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RESEARCH ARTICLE

EFFECT OF CADMIUM WATERBORNE EXPOSURE ON HISTOLOGICAL STRUCTURE IN GUT TISSUE OF MOSQUITOFISH *GAMBUSIA AFFINIS*

***Annabi, A., Said, K. and Messaoudi, I.**

Laboratoire de Recherche (LR11/ES41): Génétique, Biodiversité et Valorisation des Bioressources. Institut Supérieur de Biotechnologie, Université de Monastir, 5000 Monastir, Tunisia

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ABSTRACT

In this study, cadmium (Cd) accumulation in mosquitofish *G. affinis* tissue and histological investigation of gut tissue was studied following water borne exposure. For this purpose fish were acutely exposed for 48 h to two concentrations of Cd (8 and 12 mg$CdCl_2$/L). Following acute Cd-exposure, metal accumulation profiles differed between the two concentrations. Indeed, Cd content in fish tissue is concentration dependent manner. The Cd levels in control tissue differ significantly than group exposed to 8 and 12 mg$CdCl_2$/L for 48 h and were respectively 0.67 ± 0.12, 3.03±1.19 and 30.14±19.18 µgCd/g of dw. Histopathological investigations revealed greater changes in gut *G. affinis* tissue. Following 48 h of exposure to 12 mg$CdCl_2$/L, the most changes in gut tissue were characterized by infiltration of mononuclear leucocyte (IML) and eosinophils (LE) toward lamina propria following dilatation of blood vessels. After 96h of Cd exposure, histological lesions become more pronounced and include much degeneration of submucosa and a large number of goblet cells within villi were also observed. In addition, a reduction of the villi length and infiltration of leucocyte was also noted. The results showed that, freshwater fish, as the case of mosquitofish *G. affinis*, are sensitive to the presence of Cd in their environment and are able to accumulate it via a digestive tract.

Key words: Cadmium, *Gambusia affinis*, Gut tissue, Histopathology.

INTRODUCTION

Cadmium (Cd) is considered to be one of most toxic heavy metals and one of the most toxicologically problematic metals in the freshwater environment (Canadian Environmental Protection Act, 1994). Cd, even at very low concentrations, can also disturb central functions of fish by affecting various basic biochemical and physiological processes (Larsoon *et al*., 1976). It enters the environment from natural and, essentially, anthropogenic sources (Burger, 2008). Cd dissolved in water or deposit in sediment constitutes a contamination source for the various aquatic food chain links (Romeo and Gnassia-Barelli, 1995). Most of the articles published on organisms as pollution bioindicators have concentrated on marine species, invertebrates, mainly molluscs and crustaceans. Fish are often at the top of aquatic food chain and may concentrate large amounts of Cd from water, sediment and diet (Mansour and Sidky, 2002). Therefore, the use of fish as indicators for aquatic pollution monitoring is widely recognized at present (Reddy *et al*., 2001).

Accumulation patterns of contaminants in fish depend both on uptake and on elimination rates and studies on Cd exposure in fish show the highest accumulation in the gills, intestine, kidney, and liver. Cd accumulates in organs such as the gills, liver, kidney and gastrointestinal tract of fish in an unregulated manner (Handy, 1992a, 1992b; McGeer *et al.*, 2000; Chowdhury *et al*., 2004) and accumulation between tissues varies depending on the source, whether food or waterborne uptake (Sorensen, 1991). Indeed, Cd can be incorporated into the aquatic organism biological systems by two main routes: ingestion and movement into gills (Handy, 1996). Fish have the ability to accumulate heavy metal in their tissues by the absorption along the gill surface and gut tract (Chevreuil *et al*., 1995). Dietary uptake of metals is a major cause of long-term contamination in wild fish (Dallinger *et al*., 1987), and there is renewed interest in the nutritional and toxicological effects of metals in the food of fishes (Handy, 1996).

