Ecotoxicology and Environmental Safety 273 (2024) 116119

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Cadmium induces physiological and behavioral changes associated with 180 kDa NCAM lower expression and higher polysialic acid, in the African clawed *Xenopus laevis* tadpoles

Guillaume Marchand, Ingrid Fliniaux, Pauline Titran, Yoshiko Takeda Uchimura, Jean-François Bodart, Anne Harduin Lepers, Katia Cailliau, Matthieu Marin *

Univ. Lille, CNRS, UMR 8576-UGSF-Unité de Glycobiologie Structurale et Fonctionnelle, Lille F-59000, France

ARTICLE INFO

Edited by Fernando Barbosa

Keywords:
Xenopus laevis tadpole
cadmium
NCAM
polysialic acid
behavior
aquatic toxicology

ABSTRACT

Heavy metals are released into the environment in increasing amounts from different natural and anthropogenic sources. Among them, cadmium contaminates aquatic habitats and represents a threat to Amphibians. To assess the risks of exposure to cadmium in the aquatic environment, we studied the survival rate of early tadpoles of *Xenopus laevis* under exposure to $CdCl_2$ for 6 days in the concentration range between 0.15 and 150 μM of Cd^{2+}. Tadpoles survived and reached stage 45 before feeding at all concentrations tested except 150 μM Cd^{2+}, which significantly induced death. With an exposure of 15 μM Cd^{2+}, tadpoles' mean body length decreased, heart rate increased, fastest swimming speed decreased, and distance traveled was greater compared to unexposed controls. Additionally, a witness of neuronal normal development, the neural cell adhesion molecules (NCAM) expression, was decreased. Moreover, this cell-surface glycoprotein exhibited higher polysialylation, a post-translational modification capable to reduce cell adhesion properties and to affect organ development. Our study highlights the effects of Cd^{2+} on a series of parameters including morphology, physiology, and behavior. They emphasize the deregulation of molecular NCAM suggesting this effector is an interesting biomarker to detect cadmic toxicity in early tadpoles.

1. Introduction

Anthropogenic pressures are causing an erosion of biodiversity (Monastersky, 2014). Various environmental contaminants from the atmospheric deposition, runoff, or exchanges end up in the aquatic environments (Beketov et al., 2013; Dudgeon, 2019; Dudley and Alexander, 2017) with serious ecological consequences (Blaustein and Kiesecker, 2002; Scott and Sloman, 2004; Sfakianakis et al., 2015). In particular, aquatic ecosystems are polluted by heavy metals (Singh et al., 2022). Among them, the contamination of cadmium (Cd^{2+}) in freshwater is a driver of amphibians decline (Patar et al., 2016). Considered one of the most threatened faunal groups amphibians are at risk of extinction (41% of known species, IUCN, 2022, The IUCN Red List of Threatened Species).

Cadmium reduces the amphibian's reproductive success by acting at different levels, and it affects behavior with changes in locomotion, feeding patterns, and predator avoidance, impacting survival in natural habitats (Sunderman et al., 1991, 1992; Flament et al., 2003; Slaby et al., 2019). Tadpoles exhibit deformities and have an impaired growth of several tissues with effects depending on the species, and exposure duration (Herkovits et al., 1998, 1997; James and Little, 2003; Lu et al., 2021; Patar et al., 2016; Sharma and Patiño, 2008). A Frog Embryo Teratogenesis Assay Xenopus (FETAX) was developed as a screening method to identify the potential developmental toxicity of small amounts of chemicals on a short period of time (96 h) (Bonfanti et al., 2004; Mouchet et al., 2006; Burlibasa and Gavrila, 2011). However, those tests are conducted from mid-blastula/early gastrula stage (stages 8–11) over the organogenesis period, and do not consider early developmental stages (fertilization and early segmentation). Therefore, data describing the impact of Cd^{2+} exposure during early development in the embryo/tadpole (before feeding) of amphibians are scarce, and specific bioindicators of Cd^{2+} toxicity at these stages are lacking.

Cadmium displays promiscuous effects affecting several cellular components (Lee and Thévenod, 2020), including the effectors involved

Abbreviations: PSA, polysalic acid; Cd^{2+}, cadmium; NCAM, Neural cell adhesion molecules; EDTA, Ethylenediaminetetraacetic acid.

* Corresponding author.
E-mail address: matthieu.marin@univ-lille.fr (M. Marin).

https://doi.org/10.1016/j.ecoenv.2024.116119
Received 9 October 2023; Received in revised form 16 February 2024; Accepted 17 February 2024
Available online 20 February 2024
0147-6513/© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

in cell adhesion (Prozialeck et al., 2002; Thévenod, 2009). Neural cell adhesion molecules (NCAM) are cell-surface glycoproteins that mediate cell-cell interactions and promote adhesive interactions between cells through homophilic binding (Jensen et al., 1999). They are required for the development of the nervous system (Balak et al., 1987), where they participate in synaptic plasticity (Muller et al., 1996), and regulate the ability of synapses to change their connectivity (Giagtzoglou et al., 2009), thereby influencing normal behavior in vertebrates. Since several adhesion molecules such as cadherin are targeted by Cd^{2+}, NCAM could constitute an interesting biotarget (Prozialeck et al., 2002).

To exert their role in the development, NCAM require a crucial posttranslational modification, a N-glycosylation where N-glycans are modified by the addition of long chains of sialic acids called polysialylation (Rutishauser, 2008; Weinhold et al., 2005). Polysialic acid (PSA) chains are long polymers of negatively charged sialic acid possessing a retaining function *via* direct binding to effectors. PSA are also highly hydrophilic structures in the extracellular medium that switch the adhesive properties of NCAM to antiadhesive. These changes consequently promote plasticity and migration of NCAM-expressing cells during development (Gascon et al., 2007). The high expression level of PSA-NCAM is a defining feature of neural progenitor cell migration and axon growth during embryogenesis (Bonfanti and Seki, 2021). Removal of PSA from NCAM inhibits neurite outgrowth (Doherty et al., 1990) and increases the adhesive properties of NCAM (Rutishauser et al., 1988; Sadoul et al., 1983). The quantity and nature of sialic acids determine the function of NCAM and are considered profoundly related to a normal nervous system function (Yang et al., 2020). To date, studies conducted in amphibians linking the effects of cadmium heavy metals and N-glycosylation are lacking.

