JOURNAL OF ENVIRONMENTAL SCIENCES 127 (2023) 495–507

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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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Toxicity mechanisms and bioavailability of copper to fish based on an adverse outcome pathway analysis

Wei Liao[1,2,3,4], *Ziwei Zhu*[2], *Chenglian Feng*[1,3,*], *Zhenfei Yan*[1], *Yajun Hong*[1], *Daqing Liu*[1], *Xiaowei Jin*[5,*]

[1] *State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China*
[2] *Wetland Research Center, Jiangxi Academy of Forestry, Nanchang 330032, China*
[3] *Key Laboratory of Poyang Lake Environment and Resource Utilization, Ministry of Education, School of Environmental and Chemical Engineering, Nanchang University, Nanchang 330031, China*
[4] *Jiangxi Irrigation Experiment Central Station, Nanchang 330201, China*
[5] *China National Environmental Monitoring Centre, Beijing 100012, China*

ARTICLE INFO

Article history:
Received 11 January 2022
Revised 30 May 2022
Accepted 1 June 2022
Available online 10 June 2022

Keywords:
Copper
Toxic effect
Adverse outcome pathway
Aquatic environment
Risk assessment

ABSTRACT

Copper (Cu) exists in a variety of forms in different aquatic environments, and affects their bioavailability. In this study we provide a systematic review on toxicity of Cu which focuses on identifying evidence in the mechanisms of Cu toxicity, and apply an adverse outcome pathway (AOP) analysis to identify multiple potential mechanisms and their interactions of Cu toxicity to fish. This analysis process included the mechanisms of behavior toxicant, oxidative toxicant, ion regulation disruption toxicity, as well as endocrine disruption toxicity. It was found that at low levels of Cu exposure, swimming, avoid predators, locating prey and other sensory functions will be impaired, and the organism will suffer from metabolic alkalosis and respiratory acidosis following the inhibition of the carbonic anhydrase active. The main pathway of acute toxicity of Cu to fish is the inhibition of the Na^+/K^+-ATPase enzyme, and lead to reduced intracellular sodium absorption, as well as Cu-induced increased cell permeability, in turn resulting in increased sodium ion loss, leading to cardiovascular collapse and respiratory insufficiency. The endocrine disruption toxicity of Cu to fish caused growth inhibition and reproductive reduction. In addition, there are several key pathways of Cu toxicity that are affected by hardness (e.g., Ca^{2+}) and intracellular DOC concentrations, including inhibiting Cu-induction, improving branchial gas exchange, altering membrane transport functions, decreasing Na^+ loss, and increasing Na^+ uptake. The results of the AOP analysis will provide a robust framework for future directed research on the mechanisms of Cu toxicity.

* Corresponding authors.
E-mails: fengcl@craes.org.cn (C. Feng), jinxw@cnemc.cn (X. Jin).

https://doi.org/10.1016/j.jes.2022.06.002
1001-0742/

Introduction

Copper (Cu) is a plentiful element found in the Earth's crust. It has been reported that the Cu existing in natural environments ranged from 0.2 to 30 μg/L in freshwater, and 0.03 to 0.23 μg/L in surface seawater (Flemming and Trevors, 1989; USEPA, 2007). The annual of Cu consumption throughout the world averaged approximately 18 million tons from 2015 to 2020 (NMIC, 2021). Many applications have involving the use of Cu have contributed to anthropogenic contaminant inputs to surface waters, such as mining, fabricated, leather and leather products, electric equipment and metal products. Furthermore, precipitation of atmospheric fallout is one significant source of Cu to surface waters in some areas (USEPA, 2007). Some locations receiving anthropogenic inputs have been reported from nature background levels to 100 μg/L of Cu concentrations, and in some cases areas near mining sites have reached an astonishing level of 200,000 μg/L of Cu concentrations (Davis and Ashenberg, 1989). Cu is a vital mineral essential for many biological processes, and plays an important role in the growth and development, and cellular function. It is also a cofactor for a number of main metabolism-related enzymes in living organisms (Puig and Thiele, 2002). Most of all Cu in biota and healthy humans is associated with enzyme prosthetic groups or bound to proteins (Festa and Thiele, 2011; Gaetke et al., 2014). It is found in a variety of cells and tissues with the highest concentrations in the liver and brain (Gaetke et al., 2014). The redox potential of Cu is utilized by a number of enzymes, including cytochrome C oxidase, a zinc-superoxide dismutase which scavenges the free radical superoxide (Solomon et al., 1993); and ceruloplasmin or ferroxidase I, which facilitates transport from the interstitial lumen and storage sites to sites of erythropoiesis (Gaetke et al., 2014). Additionally, Cu is a constituent of dopamine-beta-hydroxylase, which is a critical enzyme in the catecholamine biosynthetic pathway. However, when the Cu concentration elevates it becomes highly toxic to biota in freshwater (Flemming and Trevors, 1989; Wu et al., 2013; Donnachie et al., 2014; Keller et al., 2017; Mu et al., 2018).

It has been reported that the acute value of Cu ranged from 2.73 μg/L (*Daphnia pulicaria*) to 107,880 μg/L (*Notemigonus crysoleucas*), and the total of chronic values was 2.83 μg/L (*Daphnia pulex*) to 249 μg/L (*Cyprinodon variegatus*), based on the aquatic life ambient freshwater quality criteria (USEPA, 2007). The acute value of Cu to sensitive fish was 14.61 μg/L (*Ptychocheilus oregonensis*) and chronic value was 5.92 μg/L (*Oncorhynchus tshawytscha*) (USEPA, 2007). With increasing emissions to fresh water, and high sensitivity to aquatic organisms, Cu present in aquatic environments may pose a greater potential ecological risk to aquatic species. Evidence suggests that Cu poses greater risks to aquatic species than other priority metal/metalloid pollutants (e.g., Cd, Pb, Hg, As, etc.), along with most organic pollutants (e.g., ethinylestradiol, linear alkylbenzene sulfonate, nonylphenol) present in the surface freshwater of the UK (Donnachie et al., 2014; Johnson et al., 2017), China (Jin et al., 2015; Fu et al., 2016, 2017) and Japan (Hayashi and Kashiwagi, 2011; Hayashi, 2013). Therefore, it is very important to study "protective values for aquatic" for Cu based on the current guidelines. This is commonly manifested as establishing scientific water quality criteria or an appropriate environmental quality standard for Cu. At present, the ecological risk of Cu has drawn the attention of many scientists and government officials.

As is widely known, the toxicity of Cu in water to aquatic organisms is affected by the exposure total concentrations, and is also highly dependent on the different chemical speciation related to the water quality parameters (USEPA, 2007; Mebane et al., 2020; Adams et al., 2020). Initially, researchers recognized the fact that hardness and naturally occurring organic matter would inhibit the Cu toxicity to aquatic species (Herbert and Vandyke, 1964; Brown et al., 1974). The interactions between metal (Cu) and water chemistry reflect the metal "bioavailability." The factors affecting metal bioavailability may include the exposure medium pH value, hardness, presence of chemicals competing for binding sites (e.g., calcium [Ca^{2+}], sodium [Na^{+}], magnesium [Mg^{2+}]) and dissolved organic matters (as well as dissolved organic carbon, DOC), which can bind to metal cations, along with organism specifics, such as the number and types of binding sites at the site of action (DiToro et al., 2001; De Schamphelaere and Janssen, 2002; Ryan et al., 2010; Adams et al., 2020). Scientists around the world have developed a series of models for the prediction of Cu bioavailability, such as the biotic ligand model (BLM), generalized bioavailability model (gBAM) (DiToro et al., 2001; De Schamphelaere et al., 2003). The BLM, which is fundamentally a chemical equilibrium-based model, has gained widespread interest amongst in some developed countries and regions (Santore et al., 2001; DiToro et al., 2001; Paquin et al., 2002). However, at present the precise mechanisms of Cu to aquatic organisms remains elusive in a complex water body. A series of investigations of publication (Green et al., 2010; Al-Reasi et al., 2013; Giacomin et al., 2013) have provided evidence that Ca^{2+} and DOC molecules directly interact with biological surfaces, and in turn affect the metal toxicity.

An adverse outcome pathway (AOP) is a framework designed for effective translation of the mechanistic data information into endpoints that are meaningful to ecological risk. An AOP is a conceptual construct which portrays existing knowledge concerning the linkage between a direct molecular initiating event (MIE) and an adverse outcome at the biological level of organization relevant to risk assessment (Ankley et al., 2010; Kramer et al., 2011). It is regarded as a series of multiple sequences of MIEs, which are related to cellular response, organ response, organism response and even impacts on population. An AOP concept is typically developed in response to chemical pollutants with complex toxicity mechanism, microplastics, organophosphate esters, metal nanoparticles and metals, as has been reported several years ago (Garcia-Reyero et al., 2014; Muller et al., 2015; Brix et al., 2017a; Jeong et al., 2018; Jeong and Choi, 2019; Yan et al., 2020). Recently, five potential MIEs, namely disruption of Ca^{2+} homeostasis, disruption of Mg^{2+} homeostasis, disruption of $Fe^{2+/3+}$ homeostasis, reactive oxygen species-induced oxidative damage, and an allergic-type response of respiratory epithelia for an AOP analysis of nickel, were reviewed by Brix et al. (2017a). In the present study, an AOP analysis is developed to study the mechanisms action of toxicity for Cu to aquatic organisms based on the current research

progress. It is organized within an AOP framework, and draws information from multiple toxicity modes to provide a broad perspective. The results of this study will provide a robust framework for future directed research on the mechanisms of Cu toxicity. Therefore, the ultimate purposes of this work are to determine the mechanism of Cu toxicity in the water environment, and to provide more accepted evidence for the prediction model such as BLM, the multiple regression model (MRM) and application to environmental management.