Most of the data concerning the influence of cadmium on fish describe the effects of dietary cadmium. There is, however, some evidence that also waterborne cadmium is relatively quickly accumulated in fish tissues. Very little information is available on the histopathological alterations produced in the intestine of fishes exposed to waterborne Cd. In our study, selection of intestine was based on its direct contact with the tensioactive while gills and liver was chosen by several other study due to its direct and indirect, respectively, contact via the blood. As an indicator of exposure to contaminants, histological lesions represent a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects (Cengiz and Unlu, 2006), and have been widely used as biomarkers in the evaluation of the fish health both in the laboratory exposure (Thophon *et al.*, 2003; Au, 2004) and water criteria in field studies (USEPA, 1992). Freshwater fish are particularly vulnerable to Cd exposure (Sorensen, 1991). Indeed, freshwater fish are hypo-osmotic with their surroundings and thus a considerable movement of water into

***Corresponding author:** medannabi@yahoo.fr

their bodies occurs down an osmotic gradient and takes chemicals with it. Mosquitofish *(Gambusia affinis)* was selected for use as the test organism in our study because it is widespread in small streams in the Tunisian environment and it is also amenable to laboratory studies. *G. affinis* have the ability to accumulate and concentrate cadmium to levels several orders of magnitude above those found in their environment (Annabi *et al.*, 2009). To accomplish these goals, two acute concentration of cadmium chloride ($CdCl_2$) were selected. Metal concentrations in *G. affinis* whole body tissues were compared between both Cd concentrations after 48h of exposure. In addition, the present investigation was undertaken in order to establish histological lesions in *G. affinis* intestine following direct uptake of water borne Cd exposure.

MATERIALS AND METHODS

Origin and fish maintenance

Adult *G. affinis* were collected from an uncontaminated freshwater source "Oued El Gsil" in the town of Moknine (Annabi *et al*., 2009). A first phase of laboratory maintenance involved a period of quarantine in which the fish were acclimated to the laboratory conditions for at least two weeks (15-30 days) prior to the experiment. The animals were kept in glass aquaria (20 × 25 × 40) filled with dechlorinated tap water (pH = 7.09, salinity = 0.9 ‰), supplemented with sodium chloride (6 g/L), continuous aeration and temperature of 20 ± 1°C. During the acclimatization period, inspections were conducted twice a day in order to discard wounded, diseased and dead individuals. The photoperiod was 8:16 (8 light hours / 16 darkness hours) and fish were fed twice daily with commercially balanced fish food sticks (Tetramine, Hagen, France). The medium was renewed every two days.

Acute toxicity study

To study the accumulation of Cd in *G. affinis*, three groups of 20 fish in each group, two replicate per group, were placed in separate glass aquaria filled with dechlorinated tap water and supplemented with (6 g/L) sodium chloride. Abiotic conditions were: photoperiod of 8:16, temperature of 20 ± 1°C and continuous aeration. Metal concentrations were chosen according to our previous study (Annabi *et al.*, 2009). The acute toxicity test (48 h) consisted of a control and at least two concentrations levels (8 and 12 mg$CdCl_2$). Aquaria labeled 0, 1, 2 to correspond with groups "control", exposed to 8 mg$CdCl_2$/L and to 12 mg$CdCl_2$/L respectively. Mortality and behavior were observed daily in each concentration. Fish were starved for 24 h prior to and during the experiment.

Cadmium analysis

The determination of metal was carried out according to the duration of exposure. Fishes were sacrificed after 48 h since the start of experimentation. Fish whole tissues were dried for 48h at 60°C in Pyrex test tubes. Dried tissues were weighted and digested with concentrated nitric acid (Merck, 65 %) at 120°C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 10 mL with ultra pure water. Water samples were stabilized at pH 2 with 1 M nitric acid prior to direct determination of total metal concentrations (Bervoets and Blust, 2003). To check for possible metal loss during chronic exposure, Cd levels in water were analyzed. In the case of the control group, Cd concentrations in the acid solutions were measured by Graphite-Furnace atomic absorption spectrophotometry (AAS); while flame AAS was adopted for the exposure group. These were implemented using a ZEEnit 700-Analytik-Jena, Germany (Flame and Graphite-Furnace AAS), equipped with deuterium and Zeeman background correction, respectively, as recommended by the manufacturer. Detection limits were 0.046 µg/L for Flame AAS and 0.002 µg/L for Graphite-Furnace AAS. The accuracy and precision of the analyses for tissue Cd content were based on the analysis of Cd in a standard reference bovine liver preparation (NIST). We found 0.43 ± 0.02 µgCd/g (n=7) in bovine liver, as compared with the certified level of 0.40 ± 0.03 µg/g. These results show that the analytical results of this study are of satisfactory quality. Samples were analyzed in triplicate and Cd concentrations in tissues were calculated on a dry weight (dw) basis and expressed as µg/g of dw.