The objective of the present study was to determine the effects of a range of realistic Cd^{2+} concentrations that might be found in a polluted ecosystem on early embryo/tadpole stages. *Xenopus laevis* embryos/tadpoles are amphibians particularly suited to study potential effects of various pollutants including heavy metals in germinal cells and during early development, and to decipher the molecular effectors involved in their toxicity (Slaby et al., 2019). We therefore submitted embryos/tadpoles to cadmium exposure as soon as fertilization was realized, and studied survival, physiological and behavioral aspects to uncover potential new molecular markers for cadmium toxicity during the early development of the *Xenopus* African clawed frog.

2. Materials and methods

Reagent-grade chemicals were purchased from Sigma-Aldrich unless specified.

2.1. Animals handling and tadpole treatments

All animal experiments were performed according to the rules of the European Community Council guidelines (86/609/EEC) for laboratory animal experimentation. The vertebrate animal protocol was approved by the local institutional review board (Comité d'Ethique en Expérimentation Animale, Haut de France, G59–00913).

Mature *Xenopus laevis* adult females, purchased from the CRB-University of Rennes I, Rennes, France, and housed in PHExMAR, at the University of Lille, at 19/20 °C and subjected to a circadian 12 h /12 h light/dark rhythm.

For spawning, adult females were stimulated by injection of human chorionic gonadotropic hormone (700 U) in the dorsal lymph sacs. After 24 h mature oocytes, surrounded by a gangue, were obtained by exercising slight pressure on the animal's ovaries. The testicles from adult male were surgically removed after anesthesia by immersion in 5 g/L MS222 solution (tricaine methane sulfonate).

Oocytes were *in vitro* fertilized with a small piece of testicle for 10 minutes under slight agitation added or not with $CdCl_2$ (0.025, 0.25, 2.5, 25 mg/L, equivalent [Cd^{2+}] 0.15, 1.5, 15, or 150 μM). Eggs were afterward dejellied with a filtered (0.22 μm) water solution containing 2% L-cysteine added or not with Cd^{2+} for 10 minutes and rinsed three times.

Embryos from fertilization to tadpoles (stage 45) were kept in daily renewed sterilized tap water (pH 7.42; Ca^{2+} 44 mg.L^{-1}, Na^{+} 23 mg.L^{-1}, Mg^{2+} 24 mg.L^{-1}, K^{+} 2 mg.L^{-1}) added or not with Cd^{2+} at 19/20°C. Stage 45 tadpoles (6 days of exposure) showing closed stomodeum were chosen according to the Nieuwkoop and Faber table (Nieuwkoop and Faber, 1994).

For adult brain extraction, Xenopus were anesthetized by immersion in 5 g/L MS222 solution (tricaine methane sulfonate). The head was placed at 4°C onto a Petri dish. The skin was dissected into two flaps over the eyes, and the skull was opened to remove the brain, before biochemical analysis.

2.2. Behavioural analysis

Behavioral experiments were conducted in a dark, quiet, and temperature-controlled room maintained at 20°C. For each test, stage 45 tadpoles were calmly placed in a 9.5 cm diameter arena filled with 30 mL non-contaminated water. Larvae were exposed to a brightfield (LED platform) to be recorded by a Canon EOS 7D camera placed on top of the arena. Tadpole's behavior without stimulation was recorded for 5 minutes after 5 minutes of acclimation to reduce anxiety.

The exact position of tadpoles was determined as the center of each individual and recorded every 0.04 sec (1500 frames/min) with the iDTracker® software (Pérez-Escudero et al., 2014). The behavioral parameters - total distance moved (cm) and mean velocity (cm/sec) - were determined after further conversion from pixel to cm with ImageJ®.

2.3. Cardiac rhythm analysis

For each experimental condition ten tadpoles at stage 45 were placed individually in wells of a 96-well plate filled with non-contaminated medium. After an acclimation period of 30 min without stimulation, the tadpole's heartbeat was recorded using a Canon EOS 7D camera adapted to a stereomicroscope. The number of heartbeats was counted twice for each tadpole during a period of 1 min: first, live directly under the stereomicroscope, and then by watching the video.

2.4. Samples preparation for biochemical analysis

Five tadpoles at stage 45 or adult brain were lysed in a glass grinder at 4°C in RIPA buffer (1% Triton X-100; 50 mM TRIS-HCl pH 4; NP40 2%; 0.4% Na-deoxycholate; 0.1% SDS; 150 mM NaCl; 150 mM EDTA; 50 mM NaF) supplemented with 1% of protease inhibitor cocktail and phosphatase inhibitors (Roche SAS) under the ratio of 1 tadpole/10 μL RIPA buffer. Samples were centrifuged for 10 min at 12,000 G and the protein concentration of supernatants was determined using the Bradford assay (BioRad) at 595 nm (SPECTROstar Nano, BMG LABTECH). The supernatants were adjusted at 1 μg/μL and used for Endoneuraminidase-N treatments, immunoprecipitations or directly added with 2X Laemmli (65.8 mM TRIS-HCl pH 6.8; 26.3% glycerol; 2.1% SDS; 0.01% bromophenol blue; 4% β-mercaptoethanol, BioRad).

2.5. Endoneuraminidase-N treatments

Ten microliters of tadpole supernatants prepared in RIPA buffer were treated with 0.7 U endoneuraminidase-N (Eurobio Scientific, dilution 1/5) at 37°C for 1 hour before the addition of 2X Laemmli buffer.

2.6. Immunoprecipitations

Samples were pre-cleared with protein A Sepharose (20 μL of 50% beads/ 200 μL of cell lysate) at 4 °C for 1 h under gentle rocking. After brief centrifugation, supernatants were incubated with antibodies

against NCAM (Alomone, ANR041, 1/1500) at 4 °C for 1 h under rotation and followed by incubation with protein A Sepharose (20 μL of 50% bead slurry) at 4°C for 1 h under rotation. Samples were rinsed 3 times with RIPA buffer before bead pellets were collected by brief centrifugation, and resuspended in 2X Laemmli buffer.

