PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

GUSTAVO NASCENTE IGANSI

EFEITO DA EXPOSIÇÃO AO CÁDMIO SOBRE DANO OXIDATIVO, MORTE CELULAR E COMPORTAMENTO DE ZEBRAFISH

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

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RESUMO

O cádmio é considerado a sétima substância mais perigosa presente no ambiente e é classificado como carcinogênico tipo I, potencialmente afetando uma grande quantidade de seres vivos, incluindo os humanos. Ademais, o cádmio tem sido associado a defeitos neurocomportamentais que podem comprometer o status ecológico e a sobrevivência de animais. Apesar do potencial impacto, a compreensão dos mecanismos celulares e moleculares por trás de seus efeitos deletérios no comportamento de animais é ainda escassa. Este estudo teve por objetivo avaliar os efeitos comportamentais do cádmio por 1 e 7 dias a concentrações relevantes no ambiente (10 µg/L, 100 µg/L and 1000 µg/L) em *zebrafish* e analisar o estresse oxidativo e marcadores de apoptose no encéfalo. Animais expostos ao cádmio apresentaram aumento na atividade locomotora após 1 e 7 dias de tratamento em todas as concentrações e parâmetros avaliados, incluindo distância percorrida num período de 5 minutos, velocidade média e períodos móveis. A hiperlocomoção afetou significativamente o desempenho dos animais em explorar um novo ambiente em todos os grupos tratados, evidenciado por uma diminuição na eficiência de percurso e alterada distribuição na coluna d'água. Adicionalmente, nossos resultados confirmaram estudos prévios sobre aumento no dano oxidativo em peixes quando expostos ao cádmio e especialmente demonstraram níveis mais elevados de dano a proteínas em amostras de encéfalo em animais tratados a 100 µg/L por 1 dia e a 10 µg/L por 7 dias quando comparados aos seus respectivos controles. Lipoperoxidação aumentou significativamente no encéfalo de animais expostos por 1 dia a 100 µg/L. Análises dos marcadores p53 e bax por Real-time PCR apresentaram nenhuma alteração após 1 dia de exposição, mas significativamente aumentaram após 7 dias. Nossos resultados apresentam evidências dos efeitos deletérios do cádmio no comportamento de *zebrafish* e chama atenção para o fato de que a manifestação de seus efeitos aparece a partir de 1 dia de exposição a 10 µg/L, uma concentração aceita por muitas agências de regulamentação internacional.

ABSTRACT

Cadmium is considered the seventh most dangerous substance in the environment and is classified as carcinogen type I, potentially affecting a wide range of living organisms, including humans. In addition to its wide systemic impact and potential lethality, cadmium has been associated to neurobehavioral defects that may also compromise animals' ecological status and survival. Despite its potential impact, the comprehension of cellular and molecular mechanisms underlying cadmium deleterious effects on animals' behavior is still scarce. This study aimed to evaluate the behavioral effects of cadmium for 1 or 7 days at environmentally relevant concentrations (10 µg/L, 100 µg/L and 1000 µg/L) on zebrafish and to analyze brain oxidative stress and apoptotic markers. Cadmium-exposed zebrafish exhibited a generalized increase in locomotor activity after 1 and 7 days of treatment at all doses in all parameters evaluated, including distance travelled in a 5-min. evaluation period, mean speed and mobile periods. This hyperlocomotory effect significantly compromised animals' general performance in exploring a new environment, which was evident in all cadmium exposed animals decreased path efficiency and altered distribution on the water column. Additionally, our results confirmed previous reports of increased oxidative damage in fishes exposed to cadmium and specifically demonstrated higher levels of damaged proteins in brain samples of animals exposed to cadmium at 100 µg/L for 1 day and at 10 µg/L for 7 days when compared to their respective control groups. Lipid peroxidation was also significantly increased in animals' brain after 1 day cadmium exposure at 100 µg/L. Real-Time PCR analysis of transcripts for p53 and bax were not altered after 1 day cadmium exposure, but significantly increased after 7 days. Our results present evidence of cadmium deleterious effects on zebrafish cognitive functions and raise attention to the fact that its manifestation appears already after a one day exposure to 10 µg/L, a concentration accepted by most international regulating agencies.

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CAPÍTULO 1

1. INTRODUÇÃO

1.1 - Ecotoxicologia de Metais Pesados

A ação antrópica tem gerado uma grande variedade de substâncias que atuam diretamente sobre o meio ambiente, incluindo, por exemplo, herbicidas, pesticidas, detergentes, metais pesados e substâncias químicas de natureza diversa. A capacidade do homem de explorar e manipular metais proporcionou um importante marco no desenvolvimento de nossa sociedade (Wilson,1996). Os metais, em especial metais pesados, possuem efeitos negativos quando liberados em elevadas concentrações no meio ambiente (Han *et al*., 2002), sendo classificados como poluentes e causando impactos tanto na saúde humana (Duruibe *et al*., 2007) quanto em ecossistemas aquáticos e terrestres (Sánchez, 2008).

A maior parte dos estudos com metais pesados, como o cádmio, o mercúrio e o cobre, atenta para os efeitos negativos diretos sobre os organismos, ou seja, na sintomatologia toxicológica e mortalidade (Lefcort *et al*., 2002), enquanto pouco apontam os efeitos indiretos, por exemplo, sobre a estrutura de comunidades e de cadeias alimentares (Fleeger *et al*., 2003; Sloman, 2007). Ademais, há muitos estudos a respeito dos efeitos sobre alterações no comportamento e adaptações de organismos, que vão desde procariotos e eucariotos basais (Lass & Spaak, 2003; Challis, 2005; Schertzer *et al.*, 2009) até plantas e animais (Koricheva *et al.*, 1998; Greger, 2004; McPherson *et al*., 2004; Lürling & Scheffer, 2007; Klaschka, 2008; Liu *et al.*, 2009).

Apesar dos conhecidos efeitos negativos dos metais pesados sobre o comportamento animal, os mecanismos através dos quais os metais impactam as funções cerebrais superiores não são conhecidos, especialmente do ponto de vista celular e molecular. Esta informação, quando disponível, permitirá o melhor entendimento de seus impactos e potencialmente proporcionará substratos para estratégias terapêuticas e preventivas dos efeitos deletérios duradouros que tais elementos têm sobre o sistema nervoso central. Finalmente, Boyd (2010) alerta que para se entender o comportamento de organismos, devem ser considerados os múltiplos fatores interligados que

atuam sobre eles, como disponibilidade de recursos, estresse, condições ambientais entre outros, tornando o seu estudo realista.

1.2 – Ecotoxicologia do Cádmio

O Cádmio (Cd) é um elemento natural encontrado na crosta terrestre e geralmente está associado a outros metais, como o zinco, o cobre e o chumbo (Cardoso & Chasin, 2001). Sua concentração no ambiente varia em cada meio conforme indicado na Tabela 1**.** No Brasil, a concentração máxima permitida em água doce é de 10 µg/L (CONAMA, 2005). Sua principal fonte natural é a atividade vulcânica, seguida da exploração de minérios. Ele é amplamente utilizado pela indústria, principalmente: (1) no recobrimento de aço e ferro (parafusos, porcas, máquinas industriais); (2) como pigmento para plástico e vidro; (3) em baterias de níquel-cádmio, presentes principalmente em celulares; (4) em ligas metálicas.

Tabela 1. Níveis considerados naturais de Cd no ambiente.

MEIO	CONCENTRAÇÃO	REFERÊNCIAS
Atmosfera	0,1 a 4 ng/m^3 0,01 a 0,04 ng/m^3 (áreas remotas) 90 ng/m^3 (aerodispersóides junto ao vulcão Monte Etna)	WHO, 1992
Crosta Terrestre	0,1 a 0,4 ppm 0,01 a 1,0 ppm	WHO, 1992 ATSDR, 1997
Solos Vulcânicos	4,5 ppm	ATSDR, 1997
Rochas Sedimentares	Até 15 ppm	WHO, 1992
Fosfatos Marinhos	~1 ppm	CÁDMIUM ASSOCIATION, 2001
Sedimentos Marinhos	0,1 a 1,0 ppm	ATSDR, 1997
Água do Mar	~0,1 µg/L <0,05 ng/L (água superficial) 0,02 – 0,1 µg/L	CADMIUM ASSOCIATION, 2001 WHO, 1992 ATSDR, 1997
Água doce	0,01 – 0,06 ng/L ~0,1 µg/L	WHO, 1992 ATSDR, 1997
Gelo	5 pg/g (Ártico)/ 0,3 pg/g (Antártico)	WHO, 1992

Siglas: WHO – World Health Organization; ATSDR – Agency for Toxic Substances and Disease Registry
Fonte: CARDOSO; CHASIN, 2001, p. 28.