1. Materials and methods

In order to develop the AOP for Cu toxicity in aquatic environment, the toxicity data of Cu to organisms in this study were collected from the published studies via the Web of Science and Chinese National Knowledge Infrastructure. The keywords mainly consisted of Copper, Cu, toxicity, acute, behavior, mechanisms, development, reproduction and survival rate, which were used to search all useful research information. In total, 873 toxicity data in 89 published reports of Cu to fish, performed from 1954 to 2014, were collected. Behavior response, oxidative stress, endocrine disruption, ion regulation disruption and death were consideration as the five types of toxicity effects. Each type of Cu toxicity effect was then systematically reviewed, while considering several model organisms studied. Then the MIEs, cellular response, organ response, organism response, and ultimate population-levels were described, and the toxicity effects or mechanisms in depth with particular water quality parameters were discussed.

There are five outcome pathways of Cu which may influence the function on fish in exposed environmental aquatic freshwater: (1) sensory function and behavior influence, (2) formation of reactive oxygen species (ROS), (3) endocrine disruption, (4) ammonia excretion and acid-based balance disruption, and (5) acute narcosis toxicity. All of these were analyzed based on AOP studies.

2. Results

In general, exposure at low Cu concentrations can lead to chronic toxicity in aquatic organisms. However, most chronic toxicity effects can be gradually recovered after discontinuation of exposure. First, excessive amounts of Cu can result in adverse effects on the growth, development and metabolism (Liu et al., 2010), histological structure (Liu et al., 2010; Schmidlin et al., 2015), immunological activity (Osuna-Jiménez et al., 2009), antioxidant system and genotoxicity (Simonato et al., 2016), and egg hatchability (Khangarot and Das, 2010) of aquatic organisms. In addition, some studies have shown that Cu caused reproductive endocrine disruption in fish and teratogenic effects following long-term chronic exposure (Sfakianakis et al., 2015; Cao et al., 2019).

Homeostatic systems may become dysfunctional when intracellular Cu levels rise to the point at which protein functions become impaired following exposure to elevated Cu levels in the water. Acute toxicity of Cu to aquatic organisms mainly involves respiratory depression and interference with ion regulation (Paquin et al., 2002; Grosell, 2012). Some places that have been exposed to Cu pollutants will reach a concentration level of acute toxicity of aquatic organisms, resulting in the death of aquatic organisms (Fu et al., 2016; Johnson et al., 2017). Although the mechanism of acute toxicity and sublethalness of Cu in freshwater biotas has been surveyed in some detail, there remains a lack of effective tools or framework to summarize them. As the Cu concentration increases in the aquatic ecosystem, a range of potential targets for Cu manifest in altered ion homeostasis, physiology and toxicity, due to the macromolecular interactions, cellular responses to the organ and organismal level during Cu exposure. These targets and organismal responses will be discussed in the following section, based on an AOP analysis. Regardless of the target organ, Cu toxicity is not merely a function of the environmental Cu concentration. Factors such as complexation with organic and inorganic negatively charged molecules, and competition between Cu and cations for uptake exert a strong influence on toxicity, which are important means by which water quality parameters affect the biological toxicity of Cu (McGeer et al., 2000; Paquin et al., 2002; Taylor et al., 2000).

According to our investigation, multiple receptor pathways in aquatic organisms are impacted by Cu exposure. As shown in Table 1, we focused on five types of effects, including behavior influence, oxidative stress, endocrine disruption, disruption of cellular ion regulation and death. Then an AOP analysis was performed for Cu toxicity in aquatic systems. The mechanisms of Cu toxicity to fish will aid in establishing a toxicity prediction model of Cu in the risk assessment.

To the authors' knowledge, epithelial cells were the primary receptors in most aquatic organism. A variety of enzyme activities would cause inhibition, disruption of metabolic balance and ion homeostasis of organisms, thereby resulting in a series of toxic effects after Cu entering the cells (Grosell, 2012). In the present study, the following five potential pathways by which Cu may exert toxicity on fish in the elevating exposed Cu concentrations were identified: (1) sensory function and behavior influence, (2) formation of ROS, (3) endocrine disruption, (4) ammonia excretion and acid-base balance disruption, and (5) acute narcosis toxicity.

2.1. *PATH 1: Sensory function and behavior*

Previous research has shown that Cu has the potential to affect most behavioral aspects of representative trophic levels of aquatic animals (e.g., *Daphnia pulex, Nephelopsis obscura, Pimephales promelas*) by means of interactions with the chemosensory function of aquatic animals such as olfactory epithelia (Pyle and Mirza, 2007). Compared with other endpoints, sensory function (e.g., olfactory, lateral line hair cells and neuromasts) and behavior are the most sensitive indicators of Cu toxicity to aquatic organisms. Aquatic organisms are highly sensitive to Cu with olfactory senses and mechanisms. Over the last several decades, there has been a sharp increase in case studies demonstrating that Cu induces olfactory impairment of aquatic organisms at lower than μg/L environmentally relevant concentrations (Carreau and Pyle, 2005; Sandahl et al., 2007; Meyer and Adams, 2010). As shown in Fig. 2, Cu accumulation in the olfactory epithelium and a wide range of specific genes in the olfactory signal trans-

Table 1 – The main MIEs, KEs and AOs associated with the Cu toxicity in this study.

Effects	Molecular initiating event (MIE)	Key event (KE)	Adverse outcome (AO)	Reference
Behavior influence	Reduced g-proteins; Altered calcium channels; Formation of ROS	Olfactory impairment; Impacted lateral line hair cells; Neuromasts impact; Impaired O_2 delivery;	Inhibited swimming; Fright response	Pyle and Mirza, 2007; Olivari et al., 2009
Oxidative stress	Inhibited antioxidant enzymes; Altered mitochondrial electron-transfer chain; Formation of ROS	Increased GSH levels; Altered GSH/GSSH ratio; Increased Metallothionein; Increased HSP70 expression; Reduced catalase activity; Reduced SOD enzymatic activity; Reduced Glutathione peroxidase enzyme activity; Depletion of cellular glutathione	Cytotoxicity; Tumor formation	Krumschnabel et al., 2005; Bopp et al., 2008; Chiaverini and Ley, 2010
Endocrine disruption	Altered adrenocorticotropic hormone; Decreased 5-hydroxytryptamine; Decreased dopamine; Decreased estradiol; Increased testosterone; Decreased 11-ketotestosterone; Disturbed steroidogenesis	Increased blood sugars; Reduced appetite; Reduced food conversion; Gonads impairment; Increased metabolic rate; Decreased energy for growth	Reduced body weight; Reduced body length; Reduced growth; Reduced reproduction	Grosell et al., 2003; Teles et al., 2010; Cao et al., 2019
Ion regulation disruption	Increased plasma cortisol; Increased protein catabolism; Impacted rhesus proteins; Displacement of K^+ in ion-exchange; Inhibited carbonic anhydrase	Increased plasma ammonia/ammonium; Metaboli alkalosis; Respiratory acidosis; Impaired branchial gas exchange	Altered metabolism; Mortality	Tsui et al., 2009; Boeck et al., 2001
Ion regulation disruption and death	Inhibited Na^+/K^+- ATPase; Inhibited carbonic anhydrase; Apical H^+ pump hyperpolarizing	Increased loss of Na^+; Decreased Na^+ uptake; Disturbance of internal ions	Mortality	Paquin et al., 2002

ROS: reactive oxygen species; GSH: glutathione synthetase; GSSH: oxidized glutathione synthetase; HSP: heat shock protein; SOD: superoxide dismutase.

duction (OST), including calcium channels and ion transport, g-proteins and olfactory receptors, are downregulated in the olfactory epithelium of fish upon Cu exposure (Carreau and Pyle, 2005; Tilton et al., 2008). The ability to respond to olfactory stimulus is often very important for predator avoidance, prey localization, homing, social interaction and successful reproduction (Pyle and Mirza, 2007). Olfactory impairment would lead to a series of cellular or organ response, and resembles fright response, feeding stimulants, bile salts, catecholamines, and steroid and non-steroid pheromones, thus this response is critical for growth, survival and reproduction.

