Current Topics in Toxicology
Vol. 16, 2020

Original Article

# Toxicity of copper oxide nanoparticles to rainbow trout juveniles

**J. Auclair, P. Turcotte, C. Gagnon and F. Gagné**

Aquatic Contaminants Research Division, Environment and Climate Change Canada, 105 McGill, Montréal, Québec, H2Y 2E7, Canada.

## ABSTRACT

The increasing commercial applications of copper oxide nanoparticles (nCuO) have raised concerns about the risk to the aquatic environment. The purpose of this study was to examine the toxicity of low concentrations of nCuO and Cu(II) as copper sulfate in juvenile rainbow trout. Juveniles were exposed to a range of concentrations of nCuO (12.8, 32, 80, 200 and 500 µg/L as total Cu) and Cu(II) (12.8, 32 and 80 µg/L total Cu) for 96 h at 15 °C under constant aeration. After the exposure period, fish were removed and examined for total and labile Cu in gills, oxidative stress (lipid peroxidation and glutathione S-transferase), genotoxicity (DNA damage), and reticulum endoplasmic stress (heat shock proteins 70 and protein-ubiquitin levels). The data revealed that although nCuO did not lead to the increase in Cu accumulation in fish, the nanoparticles produced changes that were not observed with Cu(II) such as increased glutathione S-tranferase activity in gills with a strong decrease in lipid peroxidation and DNA strand breaks in the same tissues. Labile Cu levels in gills were increased and decreased by nCuO and Cu(II), respectively. Both forms of Cu were able to elicit changes in heat shock proteins and protein-ubiquitinylation. In conclusion, exposure to low concentrations of nCuO could produce change in gills and the liver in fish before the accumulation of Cu loading in tissues. The effects of nCuO differ from those of Cu(II) in rainbow trout.

**KEYWORDS:** fish, copper oxide nanoparticles, oxidative stress, genotoxicity, reticulum endoplasmic stress.

## INTRODUCTION

Nanotechnology is an intense area of research since products at the nanoscale exhibits new emerging properties some of which are of commercial interest. This trillion of dollars industry has many applications in key area such as medicine therapeutics, energy production and environmental sustainability and green chemistry such as cosmetics, personal care products, agriculture, textiles and electronics [1]. However, the production and commercial uses of nanotechnology on such a large scale by several industries led to the inadvertent release of nanomaterials in the environment thereby threatening wildlife [2, 3]. For example, their use in household products and domestic appliances release nanoparticles in urban wastewaters which are discharged in terrestrial (sludge) and aquatic environments. Recent studies revealed that nanoparticles could affect aquatic animal health at the molecular and cellular levels [4]. In carps feeding on commercial food spiked with zinc oxide nanoparticles for six weeks, a proteomic analysis of the intestines and muscle body revealed changes in protein synthesis, cytoskeleton organisation, apoptosis, cell metabolism and oxidative stress. Copper oxide nanoparticles (nCuO) have been used in cosmetics, catalysts, gas sensors and microelectronics [5]. Due to their increasing use compared to other metal oxide nanoparticles, the potential danger to aquatic organisms needs to be more closely examined. Because of the high surface area and reactivity, nanoparticles could become more toxic than the bulk counterpart [6].

Copper-based nanoparticles could accumulate in aquatic organisms and produce toxicity which involves the release of ionic Cu which, in turn, produce toxicity. However, toxicity could arise not only through the release of Cu but by other properties of nanoparticles such as surface reactivity, size and form. The 96-h acute toxicity of nCuO in rainbow trout was estimated at 680 µg/L which is circa 5.7 times less toxic than Cu(II) [7]. The toxicity of Cu(II) has been studied extensively in aquatic organisms. Exposure of 40 and 400 µg/L of Cu(II) to the Nile tilapia leads to reduced growth, oxidative stress and decreased non-specific immunity [8]. In rainbow trout, the 96-h lethal concentration (LC50%) of Cu(II) was estimated at 11 µg/L and produce a number of effects at the sublethal exposure concentration. Exposure to Cu(II) or Cu(I) led to oxidative stress in trout and induction of metallothioneins and lipid peroxidation in rainbow trout exposed to an equivalent concentration of 10 and 50 µg/L, respectively [9]. In rainbow trout larvae, exposure to low concentrations of Cu (2 and 20 µg/L) increased gene expression in metallothioneins and glutathione S-transferase (GST) [10]. However, no evidence of genotoxicity was observed using the COMET assay in erythrocytes. Recent studies showed that similar effects are obtained with nCuO although the contribution of released Cu(II) has not been determined yet. The comparative toxicity of CuO and Cu(II) was examined in rainbow trout and revealed that both Cu forms could produce similar effects but not always [11]. Although Cu(II) was more bioavailable than nCuO, both forms of Cu was able to induce DNA damage and heamolysis in erythrocytes. GST activity was induced in rainbow trout exposed to nCuO and was shown to favor internalization of Zn in tissues [12]. From the environmental protection perspective, the identification of biomarkers that could discriminate between the effects of dissolved Cu(II) and nCuO would be of value.

The purpose of this study was therefore to examine the acute toxicity and sublethal effects of nCuO and Cu(II) in juvenile *Oncorhynchus mykiss* trout. The null hypothesis is that the toxic effects between nCuO or Cu(II) are identical. Sublethal toxicity was examined by following changes in total Cu accumulation, labile or the free form of Cu in gills, oxidative stress (lipid peroxidation and GST activity), protein denaturation (heat shock proteins), protein turnover (ubiquitinylation) and genotoxicity. An attempt was made to identify biochemical responses associated to exposure to the various forms of Cu in rainbow trout.