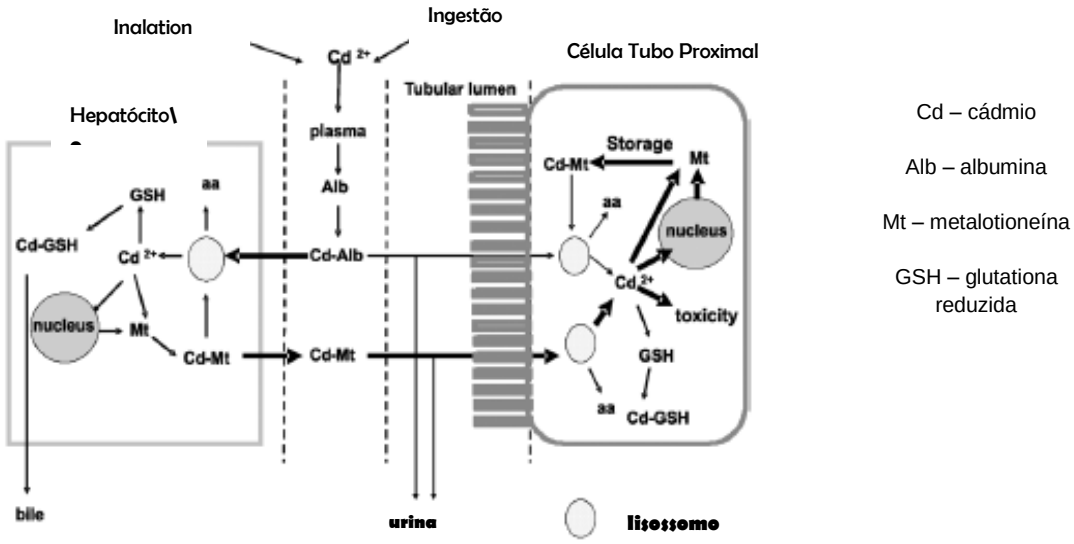
A contaminação do ambiente por Cd ocorre por fontes naturais, como as erupções vulcânicas (WHO, 1992), ou por atividade antrópica. A mineração, a produção industrial e o descarte de resíduos urbanos e industriais não tratados que contenham cádmio contaminam o ambiente (Teves, 2001). As fontes de contaminação das águas incluem a atividade de mineração de metais não ferrosos, fundições de minério não ferroso, extração de rochas fosfatadas e manufatura de fertilizantes fosfatados e a acidificação de solos e lagos (WHO, 1992 e 1998). O Cd proveniente de indústrias é rapidamente adsorvido e acumula-se no sedimento (Calmano & Förstner, 1996), associando-se a carbonatos e formando depósitos que contaminam o meio aquático e o solo entorno (WHO, 1992). No sedimento, o Cd pode sofrer ação de bactérias, que o transformam em compostos orgânicos tóxicos como, por exemplo, dimetilcádmio (Yannai & Berdicevsky, 1995). Dependendo das condições físico-químicas do ambiente, estes compostos são liberados do sedimento e podem entrar em contato com outros organismos, comprometendo toda a cadeia alimentar (Calmano & Förstner, 1996). Em água doce, o Cd está presente na forma Cd^{+2}, hidróxido de cádmio e carbonato de cádmio (ATSDR, 1997).

Cd bioacumula-se no fitoplâncton e, através de complexas teias alimentares, em animais aquáticos, tais como moluscos, peixes e crustáceos. Peixes possuem a capacidade de bioacumular Cd principalmente nas brânquias e paredes intestinais, além de outros órgãos como fígado e rins (Cardoso & Chasin, 2001). Além disso, o Cd, ao passar pelas brânquias, é transportado para as células através de vias de transporte de Ca^{+2} (Sloman *et al*., 2007). Em resposta à contaminação, o peixe passa a expressar a proteína metalotioneína (MT), que se liga ao metal, inativando-o, embora esta estratégia tenha sua eficiência limitada e dependente das concentrações do metal (De Conto Cinier *et al*., 1998).

A dieta é a principal fonte de contaminação no homem, e além dos organismos aquáticos citados acima, outros alimentos são importantes fontes potenciais de contaminação, como óleos de sementes, cereais, vegetais e raízes (Järup & Akesson, 2009). Mais de 80% do Cd em humanos vem de cereais e vegetais (Olsson *et al*., 2002), e a concentração média de ingestão diária varia de 8 a 25 µg por dia em um indivíduo adulto (Berglund *et al*., 1994;

MacIntosh *et al*.,1996; Thomas *et al*., 1999; Ysart *et al*., 2000; Larsen *et al*., 2002; Olsson *et al*., 2002; Llobet *et al*., 2003; Egan *et al*., 2007). A fumaça de tabaco é outra fonte importante de contaminação humana, sendo que crianças são mais suscetíveis (Willers *et al*., 2005; Arora *et al*., 2008), enquanto que adultos não são afetados. A Figura 1 ilustra as vias de contaminação do Cd através da ingestão alimentar ou inalação pelas vias respiratórias. No sistema nervoso, a barreira hematoencefálica oferece resistência fisiológica à passagem do Cd (Takeda *et al*., 1999), mas níveis crônicos aumentam a sua concentração no cérebro, principalmente no bulbo olfatório de peixes (Shukla *et al*., 1996). Os mecanismos moleculares responsáveis pelo transporte do Cd para o cérebro ainda permanecem obscuros (Bondier *et al*., 2008).

Figura 1. Esquema ilustrando as vias de absorção e locais de acúmulo.



Siglas: Cd – cádmio; Alb – albumina; Mt – metalotioneína; GSH – glutationa reduzida.

Fonte: BERNARND; 2008.

Alguns acidentes ambientais graves com Cd foram relatados, sendo o principal no Japão após a Segunda Guerra Mundial (Cardoso & Chasin, 2001). Plantadores de arroz e pescadores foram expostos ao metal devido ao consumo de arroz contaminado por água de irrigação proveniente de efluentes de uma indústria processadora de zinco e chumbo. Os principais sintomas nos pescadores e plantadores foram dores reumáticas, mialgias, deformidades ósseas e distúrbios renais (Cardoso & Chasin, 2001). Posteriormente, a

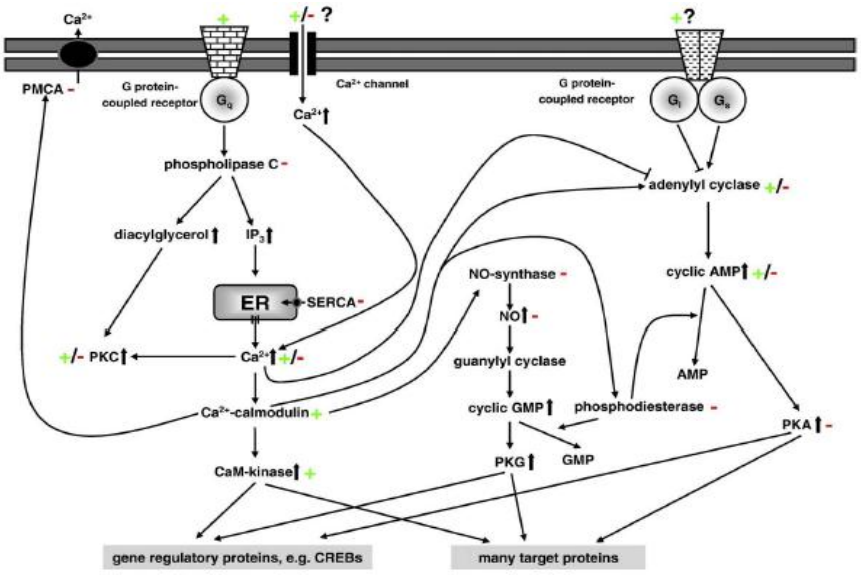
sintomatologia foi chamada de Itai-Itai (ouch-ouch). Além destes, outros sintomas foram relacionados à exposição do Cd em outros tecidos, como: (1) decréscimo da função pulmonar e enfisema; (2) aumento da pressão arterial e doenças cerebrovasculares; (3) anemia; (4) comprometimento dos ossos; (5) cálculos renais e danos nos túbulos renais (WHO, 1992; ATSDR, 1997).

1.3 – Cádmio e sua Interação com Vias de Sinalização Intracelular

Estudos têm sido publicados demonstrando a toxicologia do Cd e sua ação nos diversos processos celulares e moleculares. Porém, a forma como o Cd atua, as vias nas quais ele está envolvido e seus alvos extra e intracelulares ainda são poucos conhecidos. Ademais, muitas destas vias se interconectam e são extremamente complexas, tornando o estudo de efeitos toxicológicos desafiadores (revisado por Thévenod, 2009).

A figura 2 exemplifica algumas rotas de sinalização intracelular com as quais o Cd pode interagir e desta forma podem estar relacionadas com os efeitos deletérios da exposição a este metal.

Figura 2. Cádmio e vias de sinalização intracelular. Efeitos estimulatórios (+) e inibitórios (-) sobre segundos mensageiros e moduladores de transdução são indicados, mostrando a complexa relação entre as vias (*cross-talk*).



ER – retículo endoplasmático

IP_3 – trifosfato de inositol

PKC/PKA/PKG – proteína cinase C/A/G

Ca^+ - Cálcio

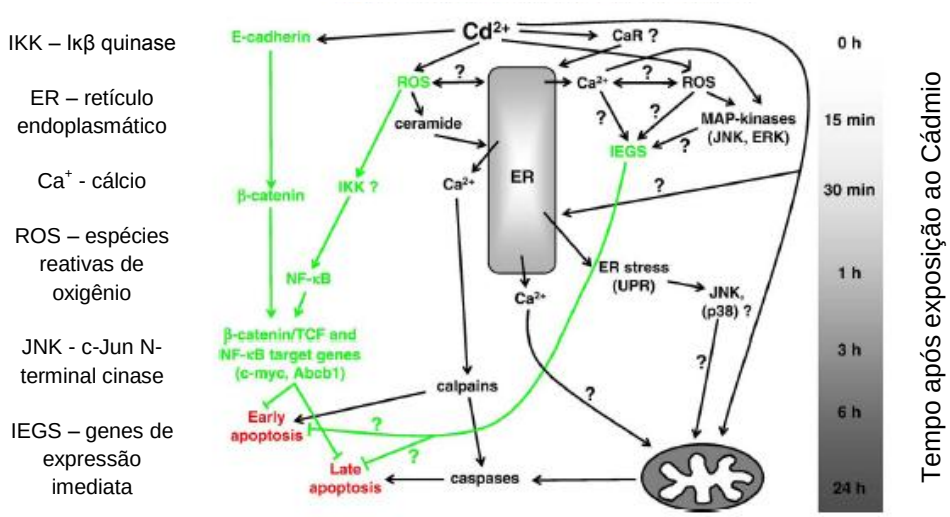
NO – óxido nítrico

Fonte: THÉVENOD; 2009.

Por exemplo, Cd altera os níveis de Ca^{+2} nas células, afetando diversas vias, principalmente a ativação/inibição de proteínas cinases (Mazzei *et al*., 1984; Kostrzewska & Sobieszek, 1990; Chao *et al*., 1995; Liu & Templeton, 2007). Ademais, Thévenod (2009) apresenta uma excelente revisão sobre as vias do cAMP, oxido nítrico, NF-*k*B, entre outras, apontando contradições nas evidências disponíveis e perspectivas futuras de investigação da influência do Cd nestas vias.