In addition to affecting olfactory neurons, mechanoreception carried out by lateral line hair cells or neuromasts in most fish has been reportedly impacted by Cu exposure at relatively low ambient Cu concentrations (Linbo et al., 2009; Olivari et al., 2009). Neuromasts are important peripheral specialized neurons, which are used for the detection of water movement relative to the fish body surface. As shown in Fig. 1, research has demonstrated that an impact of lateral line hair cells and neuromast impact is in part related to oxidative stress. This will lead to a series of cellular or organ response, such as impaired sensory function (missing relative to water currents and sensing of swimming speed) and impaired swimming. This is very important for fish for detecting water movement while swimming, avoiding predators, locating prey or conspecifics and spawning grounds, and much like olfactory

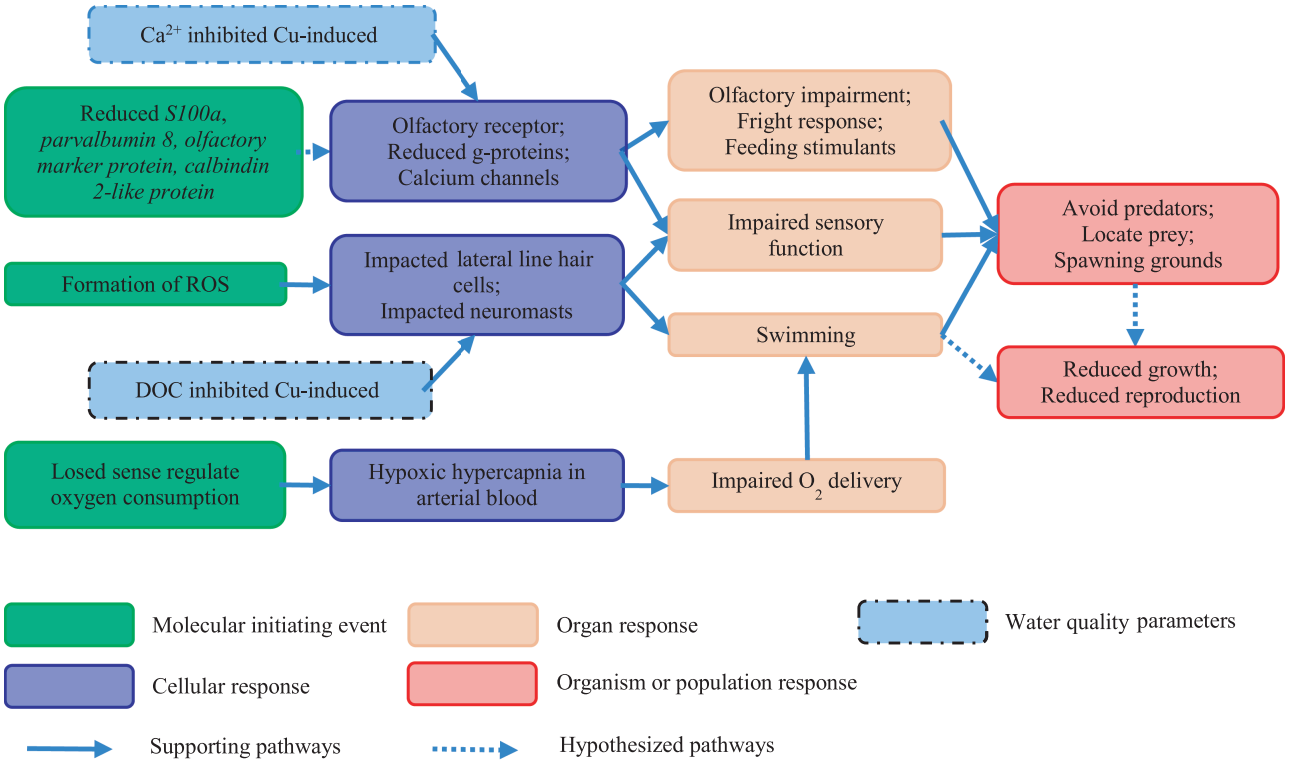

Fig. 1 – Sensory function and behavior toxicant of adverse outcome pathways in aquatic system with different Cu concentrations. Note: The green portion of the diagram represents effects on molecular initiating event, the blue and brown portion of the diagram represents effects on cellular or organ response, and the red portion of the diagram represents effects on organism or population response. The blue dashed box represents water quality parameters which potentially impact the toxicity of the pathways. The solid arrows represent pathways with supporting data, while the dashed arrows are hypothesized pathways. The development and evaluation of pathways consider multiple species, but especially those of fish.

impairments, this response is critical for growth, survival and reproduction.

Another adverse outcome pathway in sensory function and behavior manifested in the loss of the ability to sense and regulate oxygen in a moderated Cu concentration leading to impaired O_2 delivery caused by hypoxic hypercapnia in the arterial blood (De Boeck et al., 1995), and this has a marked impact on swimming for fish.

2.2. *PATH 2: Formation of ROS*

The formation of ROS and induced oxidative damage is another sub-lethal potential mechanism for Cu toxicity to both aquatic and terrestrial organisms (Krumschnabel et al., 2005). Previous research has shown that Cu-induced ROS formation has occurred in the gill cells, hepatocytes and kidney of freshwater organisms (Bopp et al., 2008). The redox properties of Cu can lead to the formation of ROS when cellular Cu levels are elevated. Current research has shown that Cu readily binds to histidine, cysteine and methionine sites in proteins, thereby potentially leading to their disfunction in mammals (Harris and Gitlin, 1996). Cu and other metals can result in oxidative stress in terms of four aspects: inhibition of antioxidant enzymes, alterations in the mitochondrial electron-transfer chain, depletion of cellular glutathione, and formation of ROS (Freedman et al., 1989; Rau et al., 2004; Wang et al., 2004).

The formation of ROS has been summarized as the Fenton reaction, which forms many hydroxyl radicals (·OH).

$$Me^{n+} + H_2O_2 \rightarrow Me^{n+1} + OH + OH^-$$

Here, Me^{n+} represents Cu^{2+}. The Cu-induced ROS was in formation in the gill cells, hepatocyte and kidney.

As shown in Fig. 2, at the beginning of the Cu exposures, Cu-induced ROS will inspire the protection against ROS system which are generated continuously during aerobic metabolism, which is normally achieved by antioxidant enzymes such as catalase, glutathione peroxidase, and Cu/Zn superoxide dismutase (SOD). At the same time, glutathione reductase, metallothionein (e.g., MT-1, MT-2) and heat shock protein 70 (HSP70) are involved in protection against oxidative stress (McDuffee et al., 1997; Evans et al., 2005; Chiaverini and Ley, 2010). The Cu will inhibit antioxidant enzymes activities, increase the ROS level formed continuing oxidative stress, and decrease the glutathione synthetase levels. Generally speaking, the cellular defense against the metals consisted of chelation and detoxification, along with scavenging

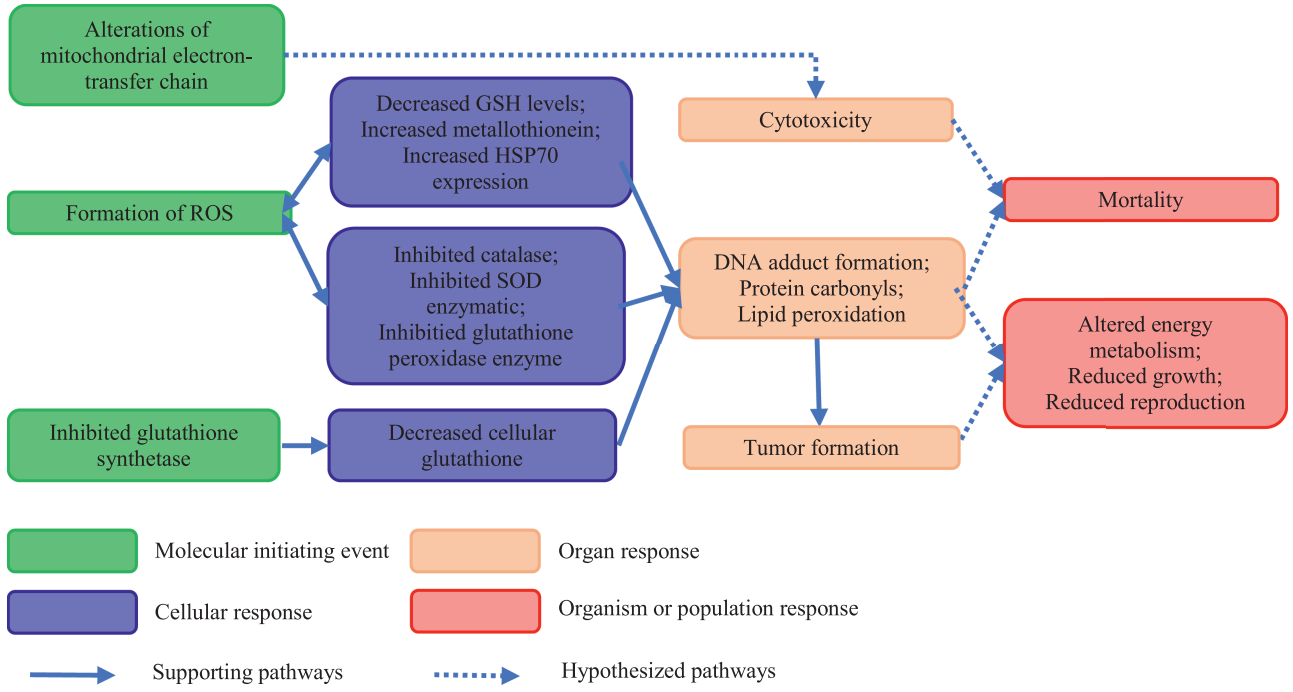

Fig. 2 – ROS toxicant of adverse outcome pathways in aquatic systems different Cu concentrations. Note: Same as Fig. 1.

of oxyradicals by glutathione reductase and antioxidant enzymes (Chiaverini and Ley, 2010). However, prolonged exposure to Cu (from days to weeks, occurring as a result of chronic Cu exposure) may result in the oxidation of DNA, protein and lipids when the cellular antioxidant capacity is exceeded. Such a response is critical for the cytotoxicity, tumor formation, potential impact growth, survival and reproduction at the organism or population level.

2.3. *PATH 3: Endocrine disruption*

An endocrine system is a network of glands, hormones and receptors. Highly-conserved endocrine systems have been found in aquatic organisms (e.g., fish, amphibians, and particularly some invertebrates). However, few studies have focused on the endocrine disruption toxicity of Cu to fish. The endocrine disruption toxicity of Cu as discussed in previously published studies has typically been related to the hypothalamic-pituitary-adrenal axis (HPA), hypothalamic-pituitary-gonad axis (HPG), in turn leading to reproductive endocrine disruption and developmental toxicity.