Histological procedure

Histopathological alterations were evaluated after 48h and 96h of exposure to 12 mg $CdCl_2$/L. Fish samples were fixed in Bouin's solution for 24 h, and prepared for histological analysis according to standard procedures (dehydrated in successive grades of ethanol series (70 and 95°) and embedded in paraffin. Serial longitudinal sections (thickness 4–5 µm) were stained with haematoxylin and eosin (H/E) for histological examination under a light microscope.

Statistics

Data related to metal concentrations are given as mean ±S.E. Statistical analyses were performed with unpaired *t*-test using STATVIEW statistical software package. Normality and homogeneity of data were confirmed before test. Differences between means were considered statistically significant when $p < 0.05$.

RESULTS

Cadmium accumulation

Following 48 h of exposure, metal accumulation profiles (Fig. 1) differed between the two concentrations of Cd exposure. Indeed, Cd accumulation in *G. affinis* tissues increases with the metal concentration in the water of aquaria (Fig. 1). The Cd contents in control fish group (0.67 ± 0.12 µgCd/g dw) was significantly different than those of group 1 and group 2 following 48h of Cd exposure. In group 1, Cd accumulation increase and reach the level of 3.03±1.19 µgCd/g of dw. Thereafter, in group 2 the increase in Cd concentration (in fish tissue) was significantly higher ($p < 0.01$) than that in group 1, sharply reaching a value of 30.14±19.18 µgCd/g of dw. This level was approximately 10 times higher than those following 48 h of exposure to 12 mg$CdCl_2$/L.

Effects of cadmium on histological structure in *G. affinis* intestine

Fig. 2 shows the normal histological structures of *G. affinis* intestine. Normal intestinal epithelium and underlying connective tissue of a control fish showing normal simple columnar morphology and basally aligned nuclei of the epithelial enterocytes. Normal mucous goblet cells are also

present in the epithelium. The lamina propria (LP) is composed of loose connective tissue and is supplied with many blood capillaries. After 48h of exposure to 12mg$CdCl_2$/L, the most common changes noticed in intestine tissue of *G. affinis* were infiltration of mononuclear leucocyte (IML) and eosinophils (LE) toward lamina propria following dilatation of blood vessels (Fig. 3). Following 96h of 12mg$CdCl_2$/L exposure, the histological alterations become very pronounced. It includes a reduction of the villi length and infiltration of leucocyte (Fig. 4). In addition, much degeneration of submucosa and a large number of goblet cells within villi was observed (Fig. 4).

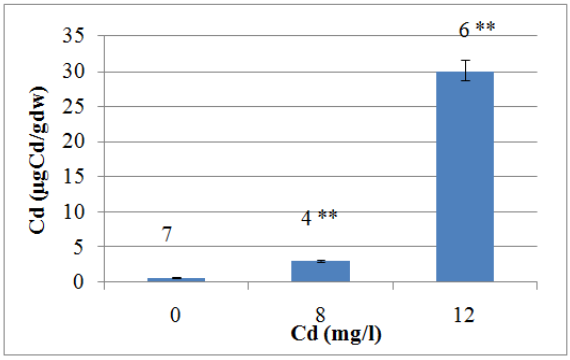

Fig. 1. Cadmium levels in *G. affinis* tissues following 48-h of exposure. ** Value significantly different than control, P < 0.01

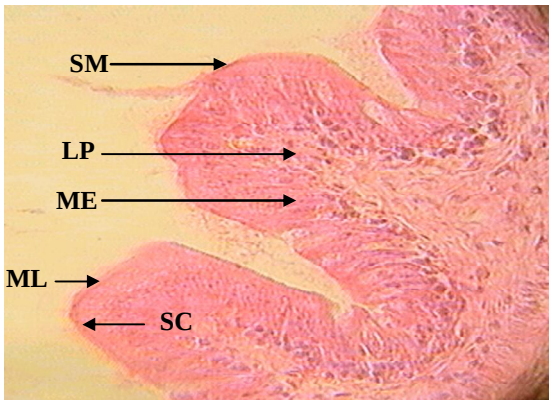

Fig. 2. Gut tissue structure of control fish. LP : Lamina Propria ; ME : Muscularis Epithelium ; ML : Muscularis Layers ; SM : Serous Membrane ; SC : Stratum Compactum (Gx40)