O Cd induz apoptose em vários tipos de células, modulando a atividade de proteínas cinases, fosfatases e fatores de transcrição (revisado por Thévenod, 2009), como exemplificado na figura 3. A formação de espécies reativas de oxigênio (ERO's), através do esgotamento e inibição de enzimas antioxidantes (glutationa, superóxido dismutase, entre outras) e inibição da cadeia transportadora de elétrons induzida pelo Cd também já foram descritas (revisado por Thévenod, 2009). Nas mitocôndrias, organelas-alvo do metal, a inibição da cadeia transportadora de elétrons acarreta a um acúmulo de semi-ubiquinonas, que são instáveis e transferem um elétron ao oxigênio molecular, formando superóxido e, consequentemente, ERO (revisado por Thévenod, 2009).

Figura 3. Envolvimento do Cd em várias vias de sinalização intracelular anti e pró-apoptóticas. A seta "→" indica estímulo, enquanto que "┤", inibição. É notável salientar que várias destas vias se interconectam (*cross-talk*).



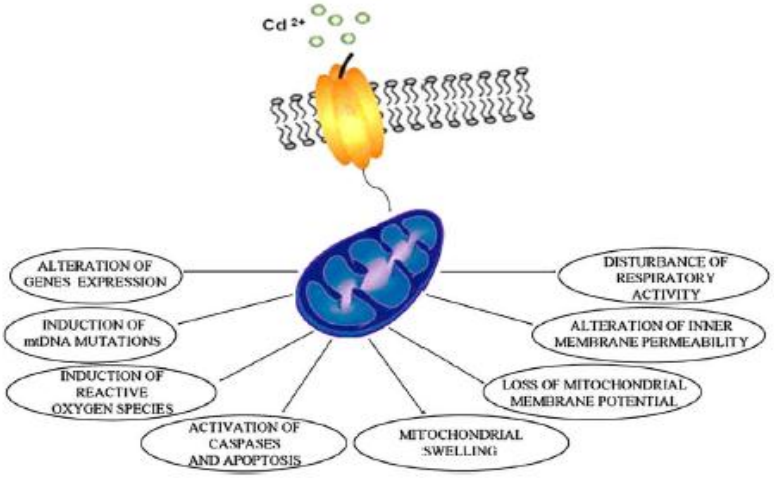
Fonte: THÉVENOD, 2009.

Ademais, Cd não é considerado um metal que participa nas reações de Fenton, mas ele afeta proteínas de membrana e citoplasmáticas que

contenham outros metais, a exemplo do ferro. Este último, quando livre no interior da célula, é capaz de formar ERO através da quebra de H_2O_2, formando o radical OH (revisado por Matovic *et al*, 2011). Por fim, exposição a longo prazo ao metal pode produzir mediadores inflamatórios, como IL-6 e IL-8, que induzem a produção de ERO (revisado por Matovic *et al*, 2011).

O acúmulo de ERO afeta o potencial de membrana da mitocôndria e ativa uma sequência de eventos moleculares. Ademais, o excesso de ERO pode levar a oxidação de macromoléculas, acarretando a lipoperoxidação e induzindo mutações no DNA mitocondrial e despolarização da membrana desta organela, culminando em morte celular por apoptose (revisado por Cannino *et al*, 2009). Os efeitos do cádmio sobre a mitocôndria são mostrados na figura 4.

Figura 4. Esquema geral sobre as consequências da intoxicação pelo Cádmio na mitocôndria.



Fonte: CANNINO *et al*, 2009.

Apoptose é um mecanismo de morte celular importante para o controle de populações celulares em condições fisiológicas e patológicas. A p53, uma fosfoproteína tetrâmera com tempo de vida entre 10-30 minutos, é considerada uma das principais proteínas regulando a apoptose iniciada por fatores relacionados com ciclo celular (Sherr *et al*, 2002; Hofseth *et al*, 2004). Estresse oxidativo e excitotoxicidade podem causar danos ao DNA e, consequentemente, ativam p53, podendo ou induzir morte celular por meio de apoptose ou ativar respostas adaptativas ao estresse, dependendo do tipo de estresse, do tipo celular que sofre o estresse e o quão longo as células ficam expostas ao dano (Joers *et al*, 2004). Ao ser fosforilada, p53 acumula-se na

célula e começa a transcrever genes específicos para morte celular, tais como Bax, NOXA, PUMA e Apaf-1 (Fridman and Lowe, 2003; Slee *et al*, 2004; Ward *et al*, 2004). Bax é uma proteina capaz de formar canais em membranas. Uma vez expressa, Bax é translocada para mitocôndria, formando poros. Este processo perturba o potencial de membrana da organela, induzindo a liberação de citocromo *c* (Dargusch *et al*, 2001; Polster & Fiskum, 2004). O citocromo *c*, a apaf-1 e a caspase-9 formam o apoptosoma, que induz apoptose ao ativar caspases efetoras, como a caspase-3 (Jayanthi *et al,* 2004). Vários estudos têm mostrado que o Cd não apenas possui a habilidade de induzir morte celular em vário tipos celulares, incluindo encéfalo e brânquias de peixes (Azzouqiel *et al,* 1994; Habeebu *et al*, 1998; Dong *et al*, 2001; López *et al*, 2003, Liu *et al*, 2011) mas também é capaz de induzir desordens neurodegenerativas em humanos, como Doença de Parkinson (O'Callaghan and Miller, 1986; Okuda *et al*, 1997) e esclerose lateral amiotrófica (Bar-Sela et al., 2001).

Por fim, Cd ocupa o sétimo lugar entre as substâncias mais perigosas (ATSDR, 2007) e é classificado como carcinogênico tipo 1 (IARC, 1993), estando associado ao câncer de pulmão, rim, próstata e pâncreas (Bernard & Lauwerys, 1984; Foulkes, 1986; Friberg *et al.*, 1986; Morselt, 1991; Goering *et al.*, 1995; Jarup *et al.*, 1998).

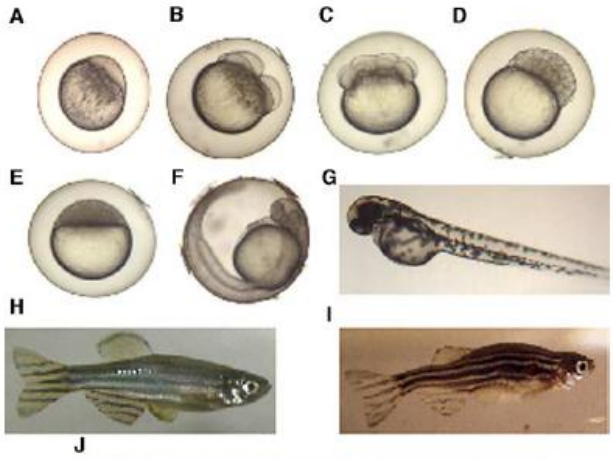
1.4 – Zebrafish como Modelo Animal

Diversos animais são utilizados para testes toxicológicos, incluindo desde invertebrados, como *Caenorhabditis elegans* e *Drosophila melanogaster*, até mamíferos, como ratos e primatas. Mamíferos sempre foram utilizados para avaliar os efeitos de tais substâncias devido a sua semelhança aos humanos. O grande número de substâncias tóxicas produzidas e liberadas pelo homem no ambiente, impactando todos os componentes dos ecossistemas, tem desviado a visão antropocêntrica do impacto de substâncias liberadas no meio, valorizando o uso de outros animais como indicadores. Adicionalmente, questões éticas quanto ao uso de animais na pesquisa e recursos limitados para manutenção e cuidados tornaram o uso de mamíferos menos atrativo. Recentemente, novos animais modelos vertebrados com conhecimento

genético e fisiológico tem sido propostos, e dentre eles tem se destacado o *zebrafish* (revisado por Peterson *et al*., 2008).

O *Zebrafish* (*Danio rerio*) é um peixe teleósteo de água doce tropical nativo da Índia (Figura 5). Ele tem sido amplamente utilizado para testes toxicológicos, farmacêuticos e terapêuticos, mutagênicos, além de estudos de desenvolvimento, envelhecimento e neurodegeneração (Spence et al., 2008). Dentre as características que vem ampliando o uso deste animal modelo estão: (1) o seu tamanho diminuto (3-5 cm quando adultos), permitindo fácil manipulação; (2) a absorção de substâncias que são adicionadas diretamente na água; (3) o baixo custo para criação e manutenção; (4) e o alto grau de similaridade com mamíferos quando comparado com outros modelos alternativos (Peterson *et al*., 2008). Além disso, as semelhanças entre o cérebro em desenvolvimento embrionário humano e de *zebrafish* tornam-no muito atraente em estudos para compreender o desenvolvimento do sistema nervoso (Tropepe & Sive, 2003). Por fim, a morfologia cerebral de *zebrafish* é muito parecida com a dos outros vertebrados, sugerindo que os mecanismos de morfogênese seguem um padrão evolutivo conservado (Tropepe & Sive, 2003).

Figura 5. Desenvolvimento em *zebrafish*. (A) embrião em estágio de 1 única célula; (B) estágio de 4 células; (C) estágio de 8 células; (D) estágio de aproximadamente 64-128 células; (E) estágio de blástula em 3 horas pós-fertilização - hpf; (F) 24 hpf; (G) 48 hpf eclodido; (H) macho adulto com 8 meses; (I) Idoso de 52 meses.



Fonte: GERHARD; 2003, p. 1334.

Estudos anteriores sobre o efeito do Cd em *zebrafish* concentraram-se em avaliar o efeito desde metal sobre o desenvolvimento inicial (Chan & Cheng, 2003; Chow et al., 2008; Chow *et al*., 2009). Poucos são os estudos que discutem a ação do cádmio em peixes adultos, e praticamente não há trabalhos que caracterizem os efeitos comportamentais e cognitivos do Cd em *zebrafish.*

2 - JUSTIFICATIVA

Considerando os pontos apresentados até aqui, acreditamos ser relevante utilizar o *zebrafish* como modelo para avaliar os efeitos da exposição ao Cd na vida adulta sobre parâmetros comportamentais e tentar identificar as vias celulares envolvidas nos eventuais efeitos desta exposição. Muito recentemente, estudos começaram a ser direcionados para a integração entre o ambiente no qual os animais estão e os diversos poluentes que interagem com eles e as implicações na fisiologia e vias de sinalização. Isto nos oferece um grande espectro para melhor entendermos como, de fato, as interações ocorrem e que são múltiplos os fatores que atuam nelas. Portanto, esta dissertação tem por finalidade caracterizar o efeito da exposição ao cádmio por 1 dia e 7 dias sobre o comportamento, dano tecidual e interação com as vias de apoptose.