## MATERIALS AND METHODS

### Copper oxide nanoparticle

A stock solution of Copper oxide nanoparticle (nCuO) from US Research Nanomaterials (USA) was used in this study. According to the manufacturer's specifications, the nCuO suspension had a size distribution between 25-55 nm. For the exposure regime, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to increasing concentrations of either nCuO or Cu(II) as $CuSO_4$ in dechlorinated tap water (controls): 0, 12.8, 32, 80 µg/L for Cu(II) and 0, 12.8, 32, 80, 200 and 500 µg/L for nCuO. Control fish were exposed to aquarium water only, which was obtained from tap water after UV-treatment and charcoal filtration. The nanoparticle size and Zeta potential of nCuO were determined in aquarium water using a dynamic light scattering instrument (Mobius Instrument, Wyatt Technologies, Santa Barbara, CA, USA) operating with a laser at a wavelength of 532 nm. The instrument was previously calibrated with standard suspensions of latex bead nanoparticles (from Polyscience, USA).

The total levels of Cu in the exposure media were determined after 1 hr of suspension of nCuO using ion-coupled plasma mass spectrometry (ICP-MS) following acidification with 1% v/v nitric acid (Seastar grade BC, Canada). Juvenile rainbow trout (n = 20) were exposed to either nCuO or Cu(II) as $CuSO_4$ for 96 h at 15 °C under constant aeration. The exposure media were not renewed and the fish were not fed during the exposure period. After the exposure period, fish mortality was recorded and the remaining fish were euthanized in 50 mg/L tricaine methanesulfonate (5 min) in aquarium water buffered at pH 7 with $NaHCO_3$ and the fish immediately placed on ice. For Cu loadings in fish, a subgroup of 10 individuals were placed in clean aquarium water overnight as a depuration step

and the fish were then utilized for total Cu determination using ICP-mass spectrometry as described above. The tissues were acid-digested in 10% $HNO_3$ at 70-80 °C for 12 h and diluted to 1% with MilliQ water. For the biomarker analyses, the remaining group of 10 fish was evaluated for total weight and fork tail length. The livers and gills were dissected on ice, weighted and immediately stored at -85 °C with 5 volumes of homogenization buffer. The homogenization buffer consisted of 140 mM NaCl containing 5 mM $KH_2PO_4$, 1 mM $NaHCO_3$, 25 mM Hepes-NaOH, pH 7.4, 1 µg/mL apoprotinin and 1 mM dithiothreitol.

**Toxicity and biomarker responses**

The acute toxicity of nCuO and Cu were determined in rainbow trout juveniles following Environment and Climate Change Canada's standard procedure [13]. In surviving fish, the livers and gills were thawed on ice for 15-30 min and homogenized using a Teflon pestle tissue grinder at 4 °C. A portion of the homogenate was set aside for lipid peroxidation (LPO), DNA damage and total proteins assessments. The remainder of the homogenate was centrifuged at 15 000 x g for 20 min at 4 °C and the supernatant (S15) was removed for the estimation of glutathione S-transferase (GST), labile Cu(II), heat shock proteins 70 and protein-ubiquitin levels. Total proteins were determined in the homogenate and S15 fraction using the protein-dye binding principle using standard solutions of serum bovine albumin for calibration [14].

Lipid peroxidation (LPO) was determined in soft tissue homogenates using a spectrofluometric methodology [15]. A volume of 25 µL of the homogenate was mixed with 175 µL of 10% trichloroacetic acid containing 1 mM $FeSO_4$ and 50 µL of 0.7% thiobarbituric acid. The mixture was heated at 75 °C for 10 min, cooled to room temperature and centrifuged at 10 000 x g for 5 min to remove any precipitates. A 200 µL volume was transferred to a 96-well dark microplate, and fluorescence readings were taken at 540 nm excitation and 600 nm emission. Standard solutions of malonaldehyde (tetramethoxypropane, Sigma Chemical Company, ON, Canada) were made for calibration in the homogenization buffer. Results were expressed as µg thiobarbituric acid reactants (TBARS)/mg total proteins in the homogenate. The levels of DNA strand breaks were also determined in the homogenate using the fluorescence DNA precipitation assay [16, 17]. Briefly, 25 µL of the homogenate from each tissues was mixed with 100 µL of 50 mM NaOH, 10 mM Tris base, 10 mM ethylenediamine tetraacetate and 2% sodium dodecyl sulphate (SDS). After 5 min, one volume of 0.12 M KCl was added and heated at 60 °C for 10 min. The mixture was cooled on ice for 10 min and centrifuged at 8 000 x g for 10 min to precipitate SDS-associated protein and genomic DNA. The supernatant (DNA strands) was mixed with SYTO Green dye in 3 mM sodium cholate, 0.4 M NaCl and 100 mM Tris-acetate pH 8 to control for the traces of SDS in the supernatant which could interference the fluorescence readings [18]. Fluorescence was measured at 485 nm excitation and 530 nm emission (Microplate, Synergy-4, Bioteck, USA) using standard solutions of salmon sperm DNA for calibration. The data were expressed as µg supernatant DNA/mg proteins.

Levels of labile Cu(II) were determined using a fluorescent probe methodology [19]. Briefly, a 20 µL sample of the S15 fraction was mixed with 180 µL of 50 µM of 1,2-diaminoanthraquinone in 140 mM NaCl, 5 mM $KH_2PO_4$ and 10 mM Hepes-NaOH, pH 7.4. Fluorescence was measured at 400 nm excitation and 475 nm emission (Synergy-4, Biotek Instuments, USA) using standard solutions of $CuSO_4$ for instrument calibration. Data were expressed as relative fluorescence units (RFU)/mg proteins. The activity of glutathione S-transferase (GST) activity was determined in the S15 fraction using a microplate spectrophotometric assay [20]. The activity was determined using reduced glutathione and 2,4-dichlorodinitrobenzene as the substrates which was followed at 340 nm. The data were expressed as the increase in absorbance at 340 nm/min/mg total proteins in the $S_{15}$ fraction. The levels of heat shock proteins 70 and polyubiquitinylated proteins were determined by enzyme-linked immunosorbent assay (ELISA) in the S15 fraction as described previously [21]. Standards of polyubiquitin (Di-ubiquitin ($K^{48}$-linked) BML-UNW9800, Enzo Life Sciences, USA) and HSP70 (SPP-763, Enzo Life Sciences, USA) and the S15 fraction were used to coat the