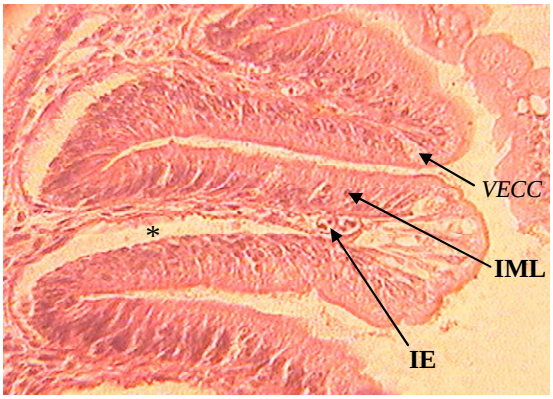

Fig. 3. Gut tissue structure of *G. affinis* exposed to 12mg$CdCl_2$/L for 48h. IE: Infiltration of eosinophils; IML: Infiltration of Mononuclear Lymphocytes; VEC: Vacuolization of epithelial cells; (*) dilatation of blood vessels in lamina propria (Gx40)

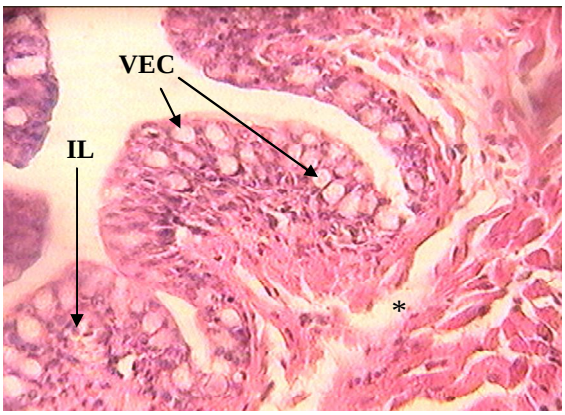

Fig. 4. Gut tissue structure of *G. affinis* exposed to 12mg$CdCl_2$/L for 96h. IL: Infiltration of leucocytes; VEC: vacuolization of epithelial cells; (*) degeneration of submucosa (Gx40)

DISCUSSION

The present study was undertaken to investigate pattern of Cd accumulation in freshwater mosquitofish (*G. affinis*) tissues following two acute waterborne Cd concentrations and to determine the histological damage in *G. affinis* intestine induced under metal exposure. In aquatic ecosystem, Cd can enter the aquatic food chain through direct consumption of water and through non-dietary routes such as absorption through epithelia. Even though, for fish, the gills, skin and digestive tract are potential sites of absorption of Cd waterborne (Norey *et al.*, 1990). Although, many studies have examined the relationship between metal exposure, accumulation and toxicity under laboratory conditions, prediction of toxic effects based on environmental or tissues concentrations remains difficult under natural exposure conditions (Barron *et al.*, 2002; Vijver *et al.*, 2004). For these reason, laboratory exposure is often combined with evaluation of biomarkers that represent early indicators of biological effects. Additionally several factors influence Cd accumulation in fish tissues and these factors include the ability for homeostatic control, detoxification and rejection system (Allen, 1995; De Conto Cinier *et al.*, 1998). During 48h of exposure, we observed higher Cd concentration in contaminated specimens with 12 mg$CdCl_2$/L in relation to those found after exposure to 8 mg$CdCl_2$/L.

During 48h of exposure, fish tissue in group 1 contained low concentrations of Cd compared to group 2 which may be due to its accumulation mainly in the detoxifying tissue. These results are in accordance with those found during our preliminary field study (Annabi *et al.*, 2009) and laboratory exposure (Annabi *et al.*, 2011). The difference between group 1 and 2 in accumulation levels for the first 48h of Cd exposure were significantly important. After 48h of exposure to 12mg $CdCl_2$/L, net accumulation of Cd was approximately 30 fold higher than that to 8mg $CdCl_2$/L. These results confirm that Cd fixation by *G. affinis* tissues depend on exposure time as well as metal concentration in the environment. Indeed, accumulation of heavy metals in aquatic animals tissues depend greatly on the concentration of this element in their natural environment, in water as well as in sediment (Hamza-Chaffai *et al.*, 1995; Mahyaoui *et al.*, 2003). Moreover, it was reported that Cd rarely distributes uniformly within the fish body tissues, but is accumulated by particular target organs