3 – OBJETIVOS

3.1 – Objetivo Geral

Caracterizar o efeito da exposição ao cádmio por 1 ou 7 dias sobre o comportamento, dano tecidual e via de morte celular em *zebrafish* adultos.

3.2 – Objetivos Específicos

- Avaliar parâmetros comportamentais em animais adultos tratados com Cd por 1 ou 7 dias através de avaliação da capacidade exploratória;

- Avaliar o dano oxidativo ocasionado pela exposição ao Cd no encéfalo de animais adultos tratados por 1 ou 7 dias através da mensuração de Carbonil e TBARS;
- Avaliar a morte celular por apoptose no encéfalo de animais adultos tratados com Cd por 1 ou 7 dias através da alteração nos níveis de expressão de p53 e bax por Real-Time PCR.

CAPÍTULO 2

ARTIGO CIENTÍFICO A SER SUBMETIDO PARA “AQUATIC TOXICOLOGY”

Título: Cadmium exposure at environmentally relevant concentrations compromises zebrafish behavior and induces cell damage and apoptosis in the brain

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Cadmium exposure at environmentally relevant concentrations compromises zebrafish behavior and increases oxidative cell damage and apoptosis markers in the brain

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Abstract

Cadmium is considered the seventh most dangerous substance in the environment and is classified as carcinogen type I, potentially affecting a wide range of living organisms, including humans. In addition to its wide systemic impact and potential lethality, cadmium has been associated to neurobehavioral defects that may also compromise animals' ecological status and survival. Despite its potential impact, the comprehension of cellular and molecular mechanisms underlying cadmium deleterious effects on animals' behavior is still scarce. This study aimed to evaluate the behavioral effects of cadmium for 1 or 7 days at environmentally relevant concentrations (10 µg/L, 100 µg/L and 1000 µg/L) on zebrafish and to analyze brain oxidative stress and apoptotic markers. Cadmium-exposed zebrafish exhibited a generalized increase in locomotor activity after 1 and 7 days of treatment at all doses in all parameters evaluated, including distance travelled in a 5-min. evaluation period, mean speed and mobile periods. This hyperlocomotory effect significantly compromised animals' general performance in exploring a new environment, which was evident in all cadmium exposed animals decreased path efficiency and altered distribution on the water column. Additionally, our results confirmed previous reports of increased oxidative damage in fishes exposed to cadmium and specifically demonstrated higher levels of damaged proteins in brain samples of animals exposed to cadmium at 100 µg/L for 1 day and at 10 µg/L for 7 days when compared to their respective control groups. Lipid peroxidation was also significantly increased in animals' brain after 1 day cadmium exposure at 100 µg/L. Real-Time PCR analysis of transcripts for p53 and bax were not altered after 1 day cadmium exposure, but significantly increased after 7 days. Our results present evidence of cadmium deleterious effects on zebrafish cognitive functions and raise attention to the fact that its manifestation appears already after a one day exposure to 10 µg/L, a concentration accepted by most international regulating agencies.

Keywords: Cadmium, behavior, oxidative stress, apoptosis, zebrafish

1. INTRODUCTION

Cadmium is a heavy metal found naturally in the crust, considered one of the most toxic substances in the environment and has no known biological function in animals (ATSDR, 1997; Soares *et al*, 2008). Environmental contamination occurs through either natural sources (WHO, 1992) or anthropogenic action (reviewed by Bernard, 2008). Aquatic ecosystems are especially vulnerable (Bhakta *et al*, 2008) and fish tend to accumulate metals on tissues such as gills, liver and kidneys (Cinier *et al*, 1999; Soares *et al*, 2008). Humans can be exposed to cadmium from different sources, including contaminated fish diet or accidental occupational exposure, with significant deleterious effects even at trace quantities (Bernard, 2008; Järup and Akesson, 2009).

Previous reports have shown evidences of the deleterious effects of cadmium exposure to brain functions in fish (reviewed by Atchison, 1987; Giusi *et al*, 2005; Scott and Sloman, 2004). Gills and olfactory rosettes are considered routes of cadmium uptake, since both structures are directly exposed to the environment. Cadmium is able to enter gills chloride cells through calcium channels (Verbost *et al*, 1987, 1989) being subsequently transported to other organs such as liver and kidneys (Szebedinsky *et al*, 2001). Cadmium crosses the olfactory epithelium and is transported through the olfactory nerves' axons, reaching the olfactory bulb. (Gottofrey and Tjälve, 1991; Tjälve and Gottofrey, 1986; Tjälve and Henriksson, 1999). Cadmium transportation to other areas of the central nervous system remains unclear (Bondier*et al*, 2008) but it reaches the striatum in rodents and humans (Fernández-Pérez *et al*, 2010; Okuda *et al*, 1997;) and telencephalic and mesencephalic areas of fish brain (Giusi*et al*, 2005).The fish and mammalian blood-brain barrier offers resistance to cadmium entrance in the brain but the olfactory route has no influence of it, being this route considered a pathway to cadmium neurotoxicity (Takeda *et al*., 1999; Tallkvist, 2002). Several studies have quantified cadmium accumulation on specific fish organs after acute and chronic exposures, demonstrating its accumulation in gills, brain and muscles (Gonzalez *et al*, 2006; Kargin and Çogun, 1999; Pretto *et al*, 2010; Rashed, 2001).

Cadmium is known to induce reactive oxygen species (ROS) in a wide range of tissues, such as brain (Kumar *et al*, 1996), gills (Pretto *et al*, 2011) and muscle (Yano *et al*, 2005) through depletion and inhibition of antioxidant enzymes, disturbances on the electron transfer chain and competition with other ions (e.g. calcium, zinc) and therefore culminating in membrane permeability alterations (reviewed by Thévenod, 2009). High levels of ROS may

induce damage of macromolecules including proteins, DNA and lipids, depolarization of mitochondrial membrane and, finally, apoptosis (reviewed by Cannino *et al*, 2009).

DNA damage caused by oxidative stress or excitotoxicity can trigger cell cycle arrest and apoptosis. The phosphoprotein p53 may induce cell death through apoptosis depending on the stress and cellular type (Joers *et al*, 2004), by activation of specific genes, including Bax (Fridman and Lowe, 2003; Slee *et al*, 2004; Ward *et al*, 2004). Once expressed, Bax is translocated to mitochondria, forming pores, disrupting mitochondrial membrane potential, releasing cytochrome *c* and triggering caspase activation and apoptosome formation (Dargusch *et al*, 2001; Jayanthi *et al*, 2004; Polster and Fiskum, 2004).

Fish have been used as excellent biomarkers for toxicological studies, since their ecological behaviors are easily observed and monitored (Scott and Sloman, 2004). Zebrafish (*Danio rerio)* is a fresh water teleost employed in many areas to different studies, including toxicology (Komjarova and Blust, 2009) and behavior (Piato *et al*, 2011). In addition to its prominent advantages, including fast development and low cost, zebrafish brain is not only neuroanatomically and functionally comparable to mammals (Guo, 2004) but also presents identical neurotransmitter systems (Kastenhuber et al., 2010; Lillesaar et al., 2007; Rosemberg et al., 2007; Yamamoto et al., 2010) and a complex range of behaviors (Buske and Gerlai, 2011; Piato*et al*, 2011).

Despite previous reports of cadmium negative effects, the mechanisms by which the metal impacts cerebral functions are poorly understood and will benefit by use of a model organism such as zebrafish. Few reports have used automated systems to evaluate effects of cadmium on fish behavior (Beauvais *et al*, 2001; Giusi*et al*, 2005) and the only two studies that used zebrafish have evaluated larvae behavior (Blechinger*et* al, 2007; Kusch*et al*, 2007). The aim of this study was to evaluate cadmium effects on adult zebrafish behavior using an automated system and analyze oxidative stress and apoptotic markers in brain samples when exposed to cadmium for 1 or 7 days.

2 MATERIALS AND METHODS

2.1. Animals and Housing

A total of 240 adult male/female wild-type zebrafish (*Danio rerio*) were purchased from a local supplier (Redfish, Porto Alegre, Brazil). Fish were acclimated for at least two weeks and housed in groups of 50 fish in 15 L tanks with reverse osmosis water equilibrated with Instant Ocean and constantly aerated. Water parameters were daily monitored (temperature: 28 ±1°C;

pH: 7-7.5; hardness: 300-450 ppm $CaCO_3$; nitrites and nitrates: 0,05 a 0,1 mg/L) and kept constant in housing tanks and in tanks used for behavioral analysis in order to preserve animals' welfare. Fish were kept on a 14–10h day/night cycle and fed three times a day with commercial flakes (Tetramin, Tetra, Melle FRG) supplemented with brine shrimp (*Artemia salina*). All protocols were approved by the Institutional Animal Care Committee (10/00214, CEUA-PUCRS) and followed Brazilian legislation, the guidelines of the Brazilian Collegium of Animal Experimentation (COBEA), Canadian Council for Animal Care (CCAC) guide on the care and use of fish in research, teaching, and testing and the recommendations by Westerfield (2000).

2.2. Treatments

Animals were assigned into eight groups, according to exposure times (1 day or 7 days) and cadmium carbonate ($CdCO_3$ – Sigma Aldrich) concentrations (0, 10 µg/L, 100 µg/L and 1000 µg/L). Animals were kept in 15 L tanks, each one containing 60 animals. $CdCO_3$ was dissolved in conditioned water as mentioned on item 2.1 and control animals were exposed to cadmium-free conditioned-water. Cadmium concentrations were based on the literature and chosen to represent environmentally tolerated concentrations (10 µg/L, according to the Brazilian Environmental Council (CONAMA, 2005) and to model release accidents (100 µg/L and 1000 µg/L) (Ansari *et al.*, 2010; Bhagure *et* al, 2009). Neither continuous exposure to the metal nor water exchange were made during treatments. Furthermore, cadmium concentrations were not measured in each treatment tank. Fish were observed twice a day during treatment (8 a.m. and 18 p.m.) for mortality and other abnormal symptoms including aggressiveness. At the end of each treatment, fish were placed in cadmium-free tanks for behavioral analysis or euthanasia for sample collection, in order to perform oxidative stress essays and Quantitative Real-Time PCR.