microplate wells (Immulon-4). The ubiquitin lys48-specific, clone Apu2, monoclonal antibody (# 05-1307; EMD Millipore, Billerica, USA) or the HSP70/HSP72 polyclonal antibody (ADI-SPA-812; Enzo Life Sciences, USA) was diluted 1/2000 in Dulbecco's phosphate buffered saline (DPBS: 137 mM NaCl, 2.7 mM KCl, 1.5 mM $KH_2PO_4$ and 8.1mM $Na_2HPO_4$, pH 7.4) containing 0.5 % albumin and was added to each wells. After incubation for 3 h at room temperature, the wells were washed with DPBS and the secondary antibody (anti-rabbit IgG-HRP conjugated; ADI-SAB-300, Enzo Life Sciences, USA) diluted 1/5000 in DPBS-0.5% albumin and incubated for another hour. After well washing, the activity of peroxidase was detected using a highly sensitive Chemiluminescence assay kit (Roche Diagnostics, QC, Canada). Data were expressed as ng of polyubiquitin or HSP70/mg proteins.

**Data analysis**

The study design examines the toxicity of two chemical forms of Cu (nCuO and Cu(II)) in rainbow trout. In this study, there were in total 9 treatments: fish exposed to aquarium water only (controls); fish exposed to 5 concentrations Cu as nCuO in aquarium water; and fish exposed to 3 concentrations of Cu as $CuSO_4$ in aquarium water. Data normality and homogeneity of variance were checked by the Shapiro-Wilk and Bartlett tests, respectively. The influence of exposure concentrations and Cu forms (control, nCuO and Cu(II)) was examined using 2-way factorial analysis of variance. Critical differences between treatments were determined using the Least Square Difference (LSD) test. The trends between the data were also analyzed using the Pearson moment correlation test. The biomarker data were also analyzed by discriminant function analysis to determine which biomarkers best discriminate between the influence of surface waters and forms of Cu. Significance was set at $p < 0.05$. All statistical analyses were performed using the SysStat software package (version 13.2, USA).

**RESULTS**

Preliminary experiments revealed that Cu(II) decreased survival of juvenile rainbow trout with an LC50 (lethal concentration that kills 50% of the fish) of 116 µg/L (95% confidence interval: 75-180). No mortality was encountered with nCuO at concentrations up to 500 µg/L. In fish exposed to Cu(II), the fish weight/fork length ratio was significantly increased while no effects was observed for nCuO (Figure 1). The hepato-somatic index (HIS; liver weight/fish weight) was significantly decreased only at 80 µg/L of Cu(II) which is close to the LC50 for this form of Cu. The levels of total Cu in fish and Cu in gills were also determined (Figure 2). The total Cu levels were significantly increased in fish exposed to Cu(II) but not to nCuO which suggests that dissolved Cu was more bioavailable than nCuO. The levels of labile Cu in gills were also increased in fish exposed to dissolved Cu(II) while a decrease was observed in gills of fish exposed to the highest concentration of nCuO (i.e., 500 µg/L). Correlation analysis revealed that the HSI was significantly correlated with Cu tot ($r = -0.24$) and Cu(II) in gills ($r = -0.26$) (Table 1).

Oxidative stress was measured by following changes in GST activity and LPO levels in gills and the liver of juvenile trout (Figure 3). With respect to GST activity, GST activity was increased for low concentrations of nCuO (12.8 to 80 µg/L) in gill tissues. With respect to LPO levels, the levels were decreased only for nCuO at concentrations > 12.8 µg/L in gills as well. A moderate decrease in LPO levels was observed at the highest concentration of Cu(II) of 80 µg/L. Gill LPO levels were significantly correlated with labile Cu(II) in gills ($r = 0.20$). GST activity was significantly correlated with condition factor ($r = -0.24$ in gills). The effects of Cu forms were also investigated at the endoplasmic reticulum stress level as determined by HSP70 and protein-ubiquitin levels (Figure 4). Hepatic HSP70 levels were significantly decreased by Cu(II) and nCuO at concentrations between 12.8-80 µg/L. Ubiquitin levels attached to proteins were significantly lower in the liver and gills in fish exposed to Cu(II). In fish exposed to nCuO, the response pattern appeared biphasis. Indeed, the protein-ubiquitin levels in gills were increased at the lowest concentration of nCuO (12.8 µg/L) and significantly decreased at 80 µg/L and increased again at higher concentrations. In the liver, the levels were