2.7. Electrophoresis and Western blots

Laemmli sample buffer (2x) was added to supernatants of stage 1 and 45 embryos/tadpoles exposed or not to Cd^{2+} or untreated adult brain before samples were heated for 15 min at 60°C. Five μg of proteins were separated on 4–20% SDS PAGE gels (mini protean TGX, BioRad), at 200 V for 1 h in denaturing buffer (0.1% SDS; 0.3% TRIS base; 1.44% glycine). Proteins were transferred onto nitrocellulose membrane (Amersham Hybond) by wet transfer (0.32% TRIS; 1.8% glycine; 20% methanol), at 100 V for 1 h. Transfer efficiency was checked using a Ponceau red reversible coloration (0.2% in 3% trichloracetic acid). Membranes were cut in two parts according to the molecular weight of proteins, and saturated in TBS added with 5% low fat dry milk and 0.05% Tween 20 (TBS-Tween). Incubation was performed overnight at 4°C with specific primary antibodies: rabbit polyclonal antibodies were against β-actin (Santa Cruz, 1/2000), NCAM (Alomone, ANR041, 1/1500), and polysialic acid (ENZO COVALB, ENZ ABS559–0100, 1/2000). After three washes of 10 min in TBS-Tween, nitrocellulose membranes were incubated for 1 h with anti-rabbit horseradish peroxidase-labelled secondary antibodies (Invitrogen, 1/30,000). Membranes were washed in TBS-Tween three times for 10 min and the signals were revealed with a chemiluminescent assay (ECL Select, GE Healthcare) on hyperfilms (Amersham hyperfilm MP). β-actin in Western Blots or NCAM in immunoprecipitations were used as loading controls. For immunoprecipitations, membranes were stripped with a ready-to-use stripping buffer (Clinisciences) and after ensuring the first chemiluminescent signal had faded reblotted with the anti-polysialic acid antibody. Signals were quantified with Image J (Fiji Software) and NCAM and PSA signals were normalized by β-actin and immunoprecipitated NCAM respectively. The means of six independent experiments were performed.

2.8. Morphology

Tadpoles were fixed in ethanol at −20°C for 2–3 days. They were stained in Alcian Blue solution (0.1 mg/mL Alcian blue 8GX in 1 vol of glacial acetic acid/4 volumes of ethanol) for 3–4 days at room temperature, before clearing in ethanol 70% added with 1% of HCl for 1–2 days.

For each tadpole, several pictures were made on lateral, ventral, and dorsal views and treated with Adobe Photoshop CS6® (v. 13.0×64, Adobe ® Photoshop Systems Incorporated, USA) software to obtain sharp images. The eye gap and length of the tadpoles were measured with ImageJ® software.

2.9. Data and statistical analysis

The number of fertilization and embryos/tadpoles included in each experiment and statistical analysis are summarized in the Table 1.

3. Results

3.1. Tadpole survival

After 6 days of contamination or not, embryos have reached stage 45 from the Nieuwkoop and Faber table of *Xenopus laevis* development (Nieuwkoop and Faber, 1994). The survival rate of *Xenopus* tadpoles exposed to Cd^{2+} (0.15, 1.5, or 15 μM) was not significantly different from the unexposed controls. On the contrary tadpole survival rate was significantly lowered after treatment with Cd^{2+} 150 μM ($p < 0.05$), (Fig. 1).

3.2. Morphology, cardiac rhythm, and behavior

To assess tadpole morphology after 6 days of exposure, biometric parameters (length and eye gap) were measured after Alcian blue staining (Fig. 2F). Stage 45 tadpoles exposed to Cd^{2+} (0.15, 1.5, and 15 μM) were smaller in size for all tested concentrations with a significantly lower length ($p < 0.001$ for 0.15 μM, 1.5 and 15 μM Cd^{2+}) and had a smaller eye gap ($p < 0.01$ for 0.15 μM and $p < 0.001$ for 1.5, and 15 μM Cd^{2+} compared to unexposed controls (Figs. 2A, 2B respectively).

In addition, for all concentrations tested, the small body size of treated tadpoles was accompanied by significant higher heartbeats per minute ($p < 0.001$ for 0.15 μM, 1.5, and 15 μM Cd^{2+}), (Fig. 2C).

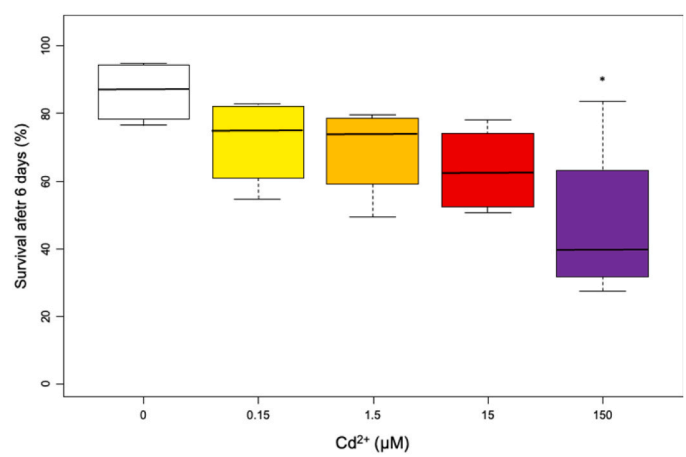

Fig. 1. Survival after 6 days of continuous exposure to Cd^{2+}. Surviving embryos 6 days after fertilization (Stage 45) unexposed (white) or exposed to contaminated medium (increasing concentrations of Cd^{2+} 0.15 μM (yellow), 1.5 μM (orange), 15 μM (red), and 150 μM (purple)). Statistical analysis was performed with the Kruskal-Wallis test and post-hoc: pairwise multiple comparison (*: $p < 0.05$, nf = 5, n=100).

Table 1
number of fertilizations and individuals studied per experiment.