As shown in Fig. 3, Cu exposure at sublethal concentrations will result in altered energy metabolism caused by adrenocorticotropic hormone changing, and decreased plasma cortisol and mobilized stress response, in turn leading to decreased energy for growth, reduce development and reproduction (Teles et al., 2010).

Recent work has reported that Cu induced adversely affects the development, growth and reproductive endocrine system in fish. Cu induction also leads to the cyp11a, cyp19b, 3βhsd, 17βhsd expressions being reduced, along with the inhibition of steroidogenesis, following the decrease in 5-hydroxytryptamine and dopamine levels and impairment gonads, which are an important pathway of reduced development, growth and reproduction (Cao et al., 2019).

2.4. *PATH 4: Ammonia excretion and acid-base balance*

The acid-base balance plays an important role in ensuring the normal metabolism of organisms. Sublethal Cu exposure may elevate plasma ammonia/ammonium, thereby resulting in modest osmoregulatory disturbances, and even acid-base balance disturbances (Wang et al., 1998; Grosell et al., 2003; Grosell et al., 2004). As shown by the yellow and brown boxes in Fig. 4, Cu-induced four key MIEs to cause reduced ammonia excretion, and led to increased plasma ammonia/ammonium. For example, elevated plasma cortisol has been reported from Cu-exposed fish, which would stimulate protein catabolism and increase protein catabolism (Boeck et al., 2001). At the same time, as is widely known, the primary route of excretion on the gill is thought to be across the epithelial cells. Cu-induced branchial carbonic anhydrase (CA) likely explains the reduced ammonia excretion (Vitale et al., 1999). In addition, the rhesus (Rh) proteins have recently been shown to be involved in ammonia excretion (Tsui et al., 2009).

Branchial Cl^-/HCO_3^- exchange was significantly dependent on the function of branchial CA. Cu induction inhibiting the branchial CA will cause increases in the CO_2 excretion inhibition and extracellular HCO_3^- concentrations. In particular, the fish will face both metabolic alkalosis and respiratory acidosis in an elevated Cu-exposed or low hardness solution. The branchial gas exchange probability impaired in this condition leads to death.

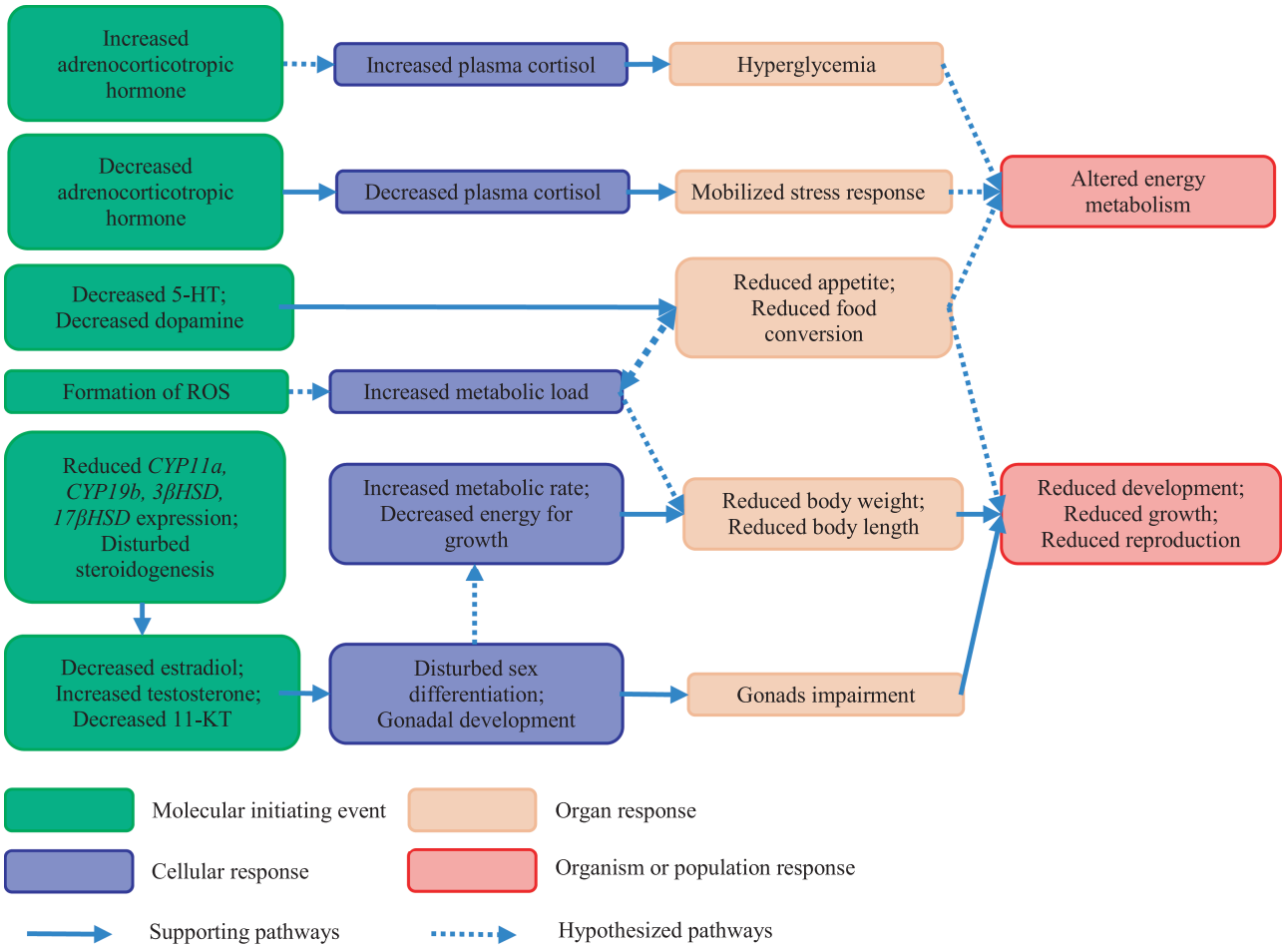

Fig. 3 – Endocrine disruption toxicant of adverse outcome pathways in an aquatic system with different Cu concentrations. Note: Same as Fig. 1.

2.5. *PATH 5: Acute narcosis toxicity*

Among all toxic effects, death is the most severe. The tolerance of aquatic organisms to Cu differs. In general, crustacea species are typically more sensitive than fish. The acute value ranged from 2.73 µg/L (*Daphnia pulicaria*) to 107,880 µg/L (*Notemigonus crysoleucas*) in the United States Environmental Protection Agency report (USEPA, 2007). It has also been demonstrated that disrupting the ability of aquatic organisms to regulate its internal osmoregulatory system causes a significant departure from the norm, in turn resulting adverse consequences, and possibly death. Evidence has suggested that most of the acute metal toxicity to fish in fresh water is caused by increasing loss of ions (e.g., Na^+, Cl^-) and gross morphological damage to gills (Paquin et al., 2002). In the molecular initiating event (Fig. 5), Cu causes interference Mg^{2+} binding for the inhibition of Na^+/K^+- ATPase active, a direct result of which is the inhibition of Na^+ and Cl^- uptake. At the same time, the increasing the paracellular permeability will increase the loss of Na^+ and Cl^-, and the organ response is reflected in reduced blood plasma osmolality and increased vascular resistance. In another pathway, Cu induces catecholamine increase and loss of sensory-regulated oxygen consumption, gill histopathology and hypoxic hypercapnia in the arterial blood, as well as vasoconstriction, resulting in cardiovascular collapse.

3. Discussion

3.1. *Water chemistry affected by Cu toxicity*

Considering Cu toxicity based on water chemical characteristics is not a novel concept. Previous research has shown a reduction in toxicity of some metals (e.g., Pb, Zn and Cu) with the increase Ca concentration solution or hardness as early as the 1930s (Adams et al., 2020; Mebane et al., 2020). Later, Zitko et al. (1973) recognized that complexing Cu with compounds such as naturally organic matter (containing humic and fulvic acids) greatly reduced the toxicity of metals. As for analytical and computational chemistry development, our understanding of mechanisms to describe the relationship between water chemistry and metal toxicity has become quite precise. Considerable research has been performed to study metal bioavailability as a function of total metal or dissolved metal, hardness, natural organic matters (including dissolved organic carbon (DOC)), pH, sodium and other