2.3. Behavioral Analysis

Twenty-four hours after cadmium exposure, animals were subjected during 5 min to behavioral analysis. Individual animals were placed and recorded in a 2.7 L tank (24 cm × 8 cm × 20 cm, length × width × height) with 15 cm high water level, divided equally in three portions (bottom, medium and top) (adapted from Egan, 2004). Videos were analyzed using the software ANY-maze (Stoelting Co, Wood Dale, IL, USA) for the following parameters:

traveled distance (m), mean speed (m/s), mobile time (s), number of rotations, path efficiency (defined as an index of efficiency of the path taken by the animal to get from the entrance in the test tank to the last position during the 5 min task, expressed as an arbitrary unit) and time spent (s) in the bottom, middle and top portions of the test tank.

2.4. Oxidative Stress

Whole brains (pool of 5, in triplicate) were dissected, frozen in liquid nitrogen and stored at -80°C until analysis. The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine as previously described (Levine et al., 1990). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in dinitrophenylhydrazine and the absorbance read at 370 nm.

As an index of lipid peroxidation we used the formation of Thiobarbituric Acid Reactive Species (TBARS) during an acid-heating reaction as previously described (Draper and Hadley, 1990). Briefly, the samples were mixed with 1 ml trichloroacetic acid 10% and 1 ml thiobarbituric acid 0.67%, then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm.

All the results were normalized by protein concentration measured by the Lowry assay (Lowry et al., 1951).

2.5. Quantitative Real-Time PCR

RNA isolation and cDNA synthesis were performed as described in Rosemberg and collaborators (2007). Briefly, whole brains (pool of 5, in duplicate) were frozen in liquid nitrogen and RNA was isolated with TRIzol reagent (Invitrogen), according to the manufacturer's instruction. RNA purity was quantified spectrophotometrically calculating the ratio between the absorbance values at 260nm and 280nm and the integrity of the samples were tested by electrophoresis in a 1.0% agarose gel with gelRed nucleic acid stain (Biotium). cDNA species were synthesized with SuperScript^{TM} III First-Strand Synthesis SuperMix (Invitrogen, USA) and we used random hexamers for the priming method (Rosemberg, 2007).

For all genes, qRT-PCRs were performed using SYBR green (Tang et al., 2007). Standard reactions (25 µl) were assembled as follows: 4 µl of SYBR green qPCRsupermix-UDG (Invitrogen), 0.25 µl of forward primer (10 µM), 0.25 µl of reverse primer (10 µM), 0.25µl of dNTPs (10mM), 1.5µl of MgCl (50mM), 2.5µl of PCR buffer (10X), 3.7 µl DEPC

water, 0.05µl of Platinum TaqDNA 0.5U (Invitrogen) and 12.5µl of template. Templates were 1:50 diluted cDNA samples, and in the case of negative controls, cDNAs were replaced by DEPC water. The primers for the constitutive genes beta actin1, ef1a and rpl13a (Table 1) were according from Tang (2007). Zebrafish gene transcripts were obtained from Jung *et al* (2011).

All real time assays were carried out in triplicate using an Applied Biosystems 7500 real-time PCR system. Forty amplification cycles were performed, with each cycle consisting of 94ºC for 15 seconds followed by 60ºC for 35 seconds. Dissociation curves were used for the analysis of reaction specificity, and amplification curves generated by the software Applied Biosystems 7500 were used for gene expression analysis.

Threshold cycle (Ct) values were obtained for each gene. Following the removal of outliers, raw fluorescence data were exported to the LinRegPCR 12.x (http://LinRegPCR.HFRC.nl) to determine the PCR amplification efficiency of each sample. PCR efficiency of each sample, together with Ct values, was used to calculate a relative gene expression value for each transcript, according to the equation: $R = (E_{ref})^{CT\ Target}\ X\ (E_{Target})^{-CT\ Target}\ X\ (E_{Target})^{CT\ Ref}\ X\ (\ Eref)^{-CT\ Ref}$, where E_{Ref} refers to PCR efficiency of the reference Gene, E_{Target} refers to PCR efficiency of the target gene, Ct_{Ref} is the Ct value for each amplification of the reference gene and Ct_{Target} to the Ct value for each amplification of the target gene in question (Pfaffl, 2001). This equation was calculated for each gene with each reference gene, and a mean of this values was obtained for the evaluation of target gene expression.

2.6. Statistical Analysis

All data were parametrically analyzed using One-Way ANOVA followed by Tukey's post-hoc test. A $p<0.05$ was considered to be significant and all data are expressed as Mean $\pm$ S.D.

3. RESULTS

3.1. Survival

Cadmium-treated groups showed high survival after 1-day exposure to cadmium (control: 93.33%; 10 µg/L: 96.66%; 100 µg/L: 100.0%; 1000 µg/L: 96.66%). Similarly,

survival was high after 7 days of cadmium exposure (control: 100.0%; 10 µg/L: 96.66%; 100 µg/L: 100.0%; 1000 µg/L: 100.0%).

3.2. Behavioral Analysis

Results for the behavioral analysis are presented in Figure 1. Cadmium-treated groups demonstrated a general increase in locomotion when compared to controls. Animals exposed for 1 day to 10 and 1000 µg/L showed increased traveled distance and mean speed when compared to their control group ($p<0.05$ and $p<0.05$, respectively; Figures 1A and 1B). All 1-day cadmium-treated animals spent significantly more time mobile ($p<0.001$; Figure 1C) and made more full body turns ($p<0.01$; Figure 1D) during the analyzed 5-minutes period than their control. Interestingly, despite hyperlocomotion, animals path efficiency was decreased in all 1-day treated groups when compared to the control group ($p<0.001$; Figure 1E).

Accordingly, after 7-day cadmium exposure at 10, 100 and 1000 µg/L, a significantly dose-dependent increase in locomotion accompanied by a dose-dependent decrease in path efficiency was observed in treated animals when compared to controls. All 7-day cadmium-treated groups showed a dose-dependent increase in traveled distance and mean speed when compared to controls ($p<0.001$ and $p<0.001$, respectively; Figures 1F and 1G). Besides, all 7-day cadmium-treated animals spent more time mobile ($p<0.001$; Figure 1H) and made more full body turns ($p<0.001$; Figure 1I) while path efficiency was dose-dependently decreased ($p<0.001$; Figure 1J).

Figure 2 shows mean time spent in the bottom, middle and top portions of the test tank during the 5-minutes session and representative trackplots. Animals exposed for 1 day to different concentrations of cadmium showed no significant differences regarding to mean time spent in the bottom, middle and top portions when compared to their respective control ($p=0.35$; Figure 2A). Distinctively, 7 days cadmium-treated groups tended to spend less time in the bottom and more in the middle and top portions of the tank. Animals treated with 10 µg/L spent significantly less time in the bottom ($p<0.01$) and more on the top ($p<0.05$). Animals exposed to 100 and 1000 µg/L spent in average less time in the top portion ($p=0.43$ for the 1000 µg/L treated group) and consequently more time in the middle portion ($p<0.05$ for both groups; Figure 2B).

3.3. *Oxidative Stress*

Cadmium-exposed animals' brain samples showed increased carbonyl levels and TBARS depending on dose and treatment period (Figure 3). Brain samples collected from animals exposed to 100 µg/L for 1 day exhibited an increase in carbonyl levels when compared to control ($p<0.05$; Figure 3A) while those of animals treated for 7 days at 10 µg/L were also increased (Figure 3B).

TBARS levels in brain samples from 1-day treated animals at 100 µg/L were increased when compared to controls ($p<0.05$; Figure 4A) while no significant difference was observed at 7 days of exposure.

3.4. Real-Time PCR

While no significant effects of cadmium exposure were observed in animals treated for 1 day (Figures 5A and 5C), significant increases of p53 were observed in animals treated with 10 and 1000 µg/L ($p<0.001$ when compared to controls; Figure 5B), and of bax in animals exposed to 1000 µg/L ($p<0.05$; Figure5D).

4. DISCUSSION

The increased locomotor activity observed has been previously described in fish, such as rainbow trout (Giusi*et al*, 2005) as well in rats (Fernández-Pérez *et al*, 2010; Rastogi*et al*, 1977). It has been shown that cadmium increases levels of catecholamines and amino acid neurotransmitters in rat central nervous system, culminating in augmented locomotor activity (Fernández-Pérez *et* al, 2010; Rastogi *et al*, 1977), which could account for the observed effects in our study. Our data also demonstrates, in addition to a robust increase in all locomotor parameters, decreased path efficiency, suggesting that cadmium-treated animals have impaired orientation and exploratory performance. Many studies in humans have shown the deleterious effects of cadmium on central nervous system, such as learning disability and hyperactivity (Pihl and Parkes, 1977; reviewed by Schoeters *et al*, 2006) in children as well neuropsychological disturbances in adults (Hart *et* al, 1989; Viaene *et al*, 2000). Furthermore, it is known that cadmium is capable of inducing aggressiveness in rats (Galbiati *et al*, 2011; Salvatori*et al*, 2004), which could be related to hyperactivity.

The deleterious effects of cadmium on overall animals' performance were also evident by animals altered distribution on the water column. When introduced to a novel environment, zebrafish tend to spend more time at the bottom portion of the test tank and gradually start to explore the top portions of the tank (Levin *et al*, 2007). Longer time spent in the bottom portion and less time spent in higher levels indicates anxiety (Gebauer *et al*, 2011; Levin *et al*, 2007; Piato *et al*, 2011). Thus, our data suggest that animals were less anxious in relation to controls, increasing their exploratory activity in the novel environment (Figure 2B). This can be considered a negative effect of cadmium exposure, since zebrafish naturally spends more time in the bottom portion as a protective behavior to escape predation. Furthermore, it is known that cadmium affects olfaction on fish, impairing the ability of fish to detect predators (reviewed by Scott and Sloman, 2004). Thus, increased exploratory activity caused by diminished anxiety and impaired olfaction may impair animal's survival, leading to increased mortality due to predation.