Experiment	Number of fertilizations (nf)	Number of embryos / tadpoles (n)				Statistical analysis * $p < 0.05$; *** $p < 0.001$
		[Cd^{2+}] 0	[Cd^{2+}] 0.15	[Cd^{2+}] 1.5	[Cd^{2+}] 15	
Survival rate	5	100	100	100	100	Kruskal-Wallis and post-hoc: pairwise multiple comparison tests
Biometric	5	50	50	50	50	
Heartbeats	8	74	69	58	81	
Swimming activity	9	99	90	75	85	
Western Blot	6	30	-	-	30	ANOVA two-factors and a Tukey post-hoc
Immuno precipitations	5	25	-	-	25	

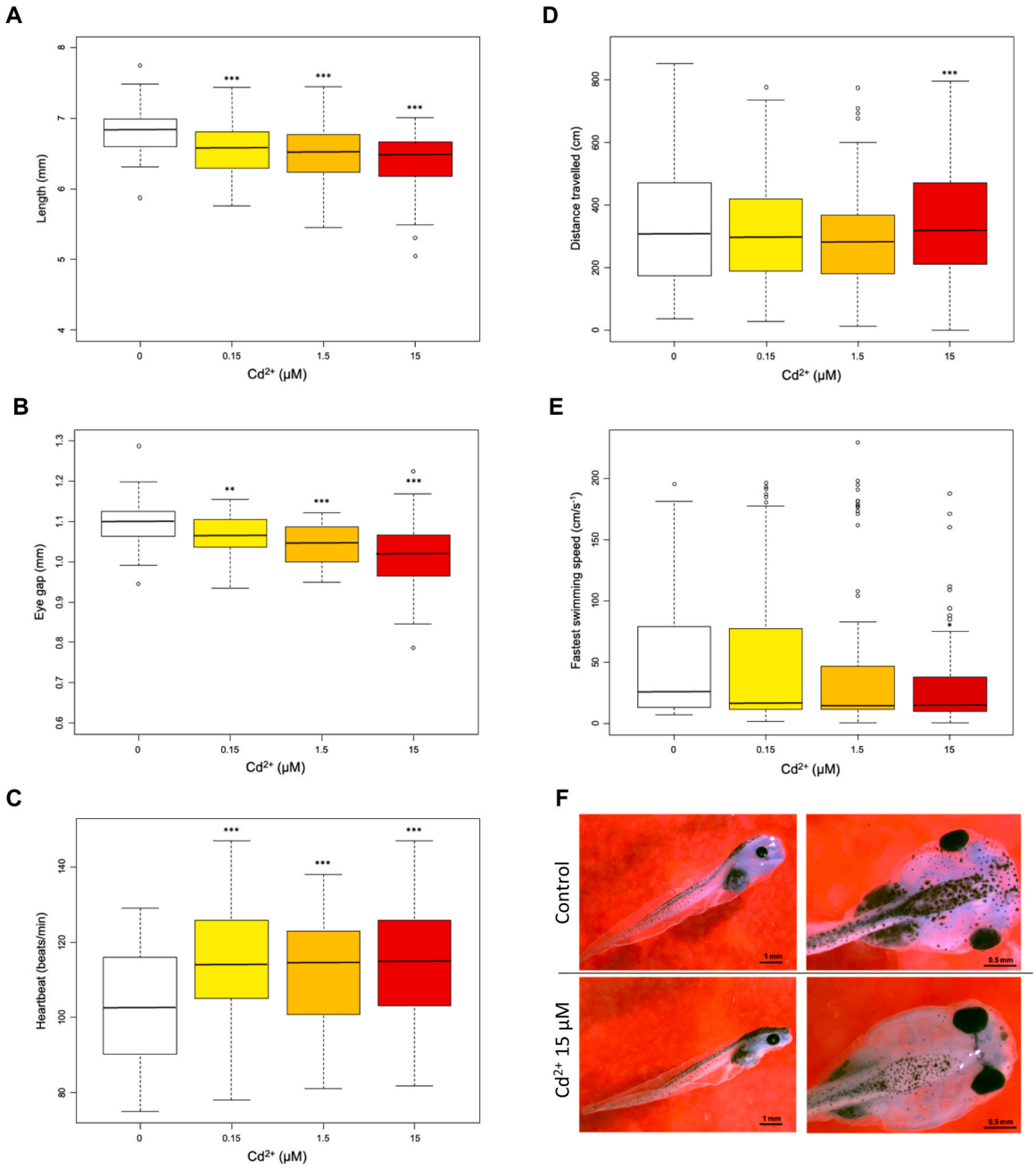

Fig. 2. Biometry, heart rate, and morphology of tadpoles continuously exposed to increasing concentrations of Cd^{2+} for 6 days. Fertilized and embryos were exposed to increasing concentrations of Cd^{2+}: 0 μM (white), 0.15 μM (yellow), 1.5 μM (orange), and 15 μM (red). A Length of tadpoles in mm. B Eye gap measurements in mm. C Heart rate (heartbeat/min) of tadpoles measured without stimuli. D Whole distance in cm covered by tadpoles was recorded during 5 minutes. E fastest swimming speed (cm/sec) of tadpoles was recorded during 5 minutes. Data are presented as boxplots. Statistical analyses are performed A, B with Kruskal-Wallis test and post-hoc: pairwise multiple comparisons (**: $p < 0.01$; ***: $p < 0.001$; nf=5, n=50); C with generalized linear mixed-effects models (***: $p < 0.001$; nf=8, 58<n<81) D, E with generalized linear mixed-effects models (***: $p < 0.001$; nf=9, 75<n<99). F Typical pictures of tadpoles at stage 45, stained with alcian blue. Up, control tadpoles, down, Cd-treated tadpoles (lateral view on the left and ventral view on the right).

Moreover, behavioral aspects were approached by measuring two motility parameters. There was a significant effect for the distance traveled (Fig. 2D) and the fastest swimming speed (Fig. 2E) only after exposure to 15 μM Cd^{2+} ($p < 0.001$).

3.3. NCAM and polysialic acid levels

To better get insight into the deregulation of swimming activity at 15 μM Cd^{2+}, we undertook the analysis of NCAM, a molecular determinant of the nervous system. Without any treatment Western Blots using NCAM antibody showed the presence of two major isoforms (180, 140 kDa) and a very low amount of 120 kDa in adult brains, whereas only one isoform (180 kDa) was detected in stage 45 tadpoles (Fig. 3A). This confirmed that in tadpole stage 45, the main NCAM isoform was 180 kDa. In stage 1 control or 15 μM Cd^{2+} exposed embryos, no NCAM 180 kDa was detected meaning it was expressed later. In stage 45 tadpoles, exposure to 15 μM Cd^{2+} showed a significant decrease in the expression of NCAM 180 kDa isoform compared to control ($p < 0.001$), (Fig. 3B).