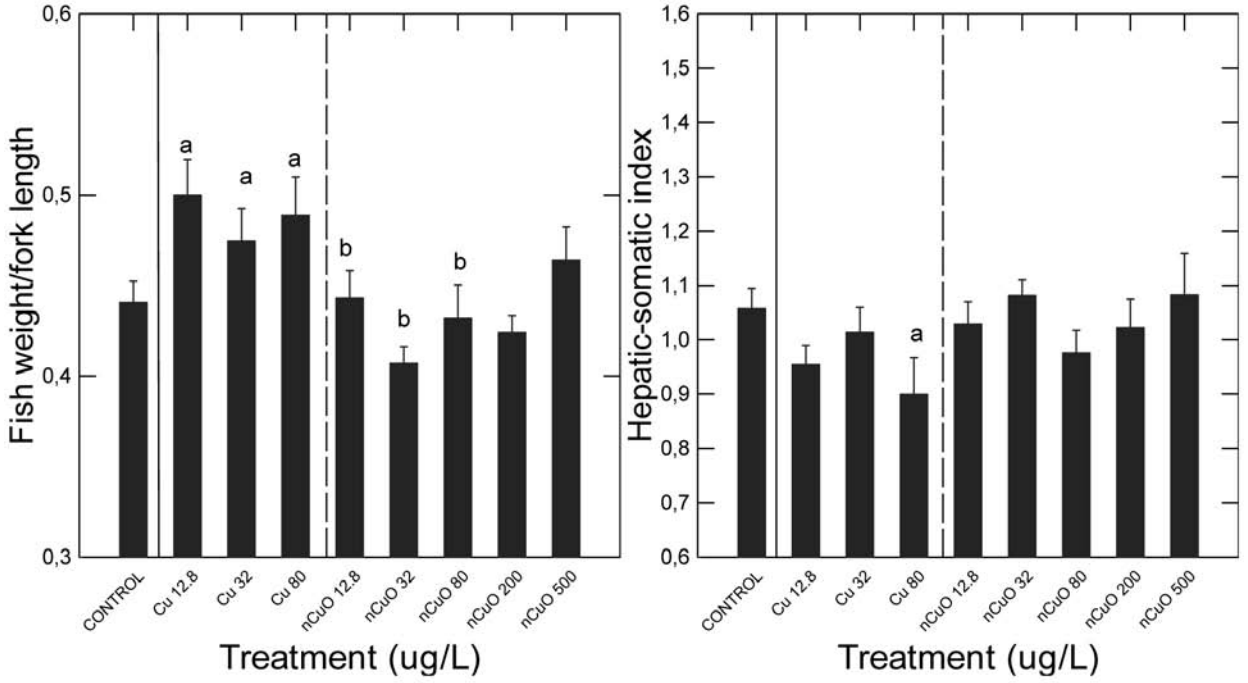


**Figure 1.** Morphological changes in juvenile trout exposed to Cu forms.
The letters a and b indicate significance from the control and between Cu forms at the same concentration, respectively.

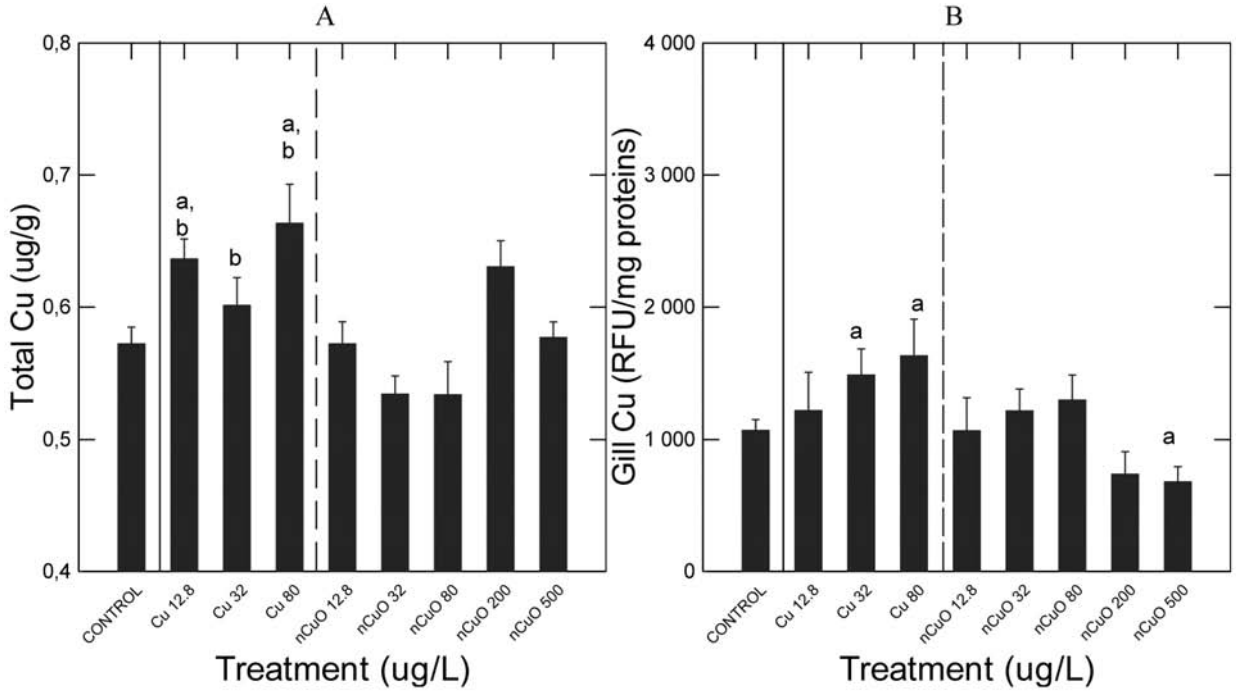


**Figure 2.** Total and gill labile copper levels of trout exposed to both forms of Cu.
The letters a and b indicate significance from the control and between Cu forms at the same concentration, respectively.

**Table 1.** Pearson Correlation Matrix.

| | **Liver** | | | | | | | **Gill** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CF | HSI | GST | LPO | DNA | UB | HSP70 | gst | lpo | dna | ub | Cu(II) |
| **Liver** | | | | | | | | | | | | |
| HSI | -0.23 | 1 | | | | | | | | | | |
| GST | -0.12 | -0.18 | 1 | | | | | | | | | |
| LPO | -0.10 | -0.12 | 0.01 | 1 | | | | | | | | |
| DNA | -0.12 | -0.19 | 0.04 | **0.35** | 1 | | | | | | | |
| UB | -0.02 | 0.11 | -0.03 | **-0.30** | **-0.24** | 1 | | | | | | |
| HSP70 | -0.16 | **0.27** | 0.10 | 0.07 | -0.17 | 0.51 | 1 | | | | | |
| **Gill** | | | | | | | | | | | | |
| Gst | **-0.24** | 0.16 | -0.04 | -0.03 | -0.49 | -0.08 | 0.03 | 1 | | | | |
| lpo | 0.06 | -0.06 | -0.15 | -0.23 | -0.10 | **0.27** | -0.05 | 0.13 | 1 | | | |
| dna | 0.08 | 0.03 | -0.01 | -0.19 | -0.05 | **0.29** | 0.02 | -0.02 | **0.54** | 1 | | |
| ub | 0.08 | 0.16 | -0.12 | -0.15 | -0.13 | 0.24 | **0.26** | 0.06 | -0.05 | 0.07 | 1 | |
| Cu(II) | 0.07 | **-0.26** | 0.01 | 0.03 | -0.17 | -0.09 | **-0.28** | **0.27** | **0.19** | 0.12 | -0.08 | 1 |
| Cu(total) | 0.11 | **-0.24** | 0.02 | -0.17 | **0.33** | 0.10 | -0.14 | **0.42** | 0.15 | **0.20** | 0.12 | 0.01 |