Importantly, the lowest concentration of 10 µg/L, which is considered to be the maximum, tolerated concentration of the metal in fresh water in Brazil (CONAMA, 2005), and lies close to other international limits, including 1 µg/L in the U.S.A. (EPA, 2001) and 5 µg/L in Europe (EEC, 1983) caused deleterious behavioral effects to the animals' behavior starting at 1-day exposure. This suggests that even concentrations considered safe may impair animals' health and survival through disturbing locomotion and exploratory parameters, which may culminate in disruption of behaviors such as foraging, social interaction (Sloman *et al*, 2003) and reproduction (Baker and Montgomery, 2001) and predator avoidance (Scott *et al*, 2003).

Several studies have characterized the cellular and molecular mechanisms of cadmium toxicity (reviewed by Cannino, 2009; reviewed by Thévenod, 2009). Carbonyl is the most general marker of protein oxidation(Dalle-Donne *et al*, 2003) while lipid peroxidation inferred by TBARS is another traditional marker of the deleterious effects of increased oxidative damage. The increase in the carbonyl and TBARS levels at 1-day exposure in brain samples may be explained due to cadmium's ability to decrease antioxidant activity (reviewed by Thévenod, 2009) and this organ being sensitive to cadmium toxicity. Different from other tissues such as muscle in which efficient mechanisms of protection and detoxification are present (Gonzalez *et al*, 2005), cadmium tends to accumulate in brain (Giusi*et al*, 2005), explaining the increased susceptibility of the latter.

Extended exposure to cadmium might enhance activity of antioxidant proteins, as a consequence of adaptative induction of genes (reviewed by Thévenod, 2009), compensating

the cellular damage. Or, maybe, as long-term exposure disturbs the blood-brain barrier, accumulating more cadmium in the brain, the injury has been so intense that cadmium disrupted completely the mitochondrial status, decreasing O_2 consumption and, subsequently, decreasing the formation of ROS. Despite the lack of statistically significant difference at 7 days on TBARS levels at the highest cadmium concentration, probably due the high data variability, Cd tends to increase lipid peroxidation through interaction with proteins, mainly the ones with SH-groups, and Fenton reactions in the brain, which contains a great pool of Fe^{+2} and Cu^{+2} (López*et* al, 2006; Thévenod, 2009). Extended exposure to the metal and/or high doses disturb several intracellular pathways involved in lipid metabolism, culminating in a wide range of damage to cell, especially to the cell membrane.

Cellular injury caused through ROS may trigger apoptosis. Many reports have shown cadmium's ability to induce apoptosis in several cell types, including fish gills and brain (Azzouqiel*et al*, 1994;Dong *et al*, 2001;Habeebu*et al*, 1998; López*et al*, 2003, Liu *et al*, 2011). After 1-day cadmium exposure, p53 and Bax levels were not altered, suggesting that this short treatment, despite other effects, was not capable of inducing detectable levels of apoptosis in the brain. Tokumoto*et al* (2011) showed that NRK-52E cells treated with cadmium induce activation of p53 after 3h of treatment but p53 mRNA levels were downregulated until 12h of treatment in the study. When exposure time was increased, p53 and Bax gene expression were increased, suggesting a sustained activation of the cell apoptotic machinery. One may speculate that the depletion of antioxidant enzymes and/or the intense damage caused to mitochondria leading to DNA damage could have activated the expression of p53 in order to protect the brain against mutagenesis. Besides, it has been shown that cadmium not only induces cell death through apoptosis but also through necrosis (Krumschnabel*et al*, 2010), since cadmium is able to deplete the intracellular levels ATP and inhibit caspase-3 activity at elevated doses (López*et al*, 2003). Finally, cadmium-induced apoptosis in the central nervous system has been linked to neurodegenerative disorders, such as Parkinson's Disease (Okuda *et al*, 1997) and amyotrophic lateral sclerosis (Bar-Sela et al., 2001), being accumulated in the striatum in rodents (Fernández-Pérez *et al*, 2010) and regions responsible for motor activity in fish (Giusi*et al*, 2005), which could explain the hyperlocomotion.

In conclusion, we have shown a robust increase in fish locomotor behavior in all tested concentrations and treatment periods, starting after 1 day exposure of cadmium at 10 µg/L. The hyperlocomotion was accompanied by decreased path efficiency and a disrupted distribution on the water column. Oxidative stress was observed in brains tissue of treated

animals, and, consequently, an increased expression of apoptotic markers after 7 days of exposure suggest a persistent deleterious effect of cadmium exposure.

Taken together, these effects suggest that, despite the lack of acute lethal effects, cadmium exposure at currently legally tolerated concentrations and those observed in release accidents, even for short periods, may lead to behavioral effects that could later compromise animals' behavioral and cognitive performance and survival.

Competing interests

There are no competing interests.

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References

Ansari, M.I., Malik, A., 2010. Seasonal variation of different microorganisms with nickel and cadmium in the industrial wastewater and agricultural soils. Environ. Monit. Assess. 167(1-4), 151-63.

Atchison, G.J., Henry, M.G., Sandheinrich, M.B., 1987. Effects of metals on fish behavior: a review. Environmental Biology of Fishes. 18(1), 11-25.

ATSDR. Agency for Toxic Substance and Disease Registry. Toxicological Profile for cadmium. Atlanta: ATSDR, p. 347, 1997.

Azzouqiel, B., Tsangaris, G.T., Pellegrini, O., Manuel, Y., Benvieniste, J., Thomas, Y., 1994. Cadmium induces apoptosis in a human T cell line. Toxicology. 88, 127-39.

Bhagure, G.R., Mirgane, S.R., 2009. Heavy metal concentrations in groundwaters and soils of Thane Region of Maharashtra, India. Environ. Monit. Assess. DOI: 10.1007/s10661-010-1412-9.

Baker, C.F., Montgomery, J.C., 2001. Sensory deficits induced by cadmium in banded kokopu, Galaxias fasciatus, juveniles. Environ. Biol. Fish. 62, 455–464.

Bar-Sela, S., Reingold, S., Richter, E.D., 2001. Amyotrophic lateral sclerosis in a battery-factory worker exposed to cadmium. Int. J. Occup. Environ. Health. 7, 109–112.

Beauvais, S.L., Jones, S.B., Parris, J.T., Brewer, S.K., Little, E.E., 2001. Cholinergic and behavioral neurotoxicity of carbaryl and cadmium to larval rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol. Environ. Saf. 49(1), 84-90.

Bernard, A., 2008. Cadmium and its adverse effects on human health. Indian. J. Med. Res. 128, 557-564.

Bhakta, J.N., Munekage, Y., 2008. Role of ecosystem components in Cd removal process of aquatic ecosystem. Ecol. Eng. 32, 274–280.

Blechinger, S. R., Kusch, R. C., Haugo, K., Matz, C., Chivers, D. P., Krone, P. H., 2007. Brief embryonic cadmium exposure induces a stress response and cell death in the developing olfactory system followed by long-term olfactory deficits in juvenile zebrafish. Toxicology and Applied Pharmacology. 224(1), 72-80.

Bondier, J.R.; Michel, G.; Propper, A.; Badot, P.M. Harmful effects of cadmium on olfactory system in mice. Inhal Toxicol. 20(13), p. 1169-77, 2008.

Buske, C., Gerlai, R., 2011. Maturation of shoaling behavior is accompanied by changes in the dopaminergic and serotoninergic systems in zebrafish. Dev Psychobiol. 54(1), 28-35.

Cannino, G., Ferruggia, E., Luparello, C., Rinaldi, A.M., 2009. Cadmium and Mitochondria. Mitochondrion. 9, 377-384.

Cinier, C.C., Petit-Ramel, M., Faure, R., Garin, D., Bouvet, Y., 1999. Kinetics of cadmium accumulation and elimination in carp (*Cyprinus carpio*) tissues. Comp. Biochem. Physiol. C. 122, 345–352.

CONAMA. Brazilian Environmental Conciul. Resolution number 357 <http://www.mma.gov.br/port/conama/res/res05/res35705.pdf>. Last accession: Jan.12, 2012.

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., Colombo, R., 2003. Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta. 329:23–38.

Dargusch, R., Piasecki, D., Tan, S., Liu, Y., Schubert, D., 2001. The role of Bax in glutamate-induced nerve cell death, J. Neurochem. 76, 295–301.

Dong, S., Shen, H.M., Ong, C.N., 2001. Cadmium induced apoptosis and phenotypic changes in mouse thymocytes. Mol. Cell Biochem. 222, 11-20.

Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 186:421–431.

EEC. European Environment Council., 1983. On limit values and quality objectives for cadmium discharges. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1983:291:0001:0008:EN:PDF>. Last accession: Jan. 12, 2012.

EPA. United States Environmental Protection Agency., 2001. Update of ambient water quality criteria for cadmium. <http://water.epa.gov/scitech/swguidance/standards/upload/2001_04_13_criteria_cadmium_cad2001upd.pdf>. Last accession: Jan. 12, 2012.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioral Brain Research. 205(1), 38-44.

Fernández-Pérez B, Caride A, Cabaleiro T, Lafuente A. 2010. Cadmium effects on 24h changes in glutamate, aspartate, glutamine, GABA and taurine content of rat striatum. J Trace Elem Med Biol. 24 (3), 212-8.

Fridman, J.S., Lowe, S.W., 2003. Control of apoptosis by p53. Oncogene. 22, 9030–9040.

Galbiati, S.T., Almeida, A.A., Godinho, A.F., 2011.Cadmium and exposure to stress increase aggressive behavior. Environmental Toxicology and Pharmacology. 32, 40–45.

Gebauer, Daiane L. ; Pagnussat, Natália ; Piato, Ângelo L. ; Schaefer, Isabel C. ; Bonan, Carla D. ; Lara, Diogo R. . Effects of anxiolytics in zebrafish: Similarities and differences between benzodiazepines, buspirone and ethanol. Pharmacology, Biochemistry and Behavior, v. 99, p. 480-486, 2011.

Giusi, G., Facciolo, R.M., Alò, R., Carelli, A., Madeo, M., Brandmayr, P., Canonaco, M., 2005. Some environmental contaminants influence motor and feeding behaviors in the ornate wrasse (*Thalassoma pavo*) via distinct cerebral histamine receptor subtypes. Environ. Health. Perspect. 113(11), 1522-9.

Gonzalez, P., Baudrimont, M., Boudou, A., Bourdineaud, J.P., 2006. Comparative effects of direct cadmium contamination on gene expression in gills, liver, skeletal muscles and brain of the zebrafish (Danio rerio). BioMetals. 19:225–235.