NCAMs are among a few proteins whose functions are drastically

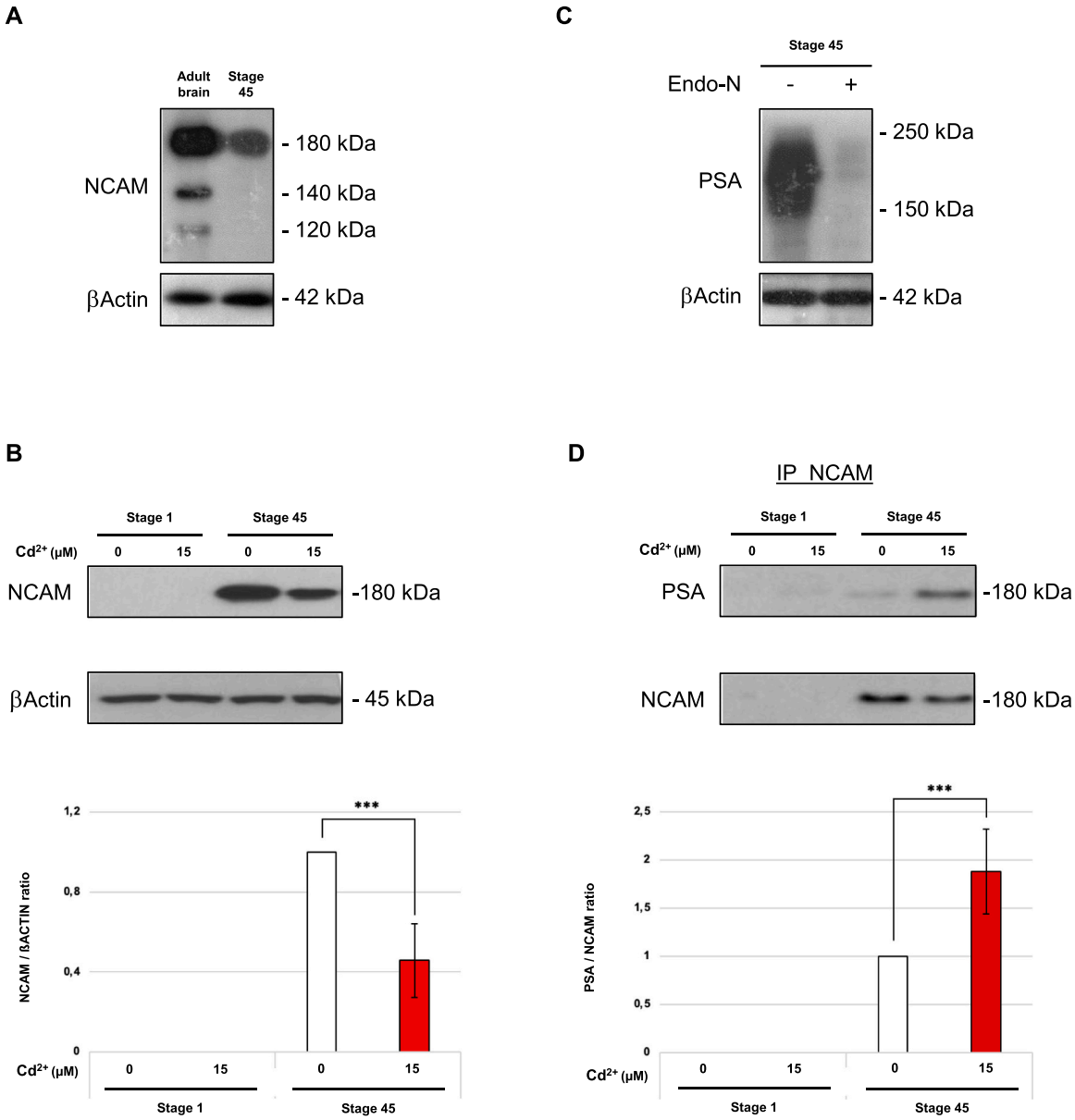

Fig. 3. Cadmium induces a decrease in NCAM expression and an enrichment in polysialic acid content in 6 days treated tadpoles. A Western blots were performed on *Xenopus* adult brains and stage 45 tadpoles using anti-NCAM and anti-β-actin antibodies. B Tadpoles at stage 1 and stage 45 were untreated or treated with 15 μM Cd^{2+} before electrophoresis and Western blot analysis with anti-NCAM or anti-β−actin antibodies. The upper part depicts a typical result of NCAM, the middle part the expression profiles of β−actin, and the lower part an analysis by Image J® normalized with respective β-actin values. C Stage 45 tadpole extracts were treated or not with endoneuraminidase-N (0.7 U) before Western blots using anti-PSA and anti-β-actin antibodies. D Stage 45 tadpoles were treated or not with 15 μM Cd^{2+} and immunoprecipitated with an anti-NCAM antibody before Western blot analysis with anti-PSA antibody. The upper part shows representative results of PSA, the middle part the same membranes reblotted with anti-NCAM antibody, and the lower part the analysis of the expression profiles of PSA by Image J® normalized with respective values of immunoprecipitated NCAM. Data are presented as mean +/- standard deviation. The significance of the results was assessed by an ANOVA two-factors and a Tukey post-hoc test (*** $p < 0.001$).

modified by polysialylation, a modification of N-glycans made of sialic acid long chains. These long chains create biophysical characteristics modifying the hydrated volume, their negative charges strongly modulate the repulsion/attraction between cells. To ensure the PSA antibody was specific toward PSA chains we used endoneuraminidase-N treatment that removes all PSA chains from NCAM 180 kDa. As expected the use of endoneuraminidase-N abolished the recognition of PSA in stage 45 embryos (Fig. 3C). We further Immunoprecipitated NCAM 180 kDa to assess the quantity of PSA chains present on stage 1 and 45 tadpoles with and without 15 μM Cd^{2+} exposure. For the same amount of NCAM immunoprecipitated in tadpoles at stage 45 (with or without 15 μM Cd^{2+} exposure) the amount of PSA present in tadpoles exposed to 15 μM Cd^{2+} was higher compared to control without Cd^{2+}, (Fig. 3D). Statistical analysis confirmed a significant increase in the quantity of polysialylation present on NCAM 180 kDa after Cd^{2+} treatment in stage 45 tadpoles ($p < 0.001$), (Fig. 3D).

