *et al*., 2002). An increase in the metabolic load or a possible reduction in the efficiency of the feed conversion probably contribute to the growth reduction, which is observed induced by Cu, but the transitory reduction of appetite also seems to be a factor (Buckley *et al*., 1982).

Early stages of development such as larvae, fingerlings and juvenile fish are more sensitive to copper exposure than embryos, according to the evaluation of the growth and survival of several species (Sauter *et al*., 1976; Mc Kim *et al*., 1978), naturally expecting in these stages a greater sensitivity to copper than in adults. In fact, tests with copper exposures in the early stages of life, lasting 60 days, have shown to provide a substitute for true chronic toxicity (McKim and Benoit, 1974; McKim, 1977; McKim *et al*., 1978). However, the possibility of a decrease in reproductive production is not taken into account, which has an ecological relevance and apparently would be very sensitive (McKim and Benoit, 1971, Horning and Neiheisel, 1979).

Other effects

We report the reduction of the two main dose-dependent neurotransmitter monoamines, serotonin (5HT) and dopamine (DA), in the telencephalon of carps exposed to sub-lethal Cu concentrations. In addition, copper causes a reduction of dopamine in the hypothalamus and brainstem (De Boek *et al*., 1995b).

At least in mammals, the synthesis of serotonin decreases even in mild hypoxia (Katz, 1981, Prioux-Guyonneau *et al*., 1982, Freeman *et al*., 1986) which, based on the concentration of lactate in the blood, probably occurred in the copper exposure showing a reduction in serotonin synthesis in the carp (De Boek *et al*., 1995b). Regardless of the reason for the reduction of serotonin levels in the brain of fish exposed to copper, the decrease of this could possibly explain the reduction in appetite shown by these fish (Johnston *et al*., 1992).

Chronic dietary copper exposure

High levels of copper in the environment not only give rise to a direct exposure of this metal, but can also lead to the copper accumulation in invertebrates and fauna that is usually prey to the fish, which translates into a potential food exposure.

In fact, high concentrations of copper have been reported in prey, which were contaminated in the natural environment, demonstrating that food exposure, and food toxicity can occur.

Most of the studies have been conducted with natural diets previously contaminated with Cu, as well as with other metals and, therefore, are difficult to interpret from the perspective of the effects induced by copper. However, previous studies with invertebrate diets collected in the field, in most cases, revealed a significant growth inhibition, observing exposure concentrations lower than those recorded in studies using artificially formulated diets (Hansen *et al*., 2004). The interpretation of this observation is that the naturally incorporated metal has greater absorption by the fish and, therefore, has more harmful effects than the metals added in artificial diets, an interpretation that agrees with a series of studies on invertebrates (Hook and Fisher, 2001, 2002; Bielmyer *et al*., 2006). However, most studies of copper toxicity in the fish diet have been and will continue to be carried out with artificially prepared diets enriched with copper salts. The copper toxicity in the fish diet has been discussed extensively in an excellent review by Clearwater *et al*., (2002). They found that the relationship between copper exposure in the diet and induced effects was much more predictable when dietary exposure is expressed in copper doses in the diet, rather than copper concentration in the diet. For example, the threshold for the effects on Rainbow trout was estimated by these authors at 44 mg Cu kg^{-1} per day, while the threshold for effects on Atlantic salmon parr and smolt was estimated at 15 mg Cu kg^{-1} per day.

Channel catfish seemed to be the most sensitive fish to the test in the 2002 study, with sub-lethal effects at 0.4-0.9 mg kg^{-1} Cu. However, similar studies showed no toxicity at a daily dose of 1 mg kg^{-1} Cu, which demonstrates the existence of additional factors such as differences between species, which influence the susceptibility to copper doses in the diet.

Such factors may include the diet composition, feed rate and water chemistry. Since the review of Clearwater *et al*. (2002), additional studies have shown effects on rainbow trout at a dose of 42 mg kg^{-1} Cu (Hansen *et al*., 2004) and 15 mg kg^{-1} Cu (Campbell *et al*., 2002, 2005), which suggests that possibly Rainbow trout and Atlantic salmon show similar sensitivity to copper exposure through diet.