Significant correlations are indicated in **bold**.

essentially decreased by nCuO. Correlation analysis revealed that protein-ubiquitin levels were negatively and positively correlated with hepatic (r = -0.3) and gill (r = 0.27) LPO levels. The levels of DNA damage were also determined (Figure 5). DNA strand breaks were significantly decreased in the liver of fish exposed to nCuO but not ionic Cu. A transient increase in DNA strand breaks was observed at 200 µg/L nCuO. Correlation analysis revealed that DNA strand breaks in gills were significantly correlated with total Cu (r = 0.20) and LPO in gills (r = 0.54). DNA strand breaks were also correlated with LPO levels in the liver (r = 0.35).

In the attempt to gain a global understanding on the toxic effects of nCuO and Cu(II), a discriminant function analysis was performed (Figure 6). The analysis revealed that the biomarker responses between controls, Cu(II) and nCuO differed from each other (classification performance of 88%). The following biomarkers were the most important i.e., had the highest factorial weights with the x and y axis components: labile Cu(II), total Cu in tissues, LPO, GST activity and DNA damage in the liver.

## DISCUSSION

The bioavailability of nCuO and Cu(II) was determined in juvenile by measuring changes in total Cu and labile Cu(II). Increased Cu levels were observed mainly in fish exposed Cu(II) and not in fish exposed to nCuO. Moreover, the labile form of Cu was also significantly increased in gills in fish exposed to Cu(II) but not to nCuO. This suggests that exposure to low levels of nCuO did not increased the Cu loadings in fish tissues. Increased copper levels in carp were observed at much higher concentrations than the one used in this study [22]. Indeed, total Cu levels were significantly increased after 5 days in carp exposed to 100 mg/L nCuO while Cu levels were increased in fish exposed to only 15 µg/L Cu(II) which was also observed in the present study. In another study, Cu accumulated preferentially in

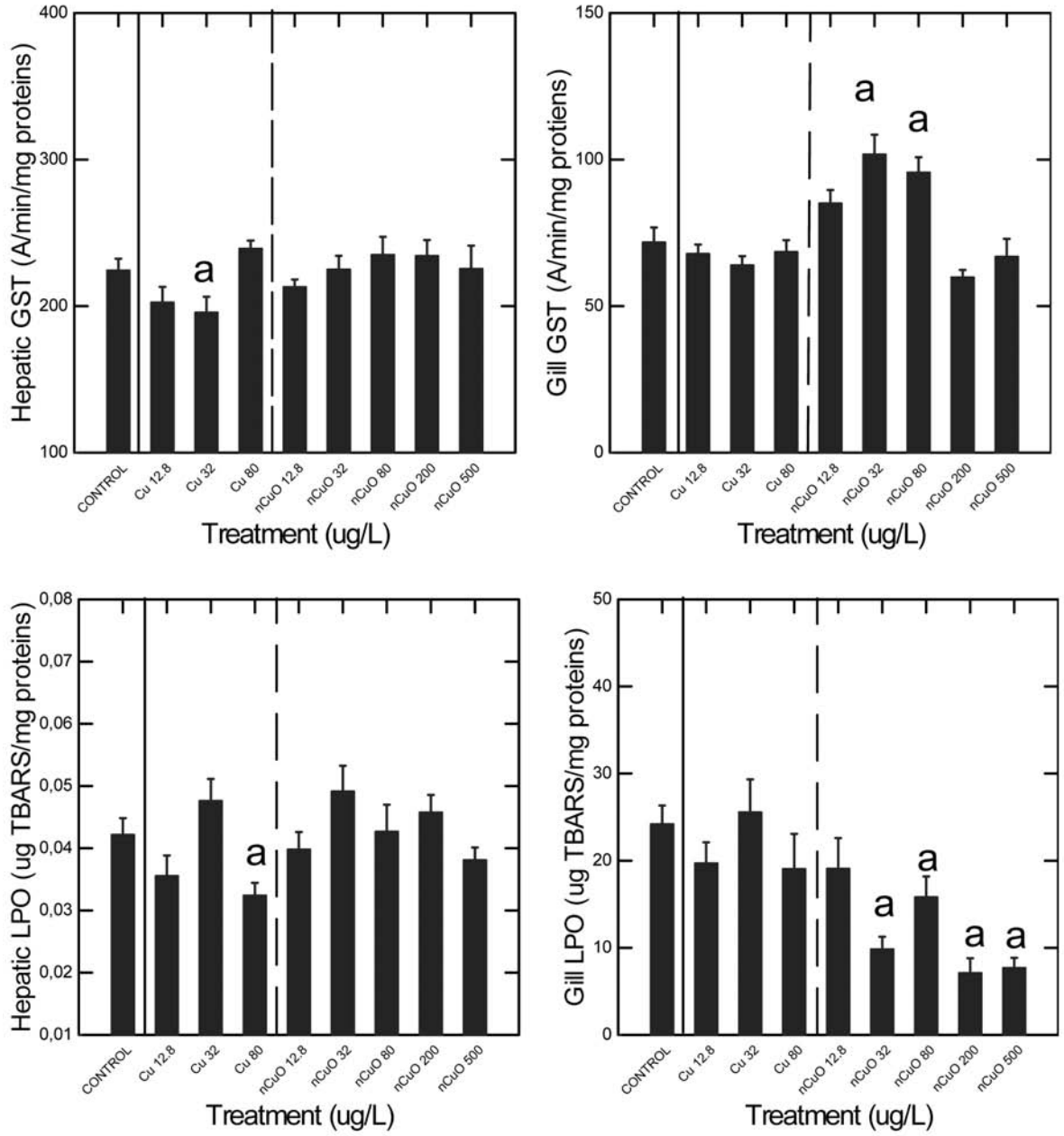


**Figure 3.** Oxidative stress in fish exposed to dissolved Cu and nCuO.
The letter a indicates significance from control. No difference was observed between the forms of Cu (at the same concentrations).

the liver of rainbow trout injected and gavaged with nCuO compared to Cu(II) [23]. This suggests that exposure of fish through the diet results in the accumulation of Cu in the liver.