4. Discussion

Our research first focused on the rate of *Xenopus laevis* early tadpoles at increasing doses of cadmium. Tadpoles exposed to high concentrations of Cd^{2+} (150 μM) in our experimental conditions died. In a former study, 100% lethality of *Xenopus laevis* tadpoles was obtained with 44.5 μM and 89 μM of Cd^{2+} applied at different stages for 3 days (stages

2–40) (Herkovits et al., 1997). Others reported that chronic exposure to Cd^{2+} decreased survival in the feeding toad larvae (*Bufo americanus*) at a concentration of 4.8 μM (James and Little, 2003) and in froglets of *Rana limnocharis* in a dose-dependent manner (0.55–2.2 μM) (Patar et al., 2016). In our experiments, Cd^{2+} exposures were realized continuously from fertilization to stage 45 for 6 days at 19/20°C. Moreover, tadpoles survived in our experimental conditions until stage 45 after 6 days of 15 μM Cd^{2+} exposure but their size was significantly lowered. Similar results of reduced length were observed on *Xenopus* tadpoles at 8.9 μM of Cd^{2+} (Sharma and Patiño, 2008). The discrepancies in these results might reflect differences in the permeability of tadpole species to cadmium, but also in duration and exposure stages.

Swimming performance represents an important feature that mediates the survival of aquatic animals regarding to prey-predator interaction and reproduction (Cano-Barbacil et al., 2020). *Swimming* around in the open water, *tadpoles* are highly vulnerable to predation. The swimming distance traveled and the fastest swimming speed after 6 days of Cd^{2+} exposure were significantly modified. These critical parameters could also be related to energy consumption defects. Below stage 45, embryos and tadpoles rely on maternal yolk as a nutritional source. Yolk stored is during oogenesis inside organelles called yolk platelets. Yolk platelets are consumed intracellularly, by proteolysis after fusion with endocytic compartments until the tadpole starts feeding after stage 45 (Fagotto and Maxfield, 1994; Jorgensen et al., 2009). Cd^{2+} is known to reduce yolk uptake leading to a smaller size of freshwater larvae (Peterson et al., 1983). It also reduces cell metabolism (Lee and Thévenod, 2020) and counteracts endocytosis in several organs (Santoyo-Sánchez et al., 2013) that are necessary for embryo/tadpole growth.

Three NCAM isoforms (180, 140, and 120 kDa) are detected in the nervous system of adult vertebrates including *Xenopus* (Cunningham et al., 1987; Sunshine et al., 1987). However, the expression of NCAM during the early development of *Xenopus* differs, as 180-NCAM is the sole form detected during early development (Krieg et al., 1989; Sunshine et al., 1987). This is in accordance with our experiments where antibodies against NCAM could reveal three isoforms of 180, 140, and 120 kDa in the adults' brains, but only the 180-NCAM form in stage 45 tadpoles. In vertebrates, NCAM is present in neurons beginning to extend neurites (Murray et al., 1986), during axon fasciculation, and myogenesis (Knudsen et al., 1990). Moreover, in embryos, NCAM carries PSA that imparts a negative charge. Positive ions such as Cd^{2+} might affect their capabilities to bind other molecular effectors and modify cell adhesion in the developing axons. Recently NCAM was also proposed to be involved in the regular function of other organs such as the gut motility in vertebrates (Weledji and Assob, 2014), or the heart contractility in *Xenopus* (Sedmera et al., 2003). Our study indicates that Cd^{2+} affects heart rhythms. These results are consistent with results obtained from developing cardiomyocytes exhibiting cardiac arrhythmias after Cd^{2+} treatment (Shen et al., 2018). The cardiovascular system is necessary for the development and proper function of every organ and anomalies could be detrimental to the survival of juveniles and adults.

5. Conclusion

In conclusion, the present study shows that Cd^{2+} 15 μM allows stage 45 *Xenopus* embryos survival. However, this dose has significant effects on major developmental points (significant decrease in length), added to physiological and behavioral dysfunctions (an increase in cardiovascular rhythm and swimming distance traveled, and a decrease in maximum swimming speed). In addition to these deleterious effects, our data demonstrate the significant decrease in the molecular neuronal 180 kDa NCAM form, associated with an increase in its PSA level. These results leave room for future studies on the role of Cd^{2+} in the development of aquatic animal species and the possibility of NCAM and PSA as possible biomarker of Cd^{2+} toxicity in amphibians and other freshwater species.

Funding sources

This work was supported by the CNRS and the University of Lille.

CRediT authorship contribution statement

Ingrid Fliniaux: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Guillaume Marchand:** Investigation, Writing – original draft, Methodology, Writing – review & editing. **Yoshiko Takeda Uchimura:** Investigation, Methodology, Writing – review & editing. **Pauline Titran:** Investigation. **Jean-François Bodart:** Conceptualization, Writing – original draft. **Katia Cailliau:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Anne Harduin Lepers:** Conceptualization, Writing – original draft. **Matthieu Marin:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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.

Data Availability

No data was used for the research described in the article.

Acknowledgements

We are sincerely indebted to A. Lescuyer (UMR-CNRS 8576) for her technical assistance, to Anne Dickes (PHexMAR), Léticia Micaletto (PHexMAR), and Corentin Spriet (TISBIO platform) for their help.

References

Balak, K., Jacobson, M., Sunshine, J., Rutishauser, U., 1987. Neural cell adhesion molecule expression in Xenopus embryos. Dev. Biol. 119, 540–550. https://doi.org/10.1016/0012-1606(87)90057-1.

Beketov, M.A., Kefford, B.J., Schäfer, R.B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. Proc. Natl. Acad. Sci. U. S. A. 110, 11039–11043. https://doi.org/10.1073/pnas.1305618110.

Blaustein, A.R., Kiesecker, J.M., 2002. Complexity in conservation: lessons from the global decline of amphibian populations Complexity in conservation: lessons from the global. Ecol. Lett. 5, 597–608. https://doi.org/10.1046/j.1461-0248.2002.00352.x.

Bonfanti, P., Colombo, A., Orsi, F., Nizzetto, I., Andrioletti, M., Bacchetta, R., Mantecca, P., Fascio, U., Vailati, G., Vismara, C., 2004. Comparative teratogenicity of chlorpyrifos and malathion on Xenopus laevis development. Aquat. tox 70, 189–200. https://doi.org/10.1016/j.aquatox.2004.09.007.

Bonfanti, L., Seki, T., 2021. The psa-ncam-positive "immature" neurons: an old discovery providing new vistas on brain structural plasticity. Cells 10. https://doi.org/10.3390/cells10102542.