(Surech *et al.,* 1993). The distribution of the metal accumulated in different organs can vary depending on the source of uptake (diet and/or waterborne) and on the animal species. Indeed, it was been reported that in *Cyprinus carpio*, gills accumulate more Cd from Cd-water contamination than from Cd-food contamination (Kraal *et al.,* 1995). Cd toxicity to fish tissue generally includes two processes: injury to gills (excess mucus production and impairment respiratory function) and damage to kidneys (impairment to renal regulation of ions such as calcium and potassium). While the rate of physiological processes that influence uptake and distribution of Cd in fish digestive tract has been considered a critical element in establishing links between toxicity and exposure in risk assessment in freshwater fish. In fish, to accelerate excretion of Cd, metal loss by sloughing of dead cellular materials and mucus from the gills and/or gut (Sorensen, 1991; Handy, 1996). When the amount of accumulated Cd exceeds the ability of the fish to synthesize the detoxifying systems (metallothioneins, MT), localization of the Cd, such as muscle, occurs (Surech *et al.,* 1993).

During exposure, Cd uptake is dependent on the interaction between cadmium and MT-transport protein which may be affected by the other factors. In fish, a large proportion of dietary Cd excretion occurs as a result of intestinal sloughing and a small quantity is probably excreted via the bile and gills (Handy, 1996). Several authors described that fish intestine is the most sensitive tissue to chronic cadmium exposure (Bay *et al.,* 1990; Brown *et al.,* 1990). Freshwater fish have two main uptake pathways for ions: the gills (waterborne ions) and the gastrointestinal tract (dietary ions), and can control the total uptake by changing the proportion of each kind of uptake according to the situation. Previous histopathological studies of fish exposed to pollutants have shown that fish gills are efficient indicators of water quality. Fish gills are vulnerable to pollutants in water because of their large surface area and external location. While, the first organ to come into contact with contaminated food particles is the gut tissue. Histologically, the overall structure of intestine in *G. affinis* is similar to other fish. However, following acute exposure to Cd waterborne, histological lesions were detected. Indeed, mucosal area of intestine was affected and several lesions of the intestine were discernable in villar region.

Infiltration of lymphocytes and dilatation of blood vessels in the lamina propria represent the most common alterations. Following 96h of exposure, dilatation of blood vessels in the lamina propria and degeneration of the submucosa were more pronounced. In addition, reduction of the villi length and a high secreting activity of goblet cells may impair blood-water exchange by reducing distances between villi leading to the reduction of the contact surface area available for cadmium uptake. These responses, whether adaptive or pathological, invariably affect homeostatic regulation of the internal environment, in particular decreasing the efficiency of dietary exchange. In this study, the high secreting activity of goblet cells following Cd-waterborne exposure was believed to have been stimulated by cadmium taken up by *G. affinis*. Enhanced secretion of mucus in Cd-exposed seems to be a defensive mechanism against Cd toxicity. Infiltration of mononuclear leucocytes and eosinophils towards lamina popria are often considered as non specific stress responses. Indeed, theses histological alterations were found in gut exposure to endosulfan, thiodan and deltamethrin (Braunbeck and Appelbaum, 1999; Cengiz *et al.,* 2001; Cengiz and Unlu, 2006). On the other hand, degeneration in the submucosal area reported in this study was similar to that observed by Banerjee and Bhattacharya (1995) in *Channa punctatus* after exposure to mercury for 7 days and by Kruatrachu *et al.* (2003) in gastrointestinal tract of *Puntius gonionotus* fed on dietary cadmium for two week. There is some evidence that cadmium disturb intestinal ionoregulatory homeostasis. Indeed, in tilapia intestine Cd inhibit Na^+/K^+-ATPase (Schoenmarkers *et al.,* 1992). The same author reveals that dietary Cd inhibits the activity of intestinal sodium–calcium exchangers. Dietary cadmium causes an oxidative damage in the intestine of Atlantic salmon (*Salmo salar*) following 1 month exposure at 204 mgCd/kg diet (Berntssen *et al.,* 2000).

Conclusion

The results of our experiment strongly support that waterborne Cd can be one of the sources of this metal in mosquitofish (*G. affinis*) tissues. It can be conclusively deduced, from intestine histopathology, that intestine *G. affinis* has the tendency to accumulate heavy metals via waterborne exposure. Histological study also found that in the gut, exposure to cadmium is associated with changes in the epithelial lining, which indicates disturbance of intestinal absorbtion. The highest accumulation of Cd in *G. affinis* tissues and the histological lesions produced in intestine make this species suitable as a test organism for toxicological research. For future research, Cd accumulation in digestive tract should be taken into consideration while estimating ecotoxicological effects of heavy metal on physiological mechanism.