Growth and mortality

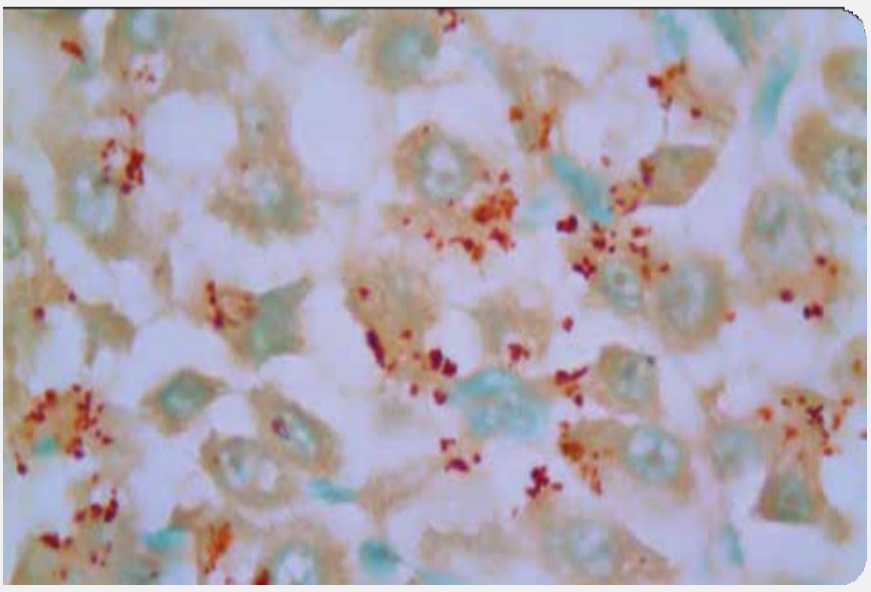
Most studies investigating growth reduction induced by dietary copper toxicity (Lanno *et al*., 1985, Baker, 1998, Berntssen *et al*., 1999a, b; Clearwater *et al*., 2002; col., 2004; Kim and Kang, 2004; Kang *et al*., 2005; Shaw and Handy, 2006) report a reduction in growth rates during exposure to copper through diet, even in cases of high dietary copper concentration there was food rejection, (Lanno *et al*., 1985) or at least, the consumption of food was reduced (Baker, 1998, Shaw and Handy, 2006). However, the growth reduction is often reported despite having no effects in terms of food intake, which seems to be caused by a reduction in the conversion of food energy to biomass (Lanno *et al*., 1985; Hansel *et al*., 2004; Kang *et al*., 2005).

The basolateral membrane of the intestinal epithelium seems to be a limiting factor in the copper uptake rate (Clearwater *et al*., 2002), and consequently copper accumulates in the intestinal tissue during dietary exposures. With this in mind, it is possible to estimate that the reduction in feed conversion is caused by the effects of copper on digestive enzymes (Li *et al*., 2007) and/or the reduction of nutrient absorption.