Burlibasa, L., Gavrila, L., 2011. Amphibians as model organisms for study environmental genotoxicity. Appl. Eco. Env. Res. 9 (1), 1–15.

Cano-Barbacil, C., Radinger, J., Argudo, M., Rubio-Gracia, F., Vila-Gispert, A., García-Berthou, E., 2020. Key factors explaining critical swimming speed in freshwater fish: a review and statistical analysis for Iberian species. Sci. Rep. 10 (1), 12. https://doi.org/10.1038/s41598-020-75974-x.

Cunningham, B.A., Hemperly, J.J., Murray, B.E.N.A., Prediger, E.A., Brackenbury, R., Edelman, G.M., 1987. Neural Cell Adhesion Molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. Science 236 (80), 799–806.

Doherty, P., Cohen, J., Walsh, F.S., 1990. Neurite outgrowth in response to transfected N-CAM changes during development and is modulated by polysialic acid. Neuron 5, 209–219. https://doi.org/10.1016/0896-6273(90)90310-C.

Dudgeon, D., 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. Curr. Biol. 29, R960–R967. https://doi.org/10.1016/j.cub.2019.08.002.

Dudley, N., Alexander, S., 2017. Agriculture and biodiversity: a review. Biodiversity 18, 45–49. https://doi.org/10.1080/14888386.2017.1351892.

Fagotto, F., Maxfield, F.R., 1994. Changes in yolk platelet pH during Xenopus laevis development correlate with yolk utilization: a quantitative confocal microscopy study. J. Cell Sci. 107, 3325–3337. https://doi.org/10.1242/jcs.107.12.3325.

Flament, S., Kuntz, S., Chesnel, A., Grillier-Vuissoz, I., Tankozic, C., Penrad-Mobayed, M., Auque, G., Shirali, P., Schroeder, H., Chardard, D., 2003. Effect of cadmium on

gonadogenesis and metamorphosis in Pleurodeles waltl (urodele amphibian). 2003 Jul. 16;64(2):143-53. Aquat. Toxicol. 16, 143–153. https://doi.org/10.1016/s0166-445x(03)00042-0.

Gascon, E., Vutskits, L., Kiss, J.Z., 2007. Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. Brain Res. Rev. 56, 101–118. https://doi.org/10.1016/j.brainresrev.2007.05.014.

Giagtzoglou, N., Ly, C.V., Bellen, H.J., 2009. Cell adhesion, the backbone of the synapse: "vertebrate" and "invertebrate" perspectives. Cold Spring Harb. Perspect. Biol. 1, 1–25. https://doi.org/10.1101/cshperspect.a003079.

Herkovits, J., Cardellini, P., Pavanati, C., Perez-Coll, C.S., 1997. Susceptibility of early life stages of Xenopus laevis to cadmium. Environ. Toxicol. Chem. 16, 312–316. https://doi.org/10.1002/etc.5620160229.

Herkovits, J., Cardellini, P., Pavanati, C., Pérez-Coll, C.S., 1998. Cadmium uptake and bioaccumulation in Xenopus laevis embryos at different developmental stages. Ecotoxicol. Environ. Saf. 39, 21–26. https://doi.org/10.1006/eesa.1997.1586.

James, S.M., Little, E.E., 2003. The effects of chronic cadmium exposure on American toad (Bufo americanus) tadpoles. Environ. Toxicol. Chem. 22, 377–380. https://doi.org/10.1897/1551-5028(2003)022<0377:TEOCCE>2.0.CO;2.

Jensen, P.H., Soroka, V., Thomsen, N.K., Ralets, I., Berezin, V., Bock, E., Poulsen, F.M., 1999. Structure and interactions of NCAM modules 1 and 2, basic elements in neural cell adhesion. Nat. Struct. Biol. 6, 486–493. https://doi.org/10.1038/8292.

Jorgensen, P., Steen, J.A.J., Steen, H., Kirschner, M.W., 2009. The mechanism and pattern of yolk consumption provide insight into embryonic nutrition in Xenopus. Development 136, 1539–1548. https://doi.org/10.1242/dev.032425.

Knudsen, K.A., McElwee, S.A., Myers, L., 1990. A role for the neural cell adhesion molecule, NCAM, in myoblast interaction during myogenesis. Dev. Biol. 138, 159–168. https://doi.org/10.1016/0012-1606(90)90185-L.

Krieg, P.A., Sakaguchi, D.S., Kintner, C.R., 1989. Primary structure and developmental expression of a large cytoptasmic domain form of Xenopus laevis neural cell adhesion molecule (NCAM). Nucleic Acids Res. 17, 10321–10335. https://doi.org/10.1093/nar/17.24.10321.

Lee, W.K., Thévenod, F., 2020. Cell organelles as targets of mammalian cadmium toxicity. Archives of Toxicology. Springer Berlin Heidelberg,. https://doi.org/10.1007/s00204-020-02692-8.

Lu, H., Hu, Y., Kang, C., Meng, Q., Lin, Z., 2021. Cadmium-induced toxicity to amphibian tadpoles might be exacerbated by alkaline not acidic pH level. Ecotoxicol. Environ. Saf. 218, 112288 https://doi.org/10.1016/j.ecoenv.2021.112288.

Monastersky, R., 2014. Life-a status report. Nature 516, 159–161. https://doi.org/10.1038/516158a.

Mouchet, F., Baudrimont, M., Gonzalez, P., Cuenot, Y., Bourdineaud, J.P., Boudou, A., Gauthier, L., 2006. Genotoxic and stress inductive potential of cadmium in Xenopus laevis larvae. Aquat. Tox 78, 157–166. https://doi.org/10.1016/j.aquatox.2006.02.029.

Muller, D., Wang, C., Skibo, G., Toni, N., Cremer, H., Calaora, V., Rougon, G., Kiss, J.Z., 1996. PSA-NCAM is required for activity-induced synaptic plasticity, 17, 413–422.

Murray, B.A., Owens, G.C., Prediger, E.A., Crossin, K.L., Cunningham, B.A., Edelman, G. M., 1986. Cell surface modulation of the neural cell adhesion molecule resulting from alternative mRNA splicing in a tissue-specific developmental sequence. J. Cell Biol. 103, 1431–1439. https://doi.org/10.1083/jcb.103.4.1431.