Based on the biomarker data obtained in this study, the toxicity of nCuO differed from Cu(II) with respect to oxidative stress (LPO, GST) and DNA damage and protein-ubiquitinylation. Indeed, GST activity was significantly induced in gills but not in the liver in fish exposed to nCuO but not to Cu(II). Moreover, LPO levels in gills were decreased by nCuO only which suggests the activation of antioxidant mechanisms by the Cu nanoparticles. Elevated levels of GST, superoxide

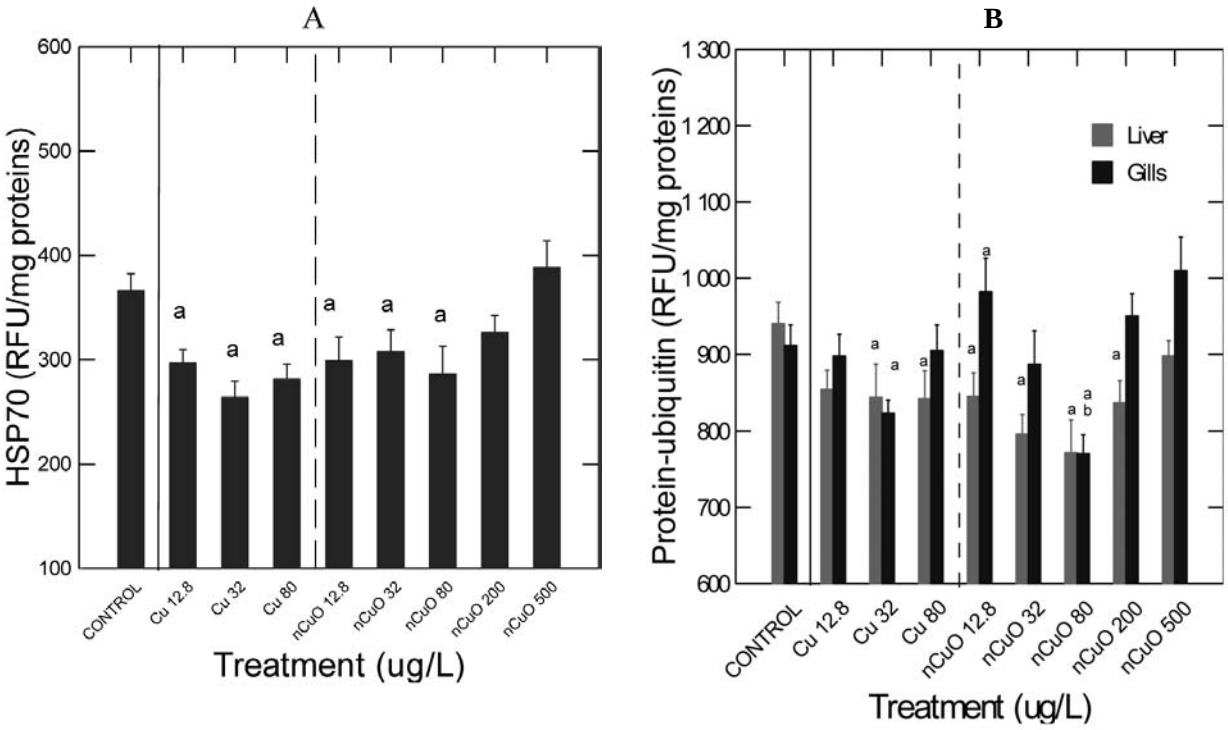


**Figure 4.** Reticulum endoplasmic stress in fish exposed to Cu forms.
The levels of hepatic HSP70 (A) and protein-ubiquitin levels in gills and the liver (B) were determined. The letters a and b indicate significance from control and between the forms of Cu (at the same concentrations).

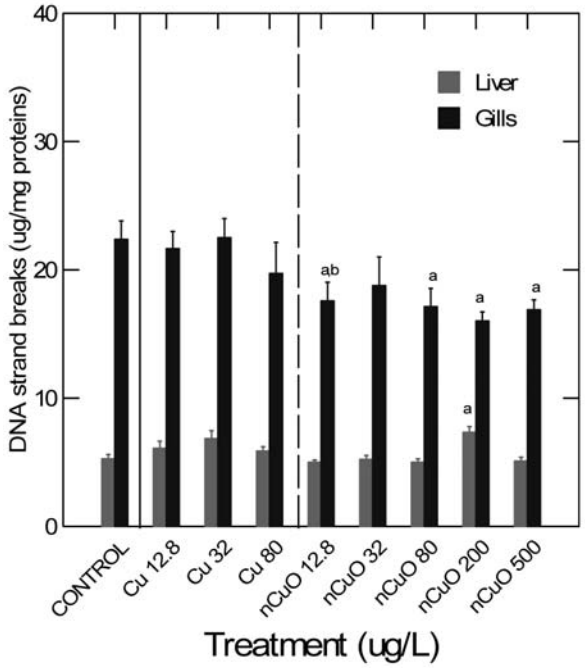


**Figure 5.** DNA damage in juvenile trout exposed to Cu forms.
The letters a and b indicate significance from control and between the forms of Cu (at the same concentrations).