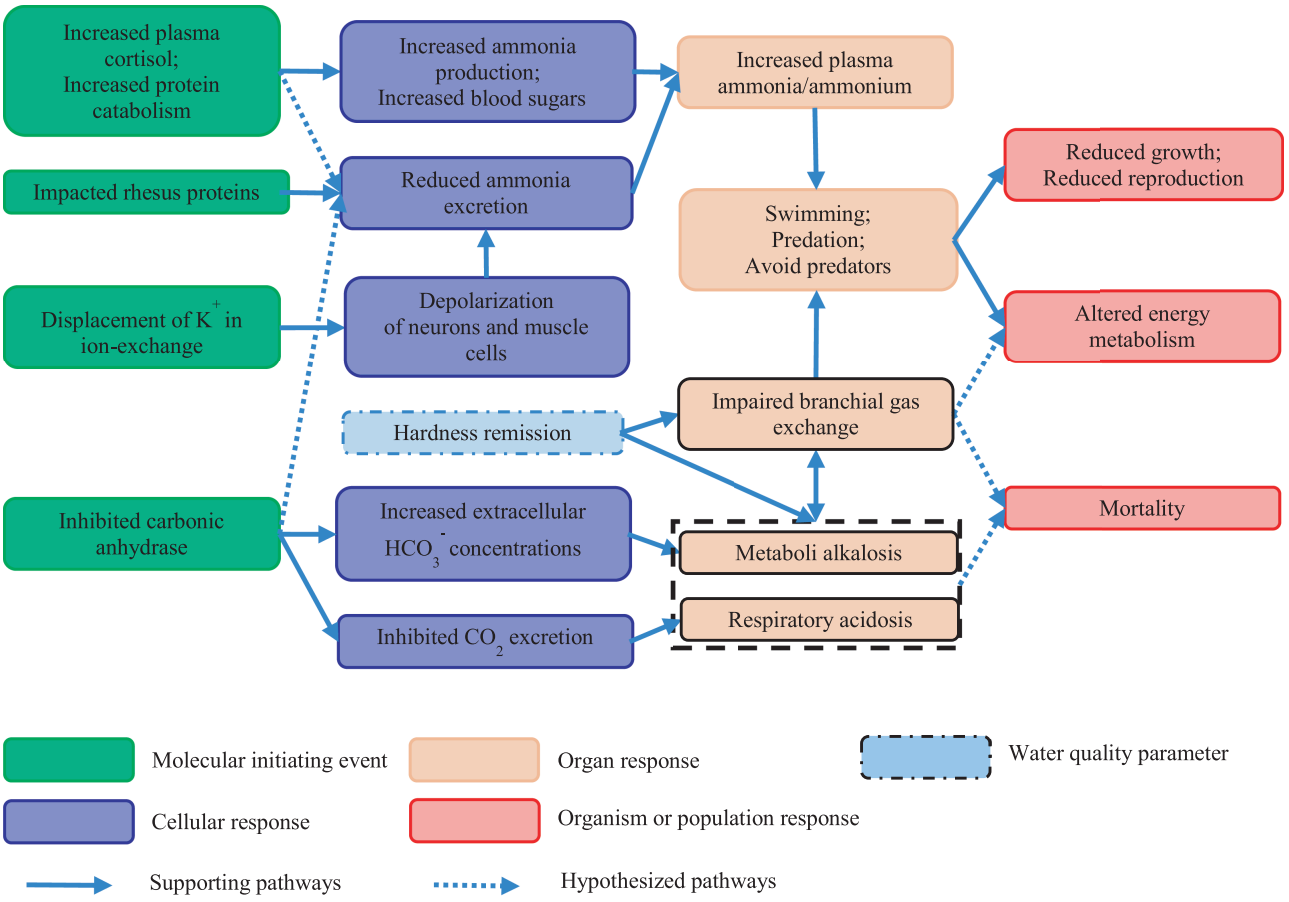

Fig. 4 – Ammonia excretion and acid-base balance toxicant of adverse outcome pathways in aquatic systems with different Cu concentrations. Note: Same as Fig. 1.

water characteristics (De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2003; Ryan et al., 2010; Liao et al., 2019). Research performed by Paquin (2002) showed that ionic is the principal toxic form (Cu^{2+}) of Cu, although $Cu(OH)_2$ and $CuOH^+$ complexes have also been demonstrated to exert toxicity (Paquin et al., 2002). Researchers have recognized that inorganic speciation of Cu in freshwater with only a small fraction of Cu are present as ionic Cu^{2+}, $Cu(OH)_2$ and $CuOH^+$. Inorganic Cu speciation applies to freshwater of high alkalinity and pH, and is dominated mainly by $CuCO_3$ and $Cu(OH)_2$ (USEPA, 2007). However, freshwater with moderate or low alkalinity and lower pH and ionic Cu^{2+} is more prevalent. The chemical speciation of Cu is very highly dependent on the water chemistry, and, likewise, the tendency for toxicity to varies widely as a function of water chemistry (Adams et al., 2020).

To the authors' knowledge, at present five potential pathways by which Cu may exert toxicity on fish have been analyzed in different exposed Cu concentrations. According to the AOP analysis for Cu toxicity, nine key pathways involving the acute toxicity and chronic toxicity of Cu in fish are shown in Fig. 6.

3.2. *Hardness cations*

It has been generally agreed that the ameliorating effects of cations (such as Ca^{2+} and Mg^{2+}) on the Cu toxicity as Cu^{2+} (and other metals such as Ni^{2+} and Zn^{2+}) to freshwater organisms is dependent on competition for uptake via the gills (Zitko and Carson, 1976; Grosell et al., 1997). For example, for the Ca^{2+}, most of the research points out a clear protection against Cu toxicity assessed as acute mortality is to compete with Cu^{2+} for uptake via the gills of fish (Welsh et al., 2000; Naddy et al., 2002). Nonetheless, some research has shown that protection from Ca^{2+} against the acute Cu toxicity may not be related to reduced Cu uptake alone, and is instead related to physiological protection against the Cu toxicity (Craig et al., 2010; Naddy et al., 2002). The results of the present review showed that increased hardness can inhibit Cu-induced olfactory impairment (as shown as the marked pathway 1 in Fig. 6, and described in Fig. 1). This can improve branchial gas exchange after respiratory acidosis (as shown as the marked pathway 3 in Fig. 6, and described in Fig. 4). A case study has indicated that a 50% reduction in olfactory epithelial Cu binding caused by a 20-fold resulted in an increase in ambient Ca^{2+} (Green et al., 2010).

3.3. *DOC concentrations*

DOC is comprised of humic acids, fulvic acids, carbohydrates, proteins and lipids. It also contains much carboxyl, the aminos have a negative charge, and Cu easily forms complexes with compounds in it (Schamphelaere, 2006; Tan and Wang, 2011).

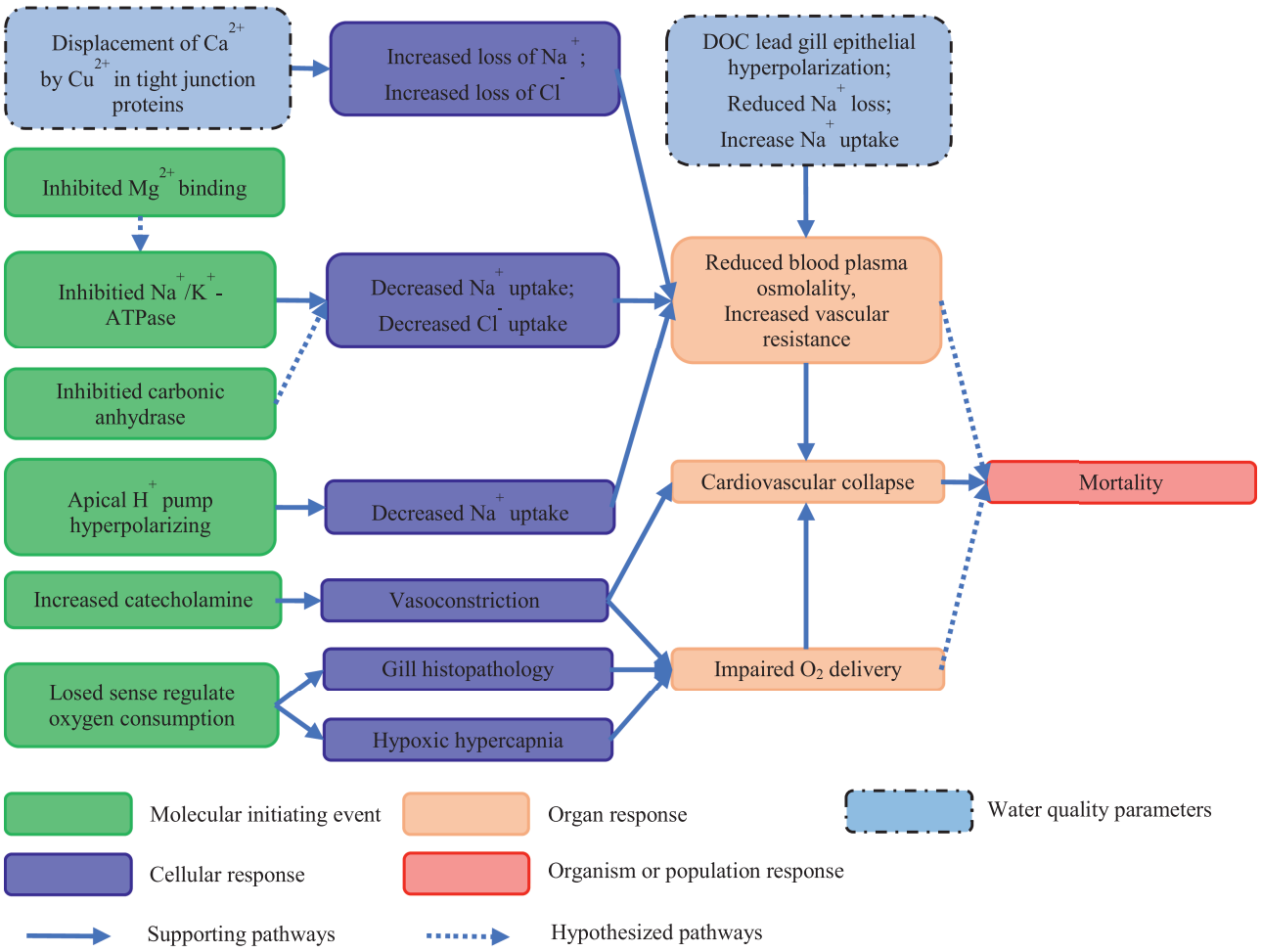

Fig. 5 – Acute toxicant of adverse outcome pathways in an aquatic system with different Cu concentrations. Note: Same as Fig. 1.