Nieuwkoop, P.D., Faber, J., 1994. A Systematical and Chronological Survey of the Development from the Fertilized Egg till the End of Metamorphosis, Edited by J. Faber, P. D. Nieuwkoop, Garland Publishing Inc, New York ISBN 0-8153-1896-0.

Patar, A., Giri, A., Boro, F., Bhuyan, K., Singha, U., Giri, S., 2016. Cadmium pollution and amphibians - studies in tadpoles of Rana limnocharis. Chemosphere 144, 1043–1049. https://doi.org/10.1016/j.chemosphere.2015.09.088.

Pérez-Escudero, A., Vicente-Page, J., Hinz, R.C., Arganda, S., De Polavieja, G.G., 2014. IdTracker: tracking individuals in a group by automatic identification of unmarked animals. Nat. Methods 11, 743–748. https://doi.org/10.1038/nmeth.2994.

Peterson, R.H., Metcalfe, J.L., Ray, S., 1983. Effects of cadmium on yolk utilization, growth, and survival of Atlantic salmon alevins and newly feeding fry. Arch. Environ. Contam. Toxicol. 12, 37–44. https://doi.org/10.1007/BF01054999.

Prozialeck, W.C., Grunwald, G.B., Dey, P.M., Reuhl, K.R., Parrish, A.R., 2002. Cadherins and NCAM as potential targets in metal toxicity. Toxicol. Appl. Pharmacol. 182, 255–265. https://doi.org/10.1006/taap.2002.9422.

Rutishauser, U., 2008. Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. Nat. Rev. Neurosci. 9, 26–35. https://doi.org/10.1038/nrn2285.

Rutishauser, U., Acheson, A., Hall, A., Mann, D., Sunshine, J., 1988. Polysialic Acid as a regulator of cell interactions. Science 240 (80), 53–57. https://doi.org/10.1007/978-1-4757-5955-6_12.

Sadoul, R., Hirn, M., Deagostini-Bazin, H., Rougon, G., C., G., 1983. Adult and embryonic mouse neural cell adhesion moleucles have differnt binding properties. Nature 304, 347–349.

Santoyo-Sánchez, M.P., Pedraza-Chaverri, J., Molina-Jijón, E., Arreola-Mendoza, L., Rodríguez-Muñoz, R., Barbier, O.C., 2013. Impaired endocytosis in proximal tubule from subchronic exposure to cadmium involves angiotensin II type 1 and cubilin receptors. BMC Nephrol. 14 https://doi.org/10.1186/1471-2369-14-211.

Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392. https://doi.org/10.1016/j.aquatox.2004.03.016.

Sedmera, D., Reckova, M., DeAlmeida, A., Sedmerova, M., Biermann, M., Volejnik, J., Sarre, A., Raddatz, E., McCarthy, R.A., Gourdie, R.G., Thompson, R.P., 2003. Functional and morphological evidence for a ventricular conduction system in zebrafish and Xenopus hearts. Am. J. Physiol. - Hear. Circ. Physiol. 284, 1152–1160. https://doi.org/10.1152/ajpheart.00870.2002.

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. https://doi.org/10.1016/j.envres.2014.12.014.

Sharma, B., Patiño, R., 2008. Exposure of Xenopus laevis tadpoles to cadmium reveals concentration-dependent bimodal effects on growth and monotonic effects on development and thyroid gland activity. Toxicol. Sci. 105, 51–58. https://doi.org/10.1093/toxsci/kfn119.

Shen, J., Wang, X., Zhou, D., Li, T., Tang, L., Gong, T., Su, J., Liang, P., 2018. Modelling cadmium-induced cardiotoxicity using human pluripotent stem cell-derived cardiomyocytes. J. Cell. Mol. Med. 22, 4221–4235. https://doi.org/10.1111/jcmm.13702.

Singh, A., Sharma, A., Verma, Rohit, K., Chopade, R.L., Pandit, P.P., Nagar, V., Aseri, V., Choudhary, S.K., Awasthi, G., Awasthi, K.K., Sankhla, M.S, 2022. Heavy metal contamination of water and their toxic effect on living organisms, in: The Toxicity of Environmental Pollutants, Edited by Daniel Junqueira Dorta and Danielle Palma de Oliveira. Intech.

Slaby, S., Marin, M., Marchand, G., Lemiere, S., 2019. Exposures to chemical contaminants: what can we learn from reproduction and development endpoints in the amphibian toxicology literature? Env. Pollut. 248, 478–495. https://doi.org/10.1016/j.envpol.2019.02.014.

Sunderman Jr, F.W., 1991. Plowman MC, Hopfer SM. Embryotoxicity and teratogenicity of cadmium chloride in Xenopus laevis, assayed by the FETAX procedure. Ann. Clin. Lab Sci. 21 (6), 381–391.

Sunderman Jr, F.W., 1992. Plowman MC, Hopfer SM. Teratogenicity of cadmium chloride in the South African frog, Xenopus laevis. IARC Sci. Publ. 118, 249–256.

Sunshine, J., Balak, K., Rutishauser, U., Jacobson, M., 1987. Changes in neural cell adhesion molecule (NCAM) structure during vertebrate neural development. Proc. Natl. Acad. Sci. U. S. A. 84, 5986–5990. https://doi.org/10.1073/pnas.84.16.5986.

Thévenod, F., 2009. Cadmium and cellular signaling cascades: to be or not to be? Toxicol. Appl. Pharmacol. 238, 221–239. https://doi.org/10.1016/j.taap.2009.01.013.

Weinhold, B., Seidenfaden, R., Röckle, I., Mühlenhoff, M., Schertzinger, F., Conzelmann, S., Marth, J.D., Gerardy-Schahn, R., Hildebrandt, H., 2005. Genetic ablation of polysialic acid causes severe neurodevelopmental defects rescued by deletion of the neural cell adhesion molecule. J. Biol. Chem. 280, 42971–42977. https://doi.org/10.1074/jbc.M511097200.

Weledji, E.P., Assob, J.C., 2014. The ubiquitous neural cell adhesion molecule (N-CAM). Ann. Med. Surg. 3, 77–81. https://doi.org/10.1016/j.amsu.2014.06.014.

Yang, Y., Murai, R., Takahashi, Y., Mori, A., Hane, M., Kitajima, K., Sato, C., 2020. Comparative studies of polysialic acids derived from five different vertebrate brains. Int. J. Mol. Sci. 21 (22), 1. https://doi.org/10.3390/ijms21228593.