Gottofrey, J., Tjälve, H., 1991. Axonal transport of cadmium in the olfactory nerve of the pike. Pharmacol. Toxicol. 69, 242-252.

Guo, S., 2004. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? Genes Brain Behav. 3(2), 63–74.

Habeebu, S.S., Liu, J., Klassen, C.D., 1998. Cadmium induced apoptosis in mouse liver. Toxicol Appl Pharmacol. 149, 203-9.

Hart, R.P., Rose, C.S., Hamer, R.M., 1989. Neuropsychological effects of occupational exposure to cadmium. J. Clin. Exp. Neuropsychol. 11,933–943.

Järup, L., Akasson, A., 2009. Current status of cadmium as an environmental health problem. Toxicology and Applied Pharmacology. 238, 201–208.

Jayanthi, S., Deng, X., Noailles, P.A., Ladenheim, B., Cadet, J.L., 2004. Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades, FASEB J. 18, 238–251.

Joers, A., Jaks. V., Kase, J., Toivo, M., 2004. p53-dependent transcription can exhibit both on/off and graded response after genotoxic stress. Oncogene. 23, 6175–6185.

Jung, H., Kim, T.H., Lee, C.R., Seok, S.H., Park, J.H., Chang, S.N., Seo, J.E., Han, H., Ko, A.S., Byun, S.H., Abdelkader, T.S., Choi, S.K., Kim, J.A., 2011. Effect of fluorescent whitening agent on the transcription of cell damage-related genes in zebrafish embryos. J. Appl. Toxicol. DOI 10.1002/jat.1665.

Kargin, F., Çogun, H.I., 1999. Metal Interactions During Accumulation and Elimination of Zinc and Cadmium in Tissues of the Freshwater Fish *Tilapia nilotica*. Bull. Environ. Contam. Toxicol. 63, 511-519.

Kastenhuber, E., Kratochwil, C.F., Ryu, S., Schweitzer, J., Driever, W., 2010. Genetic dissection of dopaminergic and noradrenergic contributions to catecholaminergic tracts in early larval zebrafish. J. Comp. Neurol. 518(4), 439–58.

Komjarova, I., Blust, R., 2009. Multimetal interactions between Cd, Cu, Ni, Pb, and Zn uptake from water in the zebrafish *Danio rerio*. Environ Sci Technol 43(19), 7225–9.

Krumschnabel, G., Ebner, H. L., Hess, M. W., Villunger, A., 2010. Apoptosis and necroptosis are induced in rainbow trout cell lines exposed to cadmium. Aquatic Toxicology. 99, 73-85.

Kumar, R., Asic, K., Agarwal, K., Seth, P.K., 1996. Oxidative stress mediated neurotoxicity of cadmium. Toxicol. Lett. 89, 65–69. M during embryonic and larval development results in the long-term hindrance of antipredator behavior in zebrafish. Environmental Toxicology and Chemistry. 27(3), 705-11.

Kusch, R. C., Krone, P. H., Chivers, D. P., 2007. Chronic exposure to low concentrations of waterbone cadmiu

Levin, E.D., Bencan, Z., Cerutti, D.T., 2007. Anxiolytic effects of nicotine in zebrafish. *Physiol Behav*, **90**, 54–58.

Levine, R.L., Garland, D., Oliver, C.N., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186:464–478.

Lillesaar, C., Tannhäuser, B., Stigloher, C., Kremmer, E., Bally-Cuif, L., 2007. The serotonergic phenotype is acquired by converging genetic mechanisms within the zebrafish central nervous system. Dev. Dyn. 236(4), 1072–84.

Liu, M., Tee, C., Zeng, F., Sherry, J.P., Dixon, B., Bols, N.C., Duncker, B.P., 2011. Characterization of p53 expression in rainbow trout. Comp. Biochem. Physiol. C., Toxicol Pharmacol. 154(4), 326-32.

López, E.; Figueroa, S.; Oset-Gasque, M.J.; González, M.P., 2003. Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture. British Journal of Pharmacology. 138, 901-11.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193(1), 265–275.

O'Callaghan, J. P., Miller, D., 1986. Diethyldithiocarbamate increases distribution of cadmium to brain but prevents cadmium-induced neurotoxicity. Brain Res. 370, 354–358.

Okuda, B., Iwamoto, Y., Tachibana, H., Sugita, M., 1997. Parkinsonism after cadmium poisoning. Clinical Neurology and Neurosurgery. 99, 263-265.

Pfaffi, M.W., 2001. A new mathematical model for relative quantification in real-time PT-PCR. Nucleic Acid Res. 29, 2003-2007.

Piato, Â.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J., Bogo, M.R., Lara, D.R., Vianna, M.R., Bonan, C.D., 2011. Unpredictable chronic stress model in Zebrafish (*Danio rerio*): Behavioral and physiological responses, Prog Neuro-Psychopharmacol Biol Psychiatry. doi:10.1016/j.pnpbp.2010.12.018

Pihl, R.O., Parkes, M., 1977. Hair element content in learning disabled children. Science. 198(4313), 204-6.

Polster, B.M., Fiskum, G., 2004. Mitochondrial mechanisms of neural cell apoptosis, J. Neurochem. 90, 1281–1289.

Pretto, A., Loro, V.L., Moraes, B.S., Clasen, B., Baldisserotto. B. Isabela, C.M., Finamor, A., Cattaneo, R., Dressler, V., 2011. Effects of Water Cadmium Concentrations on Bioaccumulation and Various Oxidative Stress Parameters in *Rhamdia quelen*. Arch. Environ. Contam. Toxicol. 60, 309–318.

Rashed, M.N., 2001. Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. Environmental Monitoring and Assessment. 68, 75–89.

Rastogi, R.B., Merali, Z., Singhal, R.L., 1977. Cadmium alters behaviour and the biosynthetic capacity for catecholamines and serotonin in neonatal rat brain. J. Neurochem. 28(4), 789-94.

Rosemberg, D.B., Rico, E.P., Guidoti, M.R., Dias, R.D., Souza, D.O., Bonan, C.D., 2007. Adenosine deaminase-related genes: molecular identification, tissue expression pattern and truncated alternative splice isoform in adult zebrafish (*Danio rerio*). Life Sci. 81(21–22), 1526–34.

Salvatori, F., Talassi, C.B., Salzgeber, S.A., Spinosa, H.S., Bernardi, M.M., 2004. Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. Neurotoxicol. Teratol. 26 (5), 673–680.

Schoeters, G., Den Hond, E., Zuurbier, M., Naginiene, R., van den Hazel, P., Stilianakis, N., Ronchetti, R., Koppe, J.G., 2006. Cadmium and children: exposure and health effects. Acta Paediatr Suppl. 95(453), 50-4.

Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology. 68, 369–392..

Slee, E.A., O'Connor, D.J., Lu, X., 2004. To die or not to die: how does p53 decide? Oncogene 23, 2809–2818.

Sloman, K.A., Scott, G.R., Diao, Z., Rouleau, C., Wood, C.M., McDonald, D.G., 2003. Cadmium affects the social behavior of rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 65, 171–185.

Soares, S.S., Martins, H., Gutiérrez-Merino, C., Aureliano, M., 2008. Vanadium and cadmium in vivo effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. Comp Bochem Physiol C. 147,168–178.

Szebedinsky, C., McGeer, J.C., McDonald, D.G. and Wood, C.M., 2001. Effects of chronic Cd exposure via the diet or water on internal organspecific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 20, 597-607.

Takeda, A.; Takefuta, S.; Ijiro, H.; Okada, S.; Oku, N. 1999. 109Cd transport in rat brain. Brain Res Bull. 49 (6), 453-7.

Tallkvist, J., Persson, E., Henriksson, J., Tjälve, H., 2002. Cadmium-metallothionein interactions in the olfactory pathways of rats and pikes. Toxicol Sci. 67(1),108-13.

Tang, R., Dodd, A., Lai, D., McNabb, W., Love, D., 2007. Validation of Zebrafish (*Danio rerio*) reference genes for quantitative real-time RT-PCR normalization. Acta Biochime. Biophys. Sin. 38, 384-90.

Thévenod, F., 2009. Cadmium and cellular signaling cascades: To be or not to be? Toxicology and Applied Pharmacology. 238, 221–239.

Tjälve, H., Gottofrey, J., 1986. Tissue disposition of in the brown trout (Salmo trutta) studied by autoradiography and impulse counting. Toxicol. Environ. Chem. 12, 31-45.

Tjälve, H., Henriksson, J., 1999. Uptake of metals in the brain via olfactory athways. Neurotoxicology 20, 181-196.

Tokumoto, M., Fujiwara, Y., Shimada, A., Hasegawa, T., Seko, Y., Nagase, H., Satoh, M., 2011. Cadmium toxicity Is caused by accumulation of p53 through the down-regulation of Ube2d family genes in vitro and in vivo. The Journal of Toxicological Sciences, 36 (2), 191-200.

Tropepe, V.; Sive, H.L., 2003. Can zebrafish be used as a model to study the neurodevelopmental causes of autism? Genes Brain Behav. 2, 268– 281.

Verbost, P.M., Flik, G., Lock, R.A.C., Wendelaar Bonga, S. E., 1987. Cadmium inhibition of Ca2+ uptake in rainbow trout gills. Am. J. Physiol. 253, 216-221

Verbost, P.M., Rooij, J.V., Flik, G., Lock, R.A.C., Wendelaar Bonga, B.S.E., 1989. The movement of cadmium through freshwater trout branchial epithelium and its interference with calcium transport. J. Exp. Biol. 145, 185-197.

Viaene, M.K., Massachelein, R., Leenders, J., De Groof, M., Swerts, L.J.V.C., Roels, H.A., 2000. Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. Occup. Environ. Med. 57:19–27 19

Ward, M.W., Kogel, D., Prehn, J.H., 2004. Neuronal apoptosis: BH3 only proteins the real killers? J. Bioenerg. Biomembr. 36, 295–298.

Westerfield, M., 2000. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). 4th ed. Eugene: Univ. of Oregon Press.

WHO. World Health Organization. Cadmium. Geneva, 1992. (Environment Health Criteria 134).