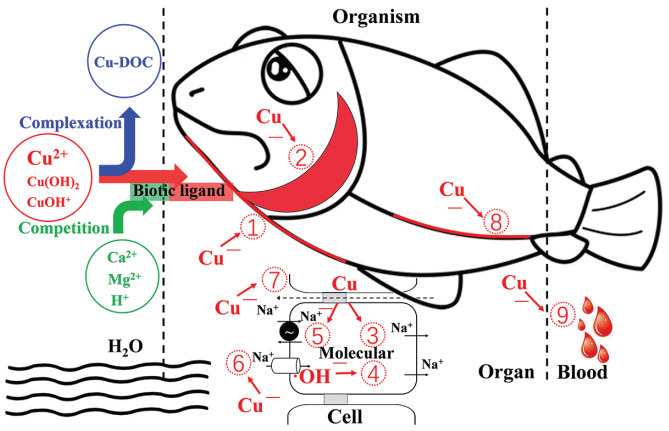

Fig. 6 – Schematic and simplified representation of Cu-sensitive freshwater fish gill transport processes relevant for salt balance, acid-base balance and ammonia excretion and oxidative stress. ① epithelial cells impairment; ② gill histopathology; ③ plasma ammonia/ammonium increase, impaired branchial gas exchange; ④ oxidative damage resulted in DNA adduct formation, protein carbonyls, lipid peroxidation; ⑤ inhibition of Na^+/K^+-ATPase active result in decrease Na^+ uptake; ⑥ apical H^+ pump hyperpolarizing resulted in decrease Na^+ uptake; ⑦ Cu-induce increased loss of Na^+; ⑧ impaired O_2 delivery; ⑨ Vasoconstriction. Note: the red minuses next to the red arrows represent the negative effects, the blue portion of the diagram represents the direct effects of DOC, and the green portion of the diagram represents the direct effects of cations.

Table 2 – Water chemistry affected to Cu toxicity.

No.	key events in effects of Cu toxicity	Influence of hardness	Influence of DOC	Reference
1	In the exposed environment	It is assumed that the main cations of relevance (Ca^{2+}, Mg^{2+}) would join competition on the toxicity of Cu as $Cu^{2+}$for reduce uptake by the gill.	Cu binds to DOC with a high affinity and this complexation prevents or reduces Cu binding and uptake, which acts to lower or prevent toxicity	Playle et al., 1992, 1993; Sciera et al., 2004
2	Olfactory	Ca^{2+} inhibited Cu -induced, via pathway 1 in Fig. 6		Carreau and Pyle, 2005; Sandahl et al., 2007
3	Lateral line hair cells and neuromasts		DOC inhibited Cu -induced, via pathway 1 in Fig. 6	Linbo et al., 2009; Olivari et al., 2009
4	Respiratory acidosis	Ca^{2+} improved branchial gas exchange, via pathway 3 in Fig. 6		Grosell et al., 2004
5	Ions loss	Ca^{2+} reduced Na^{+} loss, via pathway 7 in Fig. 6	DOC reduced Na^{+} loss and increased Na^{+} uptake, via pathways 5, 6 and 7 in Fig. 6	Paquin et al., 2002; Wood et al., 2011; Giacomin et al., 2013

Researchers have recognized that there has been a significant decrease in metal toxicity to aquatic life under an elevated DOC concentration in water environments (Brix et al., 2017b). It has been reported that 95% of Cu^{2+} binds as complexation and chelating compounds in water environments. This is the most important reason explaining how DOC reduces metal toxicity in water environments. Moreover, the DOC quality, including aspects such as allochthonous (terrestrially-derived) DOC, has been reported to be more efficient to reduced metal toxicity than autochthonous-like (algae-derived) DOC (Giacomin et al., 2013; Holland et al., 2017; Cao et al., 2019; Macoustra et al., 2019). This is because the allochthonous DOC has a greater number of negatively charged aromatic binding sites for metals.

Recent research has shown that humic acid (a component of DOC) can enhance protection against Cu-induced osmoregulatory disturbances, and reduces Cu accumulation in the gills and liver of rainbow trout (McGeer et al., 2002). Galvez et al. (2008) showed that DOC acts to hyperpolarize the gill epithelium of freshwater fish that will counteract the increased diffusive Na^{+} loss and reduced Na^{+} uptake in the presence of Cu. In addition, Linbo et al. (2009) showed that DOC offers protection against Cu-induced damage to hair cells impacting peripheral neurons, olfactory or mechanosensory functions. DOC can also change the fluidity of the lipoprotein bilayer in the transcellular pathway, and alter accessibility of the Na^{+} transport sites in short-term exposures. The number of transport sites in longer-term exposures was reported by Wood et al. (2011). In addition, Giacomin et al. (2013) demonstrated that exposure to DOM alone caused a significant a decrease in v-type H^{+}-ATPase activity and an increase in the unidirectional Na^{+} influx rate. This signifies that DOM alone can lead to alterations in membrane transport functions and whole-body Na^{+} metabolism. Therefore, it is important to explain how DOC in the environment reduces the toxicity of Cu, except with complexing Cu^{2+}.

The hardness and DOC in the aquatic ecosystem affect the Cu toxicity in terms of physiological protection against Cu toxicity, as listed in Table 2.

As a matter of fact, a series of modeling approaches, including the hardness-dependent model, free ion activity model (FIAM), gill surface interaction model (GSIM), biotic ligand model (BLM), regression models and other models were implemented to predict the relationship between metal toxicity and water chemistry. Next, these models were then applied to water quality regulations and ecological risk assessment for Cu in numerous countries and regions. The developed BLM model is a semi-mechanism model based on hydro chemical equilibrium. Its ability to predict biological toxicity has been verified by a large number of scientists. Due to the fact that it requires many input variables and is very complex, a stepwise MRM for species which have been tested over wide ranges of hardness, DOC and pH conditions has been developed. It is important to comb out the mechanism of Cu toxicity in the water environment, so as to provide a greater amount of accepted evidence for the prediction model and application to environmental management. Therefore, the AOP analysis describes the various biological toxicity of Cu with different water environment factors and degree, and its changes from the biological factors of chemical accident mechanism are expounded upon.

4. Conclusions

In conclusion, at low levels of Cu exposure, swimming, avoid predators, locating prey and other sensory functions will be impaired, and the organism will suffer from metabolic alkalosis and respiratory acidosis following the inhibition of the carbonic anhydrase active, which is an increased metabolic cost of compensating for physiological impairments by Cu induction. This will in turn result in a decrease in growth, repro-

duction and energy metabolism. The main pathway of acute toxicity of Cu to fish is the inhibition of the Na^+/K^+-ATPase enzyme. This leads to reduced intracellular sodium absorption. Cu-induced increased cell permeability, in turn resulting in increased sodium ion loss. In addition, inhibited carbonic anhydrase causes the obstruction of cellular ammonia excretion, resulting in cellular respiratory acidosis and metabolic alkalosis. The result of this is an imbalance in cellular ion regulation, in turn leading to cardiovascular failure. In addition, competition with cations and binding with DOC as complexation can sharply reduce Cu^{2+} uptake via the gills and significantly affect Cu toxicity. This demonstrates that hardness and DOC may also reduce the Cu toxicity directly affecting organism physiology, such as inhibition Cu induction, improving branchial gas exchange, and alleviating Na^+ homeostasis play important roles in altering the Cu bioavailability and Cu toxicity to the lives of aquatic organisms. Finally, when reviewing copper toxicity, it is important to evaluate the relationship between water quality parameters and reduce the Cu toxicity effects in the physiology of organisms.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Natural Scientific Foundation of China (Nos. 41773085, 41977364), the Open Foundation of State Key Laboratory of Environmental Criteria and Risk Assessment at the Chinese Research Academy of Environmental Sciences (No. SKLECRA2019OFP01), the Doctoral Scientific Research Foundation of Jiangxi Academy of Forestry (No. 2022521602) and Prof. Xiaowei Jin was supported by Beijing Outstanding Talent Training Program.

REFERENCES

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P.H., et al., 2020. Bioavailability assessment of metals in freshwater environments: a historical review. Environ. Toxicol. Chem. 39 (1), 48–59.

Al-Reasi, H.A., Wood, C.M., Smith, D.S., 2013. Characterization of freshwater natural dissolved organic matter (DOM): mechanistic explanations for protective effects against metal toxicity and direct effects on organisms. Environ. Int. 59 (9), 201–207.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., et al., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29 (3), 730–741.

Boeck, G.D., Vlaeminck, A., Balm, P.H.M., Lock, R.A.C., Wachter, B.D., Blust, R., 2001. Morphological and metabolic changes in common carp (*Cyprinus carpio*) during short-term copper exposure: interactions between Cu^{2+} and plasma cortisol elevation. Environ. Toxicol. Chem. 20 (2), 374–381.

Bopp, S.K., Abicht, H.K., Knauer, K., 2008. Copper-induced oxidative stress in rainbow trout gill cells. Aquat. Toxicol. 86 (2), 197–204.

Brix, K.V., Schlekat, C.E., Garman, E.R., 2017a. The mechanisms of nickel toxicity in aquatic environments: an adverse outcome pathway analysis. Environ. Toxicol. Chem. 36 (5), 1128–1137.

Brix, K.V., DeForest, D.K., Tear, L., Grosell, M., Adams, W.J., 2017b. Use of multiple linear regression models for setting water quality criteria for copper: a complimentary approach to the biotic ligand model. Environ. Sci. Technol. 51, 5182–5192.

Brown, V.M., Shaw, T.L., Shurben, D.G., 1974. Aspects of water quality and the toxicity of copper to rainbow trout. Water Res. 8 (10), 797–803.