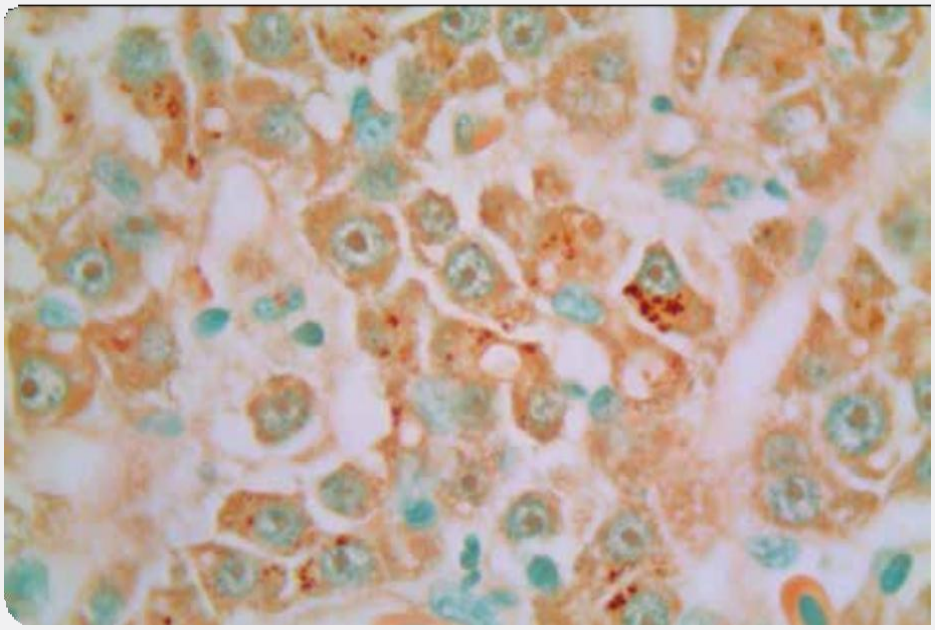
Metabolic effects

At least two studies suggest that dietary copper exposure, at high concentrations (500 to 730 mg kg-1), may have a metabolic cost. Although at rest, the metabolic rate of rainbow trout exposed to copper does not rise above the control values, fish exposed to copper swim less and therefore cover a relatively smaller distance from the oxygen consumed (Handy *et al*., 1999).

Copper-fed fish that swam at the same speed as the control fish, had higher oxygen consumption rates (Campbell *et al*., 2002) and, in general, swam at lower velocity, indicating a metabolic effect of dietary copper.



S. salar. Liver. Rhodanine A positive reaction to copper is observed, consistent with brown granules in the hepatic cytoplasm (image: Carlos Sandoval).



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In previous studies, the feeding and growth rates due to copper were not affected, therefore it seems that the reduction of activity in swimming in the laboratory is compensated, at least in part, by the increase in metabolic demand but this can have serious consequences in fish from natural environments. In fact, the impacts of dietary copper exposure on behavioural parameters are subtle but significant, and probably illustrate that such effects may affect natural populations.

A recent study revealed that social interactions between rainbow trout exposed to copper were altered compared to controls and those social interactions between control and copper-exposed fish were highly biased in favour of unexposed controls (Campbell *et al*. col., 2005). Based on the previous observation, it seems that dietary copper acts as a stressor and metabolic load factor, as is the case of water exposures.

Oxidative stress

There is evidence of oxidative stress in the intestinal and hepatic tissues, the two most important tissues that demonstrate an accumulation of copper during dietary exposure in both freshwater and seawater (Overnell and McIntosh, 1988, Berntssen *et al*., 1999a; Hoyle *et al*., 2007).

In addition to lipid peroxidation, intestinal and hepatic metallothionein levels are increased and are commonly observed during dietary copper exposure (Overnell and McIntosh, 1988, Handy *et al*., 1999, Berntssen *et al*., 1999a). Metallothionein is generally accepted as a metal binding protein, and also provides protection against ROS and thus protects against oxidative stress.

Other effects

Dietary copper exposure causes a reduction in the hepatic and lipid glycogen reserve (Hoyle *et al*., 2007), which agrees with the observations of reduction in food intake and an apparent increase in metabolic cost.

However, a marked hepatic lipidosis (increased cellular fat reserves) has also been observed in fish exposed to dietary copper (Shaw and Handy, 2006), suggesting hepatotoxicity as evident, based on observation of direct hepatic necrosis (Hansen *et al*., 2004). In addition, morphological changes in the intestine are often observed during and after dietary copper exposure (Woodward *et al*., 1995, Berntssen *et al*., 1999, Kamunde *et al*., 2001).

A recent study reports dietary copper effects on the retinoid system in zebrafish (Alsop *et al*., 2007). The metal acts on the retina, in the chromophore of rhodopsin, and is deposited inside the eggs during embryonic development (Irie and Seki. 2002). In addition, retinoids have antioxidant properties (Caiccio *et al*., 1993) and, therefore, can be depleted due to toxic substances, such as copper that can lead to ROS.
Alsop *et al*., (2007) showed that the dietary copper exposure leads to a significant depletion of retinoids, but this was without impact on reproductive performance.

There is a clear gap in the literature on the dietary copper toxicity, with the exception of the study by Alsop *et al*. (2007), which is a deficiency in the systematic evaluation of reproductive effects and to date no tests have been carried out throughout the life cycle.

Considering the apparent potential in the metabolic load stress due to the dietary copper exposure, it seems probable that the alteration of the energy distribution may occur, which leads to the reproduction reduction especially in fish fed with a fixed ration and therefore with calorie restriction.

Excerpted with modifications from:

Grossel, M. 2012. Copper. Pp: 53-133. In: C.M. Wood, A.P. Farrell and C.J. Brauner (Eds). Homeostasis and Toxicology of essential metals. Academic press. London, UK.