REFERENCES

Allen, P. 1995. Chronic accumulation of cadmium in the edible tissues of *Oreochromis aureus*: modification by mercury and lead. *Arch. Environ. Con. Tox.*, 29: 8–14.

Annabi, A., Messaoudi, I., Kerkeni, A. and Said, K. 2009. Comparative study of the sensitivity to cadmium of two populations of *Gambusia affinis* from two different sites. *Environ. Monit. Assess.*, 155: 459-465.

Annabi, A., Messaoudi, I., Kerkeni, A. and Said, K. 2011. Cadmium Accumulation and Histological Lesion in Mosquitofish (*Gambusia affinis*) tissues Following Acute and Chronic Exposure. *Int. J. Environ. Res.*, 5: 745–756.

Au, D. W. T. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. *Mar. Pollut. Bull.*, 48: 817–834.

Banerjee, S. and Bhattacharya, S. 1995. Histopathological changes induced by chronic non lethal levels of elsan, mercury and ammonia in the small intestine of *channa punctatus* (Bloch). *Ecotox. Environ. Saf.*, 31: 62–68.

Barron, M.G., Hansen, J. A. and Lipton, J. 2002. Association between contaminant tissue residues and effects in aquatic organisms. *Rev. Environ. Contam. Toxicol.*, 173: 1–37.

Bay, S. M., Greenstein, D. J., Szalay, P. and Brown, D. A. 1990. Exposure of scorpionfish (*Scorpaena guttata*) to cadmium: biochemical effects of chronic exposure. *Aquat. Toxicol.*, 16: 311–9.

Berntssen, M. H. G., Lundebye, A. K. and Hamre, K. 2000. Tissue lipid peroxidative responses in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. *Fish. Physiol. Biochem.*, 23: 35–48.

Bervoets, L. and Blust, R. 2003. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. *Environ. Pollut.*, 126: 9–19.

Braunbeck, T. and Appelbaum, S. 1999. Ultrastructural alterations in the liver and intestine of carp *Cyprinus carpio* induced orally by ultra-low doses of endosulfan. *Dis. Aquat. Organ.*, 36: 183–200.

Brown, D. A., Bay, S. M. and Hershelman, G. P. 1990. Exposure of scorpionfish (*Scorpaena guttata*) to cadmium: effects of acute and chronic exposures on the cytosolic distribution of cadmium and zinc. *Aquat. Toxicol.*, 16: 295-310.

Burger, J. 2008. Assessment and management of risk to wildlife from cadmium. *Sci. Total. Environ.*, 389: 37-45.

Canadian Environmental Protection Act 1994. Priority Substances List, Assessment Report. Cadmium and its Compounds, Government of Canada.

Cengiz, E. I. and Unlu, E. 2006. Sublethal effects of commercial deltamerthrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. *Environ. Toxicol. Phar.*, 21: 246 – 253.

Cengiz, E. I., Unlu, E. and Balci, K. 2001. The histopathological effects of Thiodan® on the liver and gut of mosquitofish, *Gambusia affinis*. *J. Environ. Sci. Health. Part B.*, 36: 75-85.

Chevreuil, M., Carru, A. M., Chesterikoff, A., Boët, P., Tales, E. and Allardi, J. 1995. Contamination of fish from different areas of the river Seine (France) by organic (PCB and pesticides) and metallic (Cd, Cr, Cu, Fe, Mn, Pb and Zn) micropollutants. *Sci. Total. Environ.*, 162: 31-42.

Chowdhury, M. J., Grosell, M., McDonald, D. G. and Wood, C. M. 2004. Gastrointestinal uptake and fate of cadmium in rainbow trout acclimated to sublethal dietary cadmium. *Aquat. Toxicol.*, 69: 149-163.

Dallinger, R., Prosi, F., Segner, H. and Back, H. 1987. Contaminate food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia.*, 73: 91-98.

De Conto Cinier, C., Petit Ramel, M., Faure, R. and Bortolato M. 1998. Cadmium accumulation and metallothionein biosynthesis in *Cyprinus carpio* tissues. *Bull. Environ. Contam. Toxicol.*, 61: 793-799.

Hamza-Chaffai, A., Cossin, R. P., Amirad-Triquet, C. and El Abed A. 1995. Physico chemical forms of storage of metals (Cd, Cu, and Zn) and metallothionein-like proteins in gills and liver of marine fish from the Tunisian coast: ecotoxicological consequences. *Comp. Biochem. Physiol. C.*, 102: 329-341.