Cao, J.L., Wang, G.D., Wang, T.Y., Chen, J.J., Guo, W.J., Wu, P.H., et al., 2019. Copper caused reproductive endocrine disruption in zebrafish (*Danio rerio*). Aquat. Toxicol. 211, 124–136.

Carreau, N.D., Pyle, G.G., 2005. Effect of copper exposure during embryonic development on chemosensory function of juvenile fathead minnows (*Pimephales promelas*). Ecotox. Environ. Safe. 61 (1), 1–6.

Chiaverini, N., Ley, M.D., 2010. Protective effect of metallothionein on oxidative stress-induced DNA damage. Free Radical Res. 44 (6), 605–613.

Craig, P.M., Wood, C.M., Mcclelland, G.B., 2010. Water chemistry alters gene expression and physiological end points of chronic waterborne copper exposure in zebrafish (*Danio rerio*). Environ. Sci. Technol. 44 (6), 2156–2162.

Davis, A., Ashenberg, D., 1989. The aqueous geochemistry of the Berkeley Pit, Butte, Montana, U.S.A. Appl. Geochem. 4 (1), 23–36.

De Boeck, G., Desmet, H., Blust, R., 1995. The effect of sublethal levels of copper on oxygen consumption and ammonia excretion in the common carp (*Cyprinus carpio*). Aquat. Toxicol. 32, 127–141.

De Schamphelaere, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for Daphnia magna: the effects of calcium, magnesium, sodium, potassium, and pH. Environ. Sci. Technol. 36 (1), 48–54.

De Schamphelaere, K.A.C., Vasconcelos, F.M., Heijerick, D.G., Tack, F.M.G., Delbeke, K., Allen, H.E., et al., 2003. Development and field validation of a predictive copper toxicity model for the green alga *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 22 (10), 2454–2465.

De Schamphelaere, K.A.C., Janssen, C.R., 2006. Bioavailability models for predicting copper toxicity to freshwater green microalgae as a function of water chemistry. Environ. Sci. Technol. 40 (14), 4514–4522.

DiToro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. technical basis. Environ. Toxicol. Chem. 20 (10), 2383–2396.

Donnachie, R.L., Johnson, A.C., Moeckel, C., Pereira, M.G., Sumpter, J.P., 2014. Using risk-ranking of metals to identify which poses the greatest threat to freshwater organisms in the UK. Environ. Pollut. 194, 17–23.

Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85 (1), 97–177.

Festa, R.A., Thiele, D.J., 2011. Copper: An essential metal in biology. Curr. Biol. 21, 877–883.

Flemming, C.A., Trevors, J.T., 1989. Copper toxicity and chemistry in the environment: a review. Water Air Soil Poll. 44, 143–158.

Freedman, J.H., Ciriolo, M.R., Peisach, J., 1989. The role of glutathione in copper metabolism and toxicity. J. Biol. Chem. 264 (10), 5598–5605.

Fu, Z.Y., Wu, F.C., Chen, L.L., Xu, B.S., Feng, C.L., Bai, Y.C., et al., 2016. Copper and zinc, but not other priority toxic metals, pose risks to native aquatic species in a large urban lake in Eastern China. Environ. Pollut. 219, 1069.

Fu, Z.Y., Guo, W.J., Dang, Z., Hu, Q., Wu, F.C., Feng, C.L., et al., 2017. Refocusing on nonpriority toxic metals in the aquatic environment in China. Environ. Sci. Technol. 51, 3117–3118.

Gaetke, L.M., Chow-Johnson, H.S., Chow, C.K., 2014. Copper: toxicological relevance and mechanisms. Arch. Toxicol. 88 (11), 1929–1938.

Galvez, F., Donini, A., Playle, R.C., Smith, D.S., O'Donnell, M., Wood, C.M., 2008. A matter of potential concern: natural organic matter alters the electrical properties of fish gills. Environ. Sci. Technol. 42 (24), 9385–9390.

Garcia-Reyero, N., Kennedy, A.J., Escalon, B.L., Habib, T., Laird, J.G., Rawat, A., et al., 2014. Differential effects and potential adverse outcomes of ionic silver and silver nanoparticles in vivo and in vitro. Environ. Sci. Technol. 48, 4546–4555.

Giacomin, M., Gillis, P.L., Bianchini, A., Wood, C.M., 2013. Interactive effects of copper and dissolved organic matter on sodium uptake, copper bioaccumulation, and oxidative stress in juvenile freshwater mussels (*Lampsilis siliquoidea*). Aquat. Toxicol. 144-145, 105–115.

Green, W.W., Mirza, R.S., Wood, C.M., Pyle, G.G., 2010. Copper binding dynamics and olfactory impairment in Fathead Minnows (*Pimephales promelas*). Environ. Sci. Technol. 44 (4), 1431–1437.

Grosell, M.H., Hogstrand, C., Wood, C.M., 1997. Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 38, 257–276.

Grosell, M., Wood, C.M., Walsh, P.J., 2003. Copper homeostasis and toxicity in the elasmobranch Raja erinacea and the teleost *Myoxocephalus octodecemspinosus* during exposure to elevated water-borne copper. Comp. Biochem. Phys. C 135 (2), 179–190.

Grosell, M., Mcdonald, M.D., Walsh, P.J., Wood, C.M., 2004. Effects of prolonged copper exposure in the marine gulf toadfish (*Opsanus beta*) II: copper accumulation, drinking rate and Na^+/K^+ -ATPase activity in osmoregulatory tissues. Aquat. Toxicol. 68 (3), 263–275.

Grosell, M., 2012. Copper. In: Wood, C.M., Farrell, A.P., Brauner, C.J. (Eds.), Homeostasis and Toxicology of Essential Metals: Volume 31A fish physilolgy. Elsevier Inc. Hamilton Ontario, pp. 53–112.

Harris, Z.L., Gitlin, J.D., 1996. Genetic and molecular basis for copper toxicity. AM. J. Clin. Nutr. 63, 836–841.

Hayashi, T.I., Kashiwagi, N., 2011. A Bayesian approach to probabilistic ecological risk assessment: risk comparison of nine toxic substances in Tokyo surface waters. Environ. Sci. Pollut. Res. 18 (3), 365–375.

Hayashi, T.I., 2013. Applying biotic ligand models and Bayesian techniques: ecological risk assessment of copper and nickel in Tokyo rivers. Integr. Environ. Assess. 9 (1), 63–69.

Herbert, D.W.M., Vandyke, J.M., 1964. The toxicity to fish of mixtures of poisons: II. Copper-ammonia and zinc-phenol mixtures. Ann. Appl. Biol. 53 (3), 415–421.

Holland, A., Wood, C.M., Scott Smith, D., Correia, T.G., Val, A.L., 2017. Nickel toxicity to cardinal tetra (*Paracheirodon axelrodi*) differs seasonally and among the black, white and clear river waters of the Amazon basin. Water Res. 123, 21–29.

Jeong, J., Song, T., Chatterjee, N., Choi, I., Cha, Y.K., Choi, J., 2018. Developing adverse outcome pathways on silver nanoparticle-induced reproductive toxicity via oxidative stress in the nematode *Caenorhabditis elegans* using a Bayesian network model. Nanotoxicology 12 (10), 1182–1197.

Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. Chemosphere 231, 249–255.

Jin, X.W., Liu, F., Wang, Y.Y., Zhang, L.S., Li, Z., Wang, Z.Z., et al., 2015. Probabilistic ecological risk assessment of copper in Chinese offshore marine environments from 2005 to 2012. Mar. Pollut. Bull. 94 (1–2), 96–102.

Johnson, A.C., Donnachie, R.L., Sumpter, J.P., Jürgens, M.D., Moeckel, C., Pereira, M.G., 2017. An alternative approach to risk rank chemicals on the threat they pose to the aquatic environment. Sci. Total Environ. 599–600, 1372–1381.

Keller, A.A., Adeleye, A.S., Conway, J.R., Garner, K.L., Zhao, L.J., Cherr, G., et al., 2017. Comparative environmental fate and toxicity of copper nanomaterials. Nanoimpact 7, 28–40.

Khangarot, B.S., Das, S., 2010. Effects of copper on the egg development and hatching of a freshwater pulmonate snail *Lymnaea luteola* L. J. Hazard Mater. 179 (1–3), 665–675.

Kramer, V.J., Etterson, M.A., Hecker, M., Murphy, C.A., Roesijadi, G., Spade, D.J., et al., 2011. Adverse outcome pathways and ecological risk assessment: bridging to population-level effects. Environ. Toxicol. Chem. 30 (1), 64–76.

Krumschnabel, G., Manzl, C., Berger, C., Hofer, B., 2005. Oxidative stress, mitochondrial permeability transition, and cell death in Cu-exposed trout hepatocytes. Toxicol. Appl. Pharm. 209 (1), 62–73.

Liao, W., Feng, C.L., Liu, N., Liu, D.Q., Yan, Z.F., Bai, Y.C., et al., 2019. Influence of hardness and dissolved organic carbon on the acute toxicity of copper to zebrafish (*Danio rerio*) at different life stages. Bull. Environ. Contam. Toxicol. 103, 789–795.

Linbo, T.L., Baldwin, D.H., Mcintyre, J.K., Scholz, N.L., 2009. Effects of water hardness, alkalinity, and dissolved organic carbon on the toxicity of copper to the lateral line of developing fish. Environ. Toxicol. Chem. 28 (7), 1455–1461.

Liu, C.S., Deng, J., Yu, L.Q., Ramesh, M., Zhou, B.S., 2010. Endocrine disruption and reproductive impairment in zebrafish by exposure to 8:2 fluorotelomer alcohol. Aquat. Toxicol. 96 (1), 70–76.