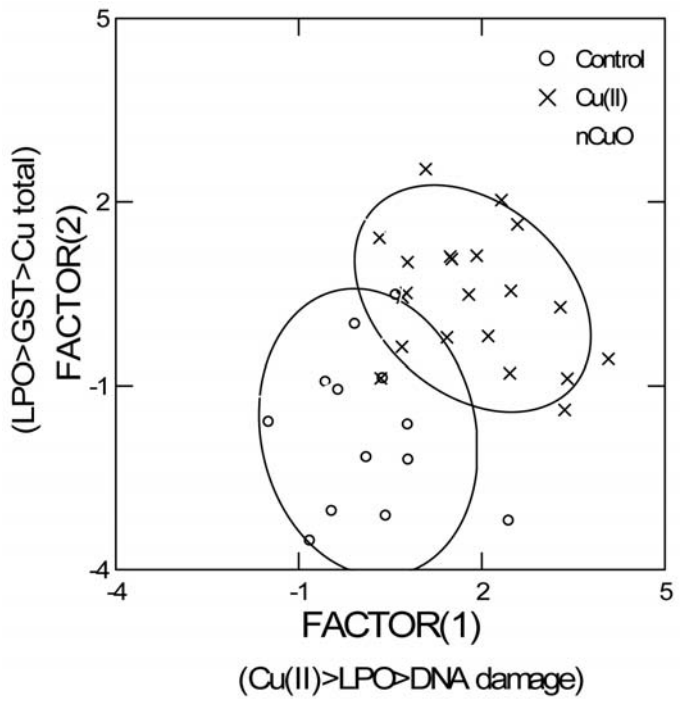


**Figure 6.** Multivariate analysis of biomarker data.
The biomarker data were analyzed using discriminant function procedure. The response pattern of the control, dissolved Cu(II) and nCuO were well discriminated with each other with an explained variance of 100% and a mean classification correctness of 88%. The 3 most important biomarkers are given under each axis (factor 1 and 2).

dismutase, catalase, 8-OHdGuanosine, ROS and LPO were observed in Chinook salmon cells exposed to nCuO [24]. However, the reported effects were at concentrations 10 times higher that the maximum concentration used in the present study, i.e. at concentrations between 5-15 mg/L. The increased levels in 8OH guanosine suggest that DNA could be harmed by nCuO. We observed a general decrease in the DNA strand breaks levels in gills which suggests decreased repair activity whereas this effect was not observed in fish exposed to Cu(II). Oxidative stress and teratogenicity were observed in zebrafish embryos exposed to nCuO at the ppm range [25]. The 48-h acute lethality (LC50) was 64 mg/L and evidence of oxidative stress and developmental malformations was observed at sublethal concentrations (5-20 mg/L) which were higher than those in the present study. Carp exposed to high concentrations of nCuO (6.25-100 mg/L) for 28 days had increased oxidative stress and DNA damage in peripheral erythrocytes as determined by the micronucleus test [26]. Waterborne nCuO was shown to impact not only the liver but the gills as well. Indeed, exposure to nCuO led to increased GST activity, protein-ubiquitin levels at low concentrations and decreased LPO levels. In carps exposed to sublethal concentrations of nCuO, catalase activity was decreased while LPO and GSH levels were increased [27]. The observed changes in the levels of protein-ubiquitin levels were also observed in blue mussel hemocytes exposed to a concentration range of 1 to 50 mg/L of iron oxide nanoparticles [28]. These changes were associated with DNA damage and oxidative stress. In this study, the exposure concentrations of nCuO were at least one order of magnitude lower (12.8-500 µg/L), which also led to oxidative stress by GST activity but not by LPO levels. LPO levels and DNA strand breaks were actually lower than controls suggesting that at low concentrations of nCuO, the decrease in DNA strands (decreased DNA repair activity) and LPO could be the result of coping mechanisms against oxidative damage where antioxidant enzymes such as GST were at play. A decrease in DNA strand breaks (repair

activity) was significantly correlated with cytogenetic damage as determined by the micronucleus test in hemocytes of *Mya arenaria* clams [29]. If this holds true, the oxidative stress and genotoxicity responses could follow a hermetic-type (cyclic) of responses as the concentration of nCuO increases.

In another study with freshwater mussels, the levels of protein-ubiquitin levels were increased by 20 and 80 nm silver nanoparticles [21]. Given that the levels of protein-ubiquitin were weakly correlated with metallothioneins ($r = 0.29$; $p < 0.05$), it was suggested that the responses were not entirely explained by the released ionic silver. However, both Cu(II) and nCuO were able to reduce protein ubiquitin levels but not oxidative stress (LPO) which suggests that both forms of Cu could produce this effect. Exposure of tilapia fish (*Oreochromis mossambicus*) to 0.5-1.5 mg/L nCuO revealed that the nanoparticles accumulated in the gills, liver and muscles [30]. Oxidative stress was increased in gills and DNA damage in circulating erythrocytes as determined by the COMET assay for the latter. It is possible that the increase in DNA strand breaks at low concentrations of Cu (20-100 µg/L for 30 days) could have resulted from apoptosis mechanisms [31] while at higher concentrations, i.e. at the ppm level, cytogenetic damage prevails as determined by the micronucleus assay. The activation of DNA repair activity was shown to prevent apoptosis in cells [32]; a cross talk mechanism exists between DNA repair and cell death since many of DNA repair factors could also influence apoptosis. However, when lesions are unrepaired or there are defects in DNA repair systems, DNA damage is often found correlated to apoptosis. Hence, the observed decrease in DNA strand breaks in gills of fish exposed to nCuO could indicate the initiation of apoptosis in gills. This is consistent with the observed change in the histological integrity of gills in tilapia fish exposed to nCuO [30]. This is consistent with a recent study with juvenile puffer fish (Takifugu fasciatus) exposed to 20 and 100 µg/L nCuO which led to increased apoptosis in the liver [31]. This was corroborated by the increase in cytochrome c in the cytosol fraction. Hepatic activities of succinate dehydrogenase and Na-K-ATPase were all decreased in mitochondria suggesting decreased respiration and energy metabolism.

## CONCLUSION

In conclusion, exposure of nCuO to juvenile rainbow trout did not increase Cu loadings in fish tissues as seen in fish exposed to Cu(II). The labile form of Cu was increased in the gills of fish exposed to Cu(II) but decreased in fish exposed to nCuO at the highest concentration (500 µg/L). Gill GST activity was increased whereas LPO levels and DNA strand breaks were decreased in fish exposed to nCuO but not to Cu(II). The data revealed that nCuO could produce specific effects in fish gills without showing Cu accumulation.