Handy, R. D. 1992a. The assessment of episodic metal pollution. I. Uses and limitations of tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*) after short waterborne exposure to cadmium or copper. *Arch. Environ. Contam. Toxicol.*, 22: 74-81.

Handy, R. D. 1992b. The assessment of episodic metal pollution. The effects of cadmium and copper enriched diets on tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.*, 22: 82-87.

Handy, R.D. 1996. Dietary exposure to toxic metals in fish, In: *Toxicology of Aquatic Pollution: Physiological, Molecular, and Cellular Approaches, Society of Experimental Biology Seminar Series 57* (Ed. Taylor, E. W.), 29-60. Cambridge University Press, Cambridge.

Kraal, M. H., Kraak, M. H. S., De Graat, C. J. and Davids, C. 1995. Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). *Ecotox. Environ. Safe.*, 31: 179-183.

Kruatrachue, M., Rangsayatorn, N., Pokethitiyook, P., Upatham, E.S. and Singhakaew, S. 2003. Histopathological changes in the gastrotestinal tract of fish, *Puntius gonionotus*, fed on dietary cadmium. *Bull. Environ. Contam. Toxicol.*, 71: 561-569.

Larsson, A., Johansson-Sjobeck, M. L. and Fange, R. 1976. Comparative study of some haematological and biochemical blood parameters in fishes from the skagerrak. *J. Fish. Biol.*, 9: 425–440.

Mahyaoui, M., Saghi, M. and Kharchaf, I. 2003. Etude de la pollution métallique (Cd- Cu-Pb et Zn) du littoral atlantique marocain entre Kenitra et Temara. *Chemosphere.*, 18: 1639-1655.

Mansour, S. A. and Sidky, M. M. 2002. Ecotoxicological studies 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food. Chem.*, 78: 15-22.

McGeer, J. C., Szebedinszky, C., McDonald, D. G. and Wood, C. M. 2000b. Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout 2: tissue specific metal accumulation. *Aquat. Toxicol.*, 50: 245–256.

Norey, C. G., Cryer, A. and Kay, J. 1990. A comparison of cadmium induced metallothioneins gene expression and Me2+ distribution in the tissues of cadmium-sensitive (rainbow trout; *Salmo gairdneri*) and tolerant (stone loach; *Noemacheilus barbatulus*) species of freshwater fish. *Comp. Bioch. Physiol. C.*, 97: 221–225.

Reddy, M. L., Dierauf, L. A. and Gulland, F. M. D. 2001. In: Marine mammals as sentinels of the ocean health, Handbook of marine mammal medicine (Eds. Dierauf, A. L. and M. D. Gulland), 3-13. Boca Raton, CRC Press.

Romeo, M. and Gnassia-Barelli, M. 1995. Metal distribution in different tissues and in subcellular fractions of the mediterranean clam *Ruditapes decussatus* treated with cadmium, copper, or zinc. *Comp. Bioch. Physiol.*, 111: 457-463.

Schoenmarkers, T. J. M., Klaren, P. H. M., Flik, G., Lock, R. A. C., Pang, P. K. T. and Wendelaar Bonga, S. E. 1992. Actions of cadmium on basolateral plasma membrane proteins involved in calcium uptake by fish intestine. *J. Membr. Biol.*, 127: 161-172.

Sorensen, E. M. 1991. Metal poisoning in fish, 175–234. CRC press, Boca Raton.

Suresh, K., Ahamed, M. S., Durairaj, G. and Nair, K. V. K. 1993. Impact of power plant heated effluent on the abundance of sedimentary organisms, off Kalpakkam, East Coast India. Hydrobiologia., 268: 109-114.

Thophon, S., Kruatrachuea, M., Upathama, E. S., Pokethitiyooka, P., Sahaphongb, S. and Jaritkhuanc, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. *Environ. Pollut.*, 121: 307–320.

USEPA (United States Environmental Protection Agency) (1992). Ground water and drinking water. [M]. 810/K-92-001.

Vijver, M. G., Van Gestel, C. A., Lanno, R. P., Van Straalen, N. M. and Peijnen-burg, W. J. 2004. Internal metal sequestration and its ecotoxicological relevance: a review. *Environ. Sci. Technol.*, 38: 4705–4712.

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