Macoustra, G., Holland, A., Stauber, J., Jolley, D.F., 2019. Effect of various natural dissolved organic carbon on copper lability and toxicity to the tropical freshwater microalga *Chlorella sp*. Environ. Sci. Technol. 53, 2768–2777.

McDuffee, A.T., Senisterra, G., Huntley, S., Lepock, J.R., Sekhar, K.R., Meredith, M.J., et al., 1997. Proteins containing non-native disulfide bonds generated by oxidative stress can act as signals for the induction of the heat shock response. J. Cell Physiol. 171 (2), 143–151.

McGeer, J.C., Playle, R.C., Wood, C.M., Galvez, F., 2000. A physiologically based biotic ligand model for predicting the acute toxicity of waterborne silver to rainbow trout in freshwaters. Environ. Sci. Technol. 34 (19), 4199–4207.

McGeer, J.C., Szebedinszky, C., Mcdonald, D.G., Wood, C.M., 2002. The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure. Comp. Biochem. Phys. C 133 (1–2), 147–160.

Mebane, C.A., Chowdhury, M.J., De Schamphelaere, K.A.C., Lofts, S., Paquin, P.R., Santore, R.C., et al., 2020. Metal bioavailability models: current status, lessons learned, considerations for regulatory use, and the path forward. Environ. Toxicol. Chem. 39 (1), 60–84.

Meyer, J., Adams, W., 2010. Relationship between biotic ligand model-based water quality criteria and avoidance and olfactory responses to copper by fish. Environ. Toxicol. Chem. 29 (9), 2096–2103.

Mu, Y.S., Wang, Z., Wu, F.C., Zhong, B.Q., Yang, M.R., Sun, F.H., et al., 2018. Model for predicting toxicities of metals and metalloids in coastal marine environments worldwide. Environ. Sci. Technol. 52, 4199–4206.

Muller, E.B., Lin, S.J., Nisbet, R.M., 2015. Quantitative adverse outcome pathway analysis of hatching in zebrafish with CuO nanoparticles. Environ. Sci. Technol. 49, 11817–11824.
Naddy, R.B., Stubblefield, May, J.R., Tucker, S.A., Hockett, J.R., 2002. The effect of calcium and magnesium ratios on the toxicity of copper to five aquatic species in freshwater. Environ. Toxicol. Chem. 21 (2), 347–352.
NMIC (National Minerals Information Center), 2021. Copper Statistics and Information. United States Department of the Interior, pp. 1–2.
Olivari, F.A., Hernández, P.P., Allende, M.L., 2009. Acute copper exposure induces oxidative stress and cell death in lateral line hair cells of zebrafish larvae. Brain Res. 1244, 1–12.
Osuna-Jiménez, I., Williams, T.D., Prieto-Álamo, M., Abril, N., Chipman, J.K., Pueyo, C., 2009. Immune- and stress-related transcriptomic responses of *Solea senegalensis* stimulated with lipopolysaccharide and copper sulphate using heterologous cDNA microarrays. Fish Shellfish Immun. 26 (5), 699–706.
Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., et al., 2002. The biotic ligand model: a historical overview. Comp. Biochem. Phys. C 133 (1), 3–35.
Playle, R.C., Gensemer, R.W., Dixon, D.G., 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill micro-environment. Environ. Toxicol. Chem. 11, 381–391.
Playle, R.C., Dixon, D.G., Burnison, K., 1993. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. Can. J. Fish Aquat. Sci. 50 (12), 2667–2677.
Puig, S., Thiele, D., 2002. Molecular mechanisms of copper uptake and distribution. Curr. Opin. Chem. Biol. 6 (2), 171–180.
Pyle, G.G., Mirza, R.S., 2007. Copper-impaired chemosensory function and behavior in aquatic animals. Hum. Ecol. Risk Assess. 13 (3), 492–505.
Rau, M.A., Whitaker, J., Freedman, J.H., Di Giulio, R.T., 2004. Differential susceptibility of fish and rat liver cells to oxidative stress and cytotoxicity upon exposure to prooxidants. Comp. Biochem. Phys. C 137 (4), 335–342.
Ryan, A.C., Tomasso, J.R., Klaine, S.J., 2010. Influence of pH, hardness, dissolved organic carbon concentration, and dissolved organic matter source on the acute toxicity of copper to Daphnia magna in soft waters: implications for the biotic ligand model. Environ. Toxicol. Chem. 28 (8), 1663–1670.
Sandahl, J.F., Baldwin, D.H., Jenkins, J.J., Scholz, N.L., 2007. A sensory dystem at the interface between urban stormwater runoff and salmon survival. Environ. Sci. Technol. 41 (8), 2998–3004.
Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S., 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*. Environ. Toxicol. Chem. 20 (10), 2397–2402.
Schmidlin, L., Fumetti, S.V., Nagel, P., 2015. Copper sulphate reduces the metabolic activity of *Gammarus fossarum* in laboratory and field experiments. Aquat. Toxicol. 161, 138–145.
Sciera, K.L., Isely, J.J., Tomasso, J.R., Klaine, S.J., 2004. Influence of multiple water-quality characteristics on copper toxicity to fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 23 (12), 2900–2905.
Sfakianakis, D.G., Renieri, E., Kentouri, M., Tsatsakis, A.M., 2015. Effect of heavy metals on fish larvae deformities: a review. Environ. Res. 137, 246–255.
Simonato, J.D., Mela, M., Doria, H.B., Guiloski, I.C., Randi, M.A.F., Carvalho, P.S.M., et al., 2016. Biomarkers of waterborne copper exposure in the Neotropical fish *Prochilodus lineatus*. Aquat. Toxicol. 170, 31–41.
Solomon, E.I., Lowery, M.D., 1993. Electronic structure contributions to function in bioinorganic chemistry. Science 259, 1575–1581.
Tan, Q.G., Wang, W.X., 2011. Acute toxicity of cadmium in *Daphnia magna* under different calcium and pH conditions: importance of influx rate. Environ. Sci. Technol. 45, 1970–1976.
Taylor, L.N., McGeer, J.C., Wood, C.M., Mcdonald, D.G., 2000. Physiological effects of chronic copper exposure to rainbow trout (*Oncorhynchus mykiss*) in hard and soft water: evaluation of chronic indicators. Environ. Toxicol. Chem. 19 (9), 2298–2308.
Teles, M., Pacheco, M., Santos, M.A., 2010. Physiological and genetic responses of European eel (*Anguilla anguilla* L.) to short-term chromium or copper exposure-Influence of preexposure to a PAH-like compound. Environ. Toxicol. 20 (1), 92–99.
Tilton, F., Tilton, S.C., Bammler, T.K., Beyer, R., Farin, F., Stapleton, P.L., et al., 2008. Transcriptional biomarkers and mechanisms of copper-induced olfactory injury in zebrafish. Environ. Sci. Technol. 42 (24), 9404–9411.
Tsui, T.K.N., Hung, C.Y.C., Nawata, C.M., Wilson, J.M., Wright, P.A., Wood, C.M, 2009. Ammonia transport in cultured gill epithelium of freshwater rainbow trout: the importance of Rhesus glycoproteins and the presence of an apical Na^+/NH_4^+ exchange complex. J. Exp. Biol. 212 (6), 878–892.
USEPA (United States Environmental Protection Agency), 2007. Aquatic Life Ambient Freshwater Quality Criteria-Copper. Office of Water United States Environmental Protection Agency, Washington D.C., pp. 1–204.
Vitale, A.M., Monserrat, J.M., Castilho, P., Rodriguez, E.M., 1999. Inhibitory effects of cadmium on carbonic anhydrase activity and ionic regulation of the estuarine crab *Chasmagnathus granulata* (Decapoda, Grapsidae). Comp. Biochem. Phys. C 122 (1), 121–129.
Wang, T., Knudsen, P.K., Brauner, C.J., Busk, M., Vijayan, M.M., Jensen, F.B., 1998. Copper exposure impairs intra- and extracellular acid-base regulation during hypercapnia in the fresh water rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B 168 (8), 591–599.
Wang, Y.D., Fang, J., Leonard, S.S., Rao, K.M.K., 2004. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radical Bio. Med. 36 (11), 1434–1443.
Welsh, P.G., Lipton, J., Chapman, G.A., Podrabsky, T.L., 2000. Relative importance of calcium and magnesium in hardness-based modification of copper toxicity. Environ. Toxicol. Chem. 19 (6), 1624–1631.
Wood, C.M., Al-Reasi, H.A., Smith, D.S., 2011. The two faces of DOC. Aquat. Toxicol. 105 (3–4), 3–8.
Wu, F.C., Mu, Y.S., Chang, H., Zhao, X.L., Giesy, J.P., Wu, K.B., 2013. Predicting water quality criteria for protecting aquatic life from physicochemical properties of metals or metalloids. Environ. Sci. Technol. 47, 446–453.
Yan, Z.F., Jin, X.W., Liu, D.Q., Hong, Y.J., Liao, W., Feng, C.L., et al., 2020. The potential connections of adverse outcome pathways with the hazard identifications of typical organophosphate esters based on toxicity mechanisms. Chemosphere 266, 128989.
Zitko, P., Carson, W.V., Carson, W.G., 1973. Prediction of incipient lethal levels of copper to juvenile Atlantic Salmon in the presence of humic acid by cupric electrode. Bull. Environ. Contam. Toxicol. 10 (5), 265–271.
Zitko, V., Carson, W.G., 1976. A mechanism of the effects of water hardness on the lethality of heavy metals to fish. Chemosphere 5, 299–303.