## ACKNOWLEDGEMENTS

The authors thank the Joanna Kowalczyk for the technical assistance for bioanalyses. This project was funded by the Chemical Management Plan of Environment and Climate Change Canada.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest of personal and financial nature.

## REFERENCES

1. Aitken, R. J., Chaudhry, M. Q., Boxall, A. B. and Hull, M. 2006, Occup. Med. (Lond.), 56, 300.
2. Moore, M. N. 2006, Environ, Int., 32, 967.
3. Gagné, F., Gagnon, C. and Blaise, C. 2007, Curr. Top. Toxicol., 4, 51.
4. Chupani, L., Niksirat, H., Lünsmann, V., Haange, S. B., von Bergen, M., Jehmlich, N. and Zuskova, E. 2018, Sci. Total Environ., 613-614, 62.
5. Mohajerani, A., Burnett, L., Smith, J. V., Kurmus, H., Milas, J., Arulrajah, A., Horpibulsuk, S. and Abdul Kadir, A. 2019, Materials (Basel), 12, E3052.
6. Bhatt, I. and Tripathi, B. N. 2011, Chemosphere, 82, 308.
7. Song, L., Vijver, M. G., Peijnenburg, W. J., Galloway, T. S. and Tyler, C. R. 2015, Chemosphere, 139, 181.

8. Gopi, N., Vijayakumar, S., Thaya, R., Govindarajan, M., Alharbi, N. S., Kadaikunnan, S., Khaled, J. M., Al-Anbr, M. N. and Vaseeharan, B. 2019, J. Trace Elem. Med. Biol., 55, 170.
9. Elia, A. C., Magara, G., Righetti, M., Dörr, A. J., Scanzio, T., Pacini, N., Abete, M. C. and Prearo, M. 2017, Environ. Sci. Poll. Res. Int., 24, 10205.
10. Santos, S. W., Cachot, J., Gourves, P. Y., Clérandeau, C., Morin, B. and Gonzalez, P. 2019, Ecotoxicol. Environ. Saf., 170, 778.
11. Isani, G., Falcioni, M. L., Barucca, G., Sekar, D., Andreani, G., Carpenè, E. and Falcioni, G. 2013, Ecotoxicol. Environ. Saf., 97, 40.
12. Hernández-Moreno, D., Valdehita, A., Conde, E., Rucandio, I., Navas, J. M. and Fernández-Cruz, M. L. 2019, Sci, Total Environ., 687, 24.
13. Environment Canada. 1990, Biological test method: acute lethality test using rainbow trout. Environmental Protection Series Environment Canada Report EPS 1/RM/9.
14. Bradford, M. M. 1976, Anal. Biochem., 72, 248.
15. Wills, E. D. 1987, Evaluation of lipid peroxidation in lipids and biological membranes. In: K. Snell and B. Mullock editors. Biochemical toxicology: a practical approach. Washington D.C: IRL Press, 127.
16. Gagné, F., Auclair, J., Turcotte, P., Fournier, M., Gagnon, C., Sauvé, S. and Blaise, C. 2008, Aquat. Toxicol., 86, 333.
17. Olive, P. L. 1988, Environ. Mol. Mutagen., 11, 487.
18. Bester, M. J., Potgieter, H. C. and Vermaak, W. J. H. 1994, Anal. Biochem., 223, 299.
19. Udhayakumaria, D., Velmathia, S., Sung, Y.-M. and Wu, S.-P. 2014, Sensors and Actuators B, 198, 285.
20. Boryslawskyj, M., Garrood, A. C., Pearson, J. T. and Woodhead, D. 1988, Mar. Environ. Res., 24, 101.
21. Gagné, F., Auclair, J., Turcotte, P. and Gagnon, C. 2013, J. Toxicol. Environ. Health A, 76l, 479.
22. Zhao, J., Wang, Z., Liu, X., Xie, X., Zhang, K. and Xing, B. 2011, J. Hazard. Mat., 197, 304.
23. Lindh, S., Razmara, P., Bogart, S. and Pyle, G. 2019, Environ. Toxicol. Chem., 38, 80.
24. Srikanth, K., Pereira, E., Duarte, A. C. and Rao, J. V. 2016, Protoplasma, 253, 873.
25. Ganesan, S., Thirumurthi, N. A., Raghunath, A., Vijayakumar, S. and Perumal, E. 2016, J. Appl. Toxicol., 36, 554.
26. Ogunsuyi, O. I., Fadoju, O. M., Akanni, O. O., Alabi, O. A., Alimba, C. G., Cambier, S., Eswara, S., Gutleb, A. C., Adaramoye, O. A. and Bakare, A. A. 2019, Environ. Sci. Pollut. Res. Int., 26, 27470.
27. Noureen, A., Jabeen, F., Tabish, T. A., Ali, M., Iqbal, R., Yaqub, S. and Chaudhry, A. S. 2019, Drug Chem. Toxicol., 15, 1.
28. Taze, C., Panetas, I., Kalogiannis, S., Feidantsis, K., Gallios, G. P., Kastrinaki, G., Konstandopoulos, A. G., Václavíková, M., Ivanicova, L. and Kaloyianni, M. 2016, Aquat. Toxicol., 172, 9.
29. Debenest, T., Gagné, F., Burgeot, T., Blaise, C. and Pellerin, J. 2013, Environ. Sci. Poll. Res. Int., 20, 621.
30. Shahzad, K., Khan, M. N., Jabeen, F., Kosour, N., Chaudhry, A. S. and Sohail, M. 2018, Environ. Sci. Poll. Res. Int., 25, 15943.
31. Wang, T., Wen, X., Hu, Y., Zhang, X., Wang, D. and Yin, S. 2019, Fish Shellfish Immunol., 84, 648.
32. De Zio, D., Cianfanelli, V. and Cecconi, F. 2013, Antioxid Redox Signal., 19, 559-571.