Yamamoto, K., Ruuskanen, J.O., Wullimann, M.F., Vernier, P., 2010. Two tyrosine hydroxylase genes in vertebrates new dopaminergic territories revealed in the zebrafish brain. Mol. Cell Neurosci. 43(4), 394–402.

Yano, C.L., Marcondes, M.C.C.G., 2005. Cadmium chloride-induced oxidative stress in skeletal muscle cells in vitro. Free Radical Biology & Medicine 39, 1378 – 1384.

TABLES

TABLE 1 - PRIMERS

Protein	Gene	Accession Number	Primers (5'-3')
Bax*	bax	BC055592	F: GAGCTGCACTTCTCAACAACTTT R: CTGGTTGAAATAGCCTTGATGAC
P53*	p53	U60804	F: CTATAAGAAGTCCGAGCATGTGG R: GGTTTTGGTCTCTTGGTCTTTCT
β-actin**	bactin1	ENSDART00000055194	F: CGAGCTGTCTTCCCATCCA R: TCACCAACGTZGCTGTCTTTCTG
Elongation factor 1 alplha**	ef1a	ENSDART00000023156	F: CTGGAGGCCAGCTCAAACAT R: ATCAAGAAGAGTAGTACCGCTAGCATTAC
Ribosomal protein L 13 alpha**	rpl13a	NM_212784	F: TCTGGAGGACTGTAAGAGGTATGC R: AGACGCACAATCTTGAGAGCAG

* Jung, H. *et al* (2011) - Effect of fluorescent whitening agent on the transcription of cell damage-related genes in zebrafish embryos

** Tang, R. *et al* (2007) - Validation of Zebrafish (*Danio reiro*) reference genes for quantitative real-time RT-PCR normalization.

Figure Legends

Figure 1. Cadmium exposure results in hyperlocomotion and exploratory efficiency deficit. Traveled distance (A, F), mean speed (B, G), mobile time (C, H), number of rotations (D, I) and path efficiency (E, J) measured during a 5-min session after 1-day (left column) and 7-day exposure (right column) periods. Data is presented as mean ± S.D. *N* = 13-15 per group. ***p<0.001; **p<0.01 and *p<0.05 when compared to respective control group using One-Way ANOVA followed by Tukey's test post-hoc.

Figure 2 Cadmium alters animals' distribution on the water column. Mean ± S.D. time in bottom, middle and top portions of test tank and representative track-plots after 1 (A) and 7 days (B) exposure periods. Track-plots represent the traveled path and distribution of animals during 5-minutes session. *N* = 13-15 per group.**p<0.01 and *p<0.05 when compared to control group using One-Way ANOVA followed by Tukey's test post-hoc

Figure 3. Effects of cadmium exposure on protein oxidative damage in the brain. Carbonyl levels (mean ± S.D.) in brain tissue of animals exposed to Cadmium for 1 (A) or 7 days (B). *N* = 3 pools of 6 individual samples per group. *p<0.05 when compared to control group using One-Way ANOVA followed by Tukey's test post-hoc.

Figure 4. Effects of cadmium exposure on lipid peroxidation in the brain. TBARS levels (mean ± S.D.) in brain tissue of animals exposed to Cadmium for 1 (A) or 7 days (B). *N* = 3 pools of 6 individual samples per group. *p<0.05 when compared to control group using One-Way ANOVA followed by Tukey's test post-hoc.

Figure 5. Cadmium increases apoptotic markers expression in the brain. Relative mRNA expression (mean ± S.D.) of p53 (A, B) and bax (C, D) in the brain of animals exposed to cadmium for 1 day and 7 days using Real-Time PCR based on specific sample efficiency independently and in triplicate. *N* = 2 pools of 5 brains per group. * indicates p<0.05 compared to Control by One-way ANOVA followed by Tukey post-hoc.

FIGURE 1

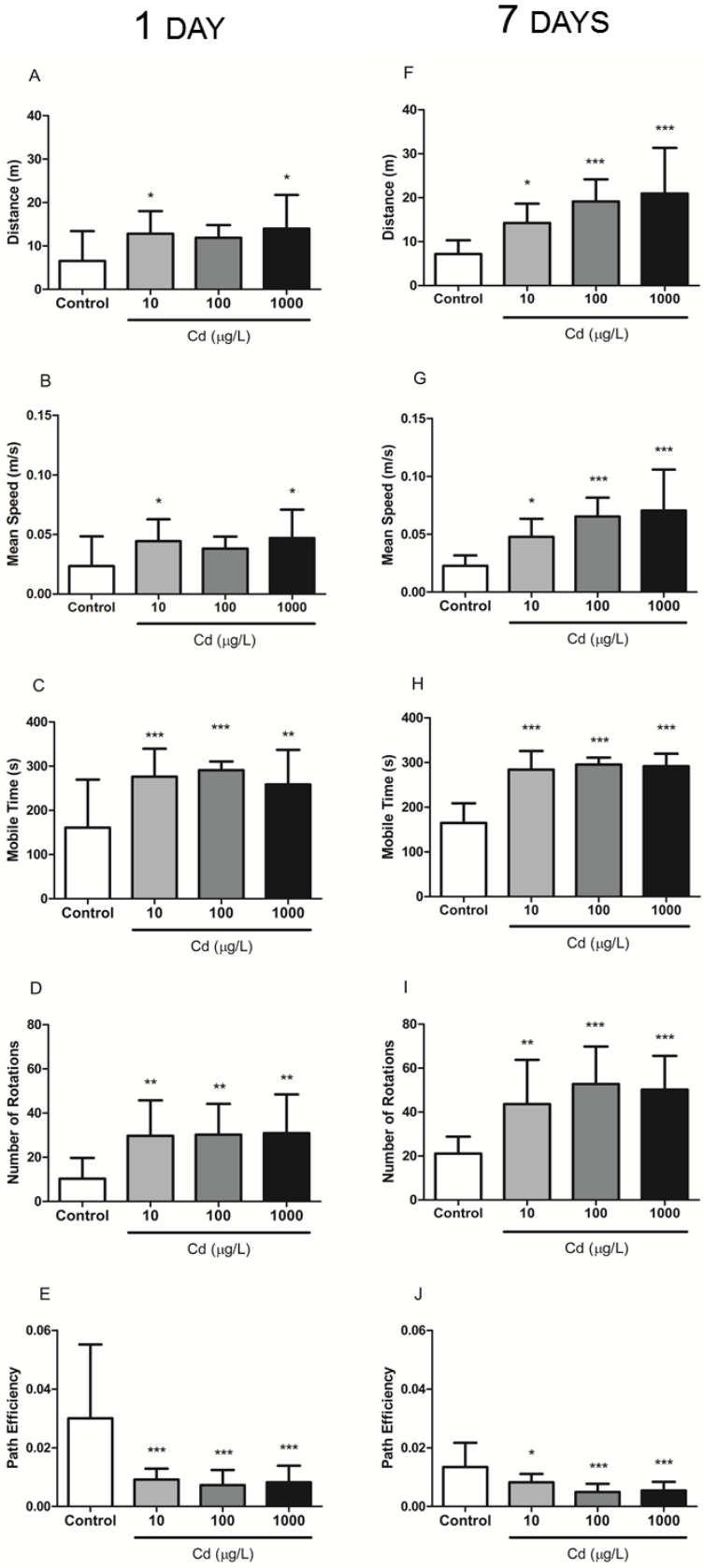

1 DAY
7 DAYS
A
F
Distance (m)
B
G
Mean Speed (m/s)
C
H
Mobile Time (s)
D
I
Number of Rotations
E
J
Path Efficiency
Control
10
100
1000
Cd (µg/L)

FIGURE 2

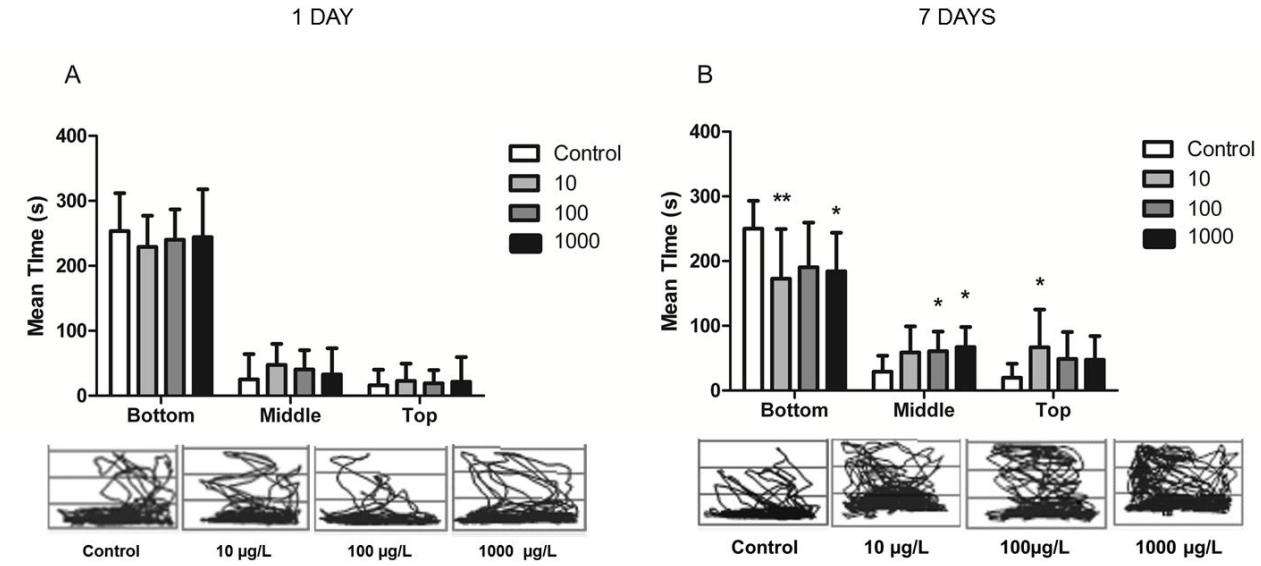

1 DAY
7 DAYS
A
B
Mean TIme (s)
400
300
200
100
0
Control
10
100
1000
Bottom
Middle
Top
**
*
*
*
*
Control
10 µg/L
100 µg/L
1000 µg/L
100µg/L

FIGURE 3

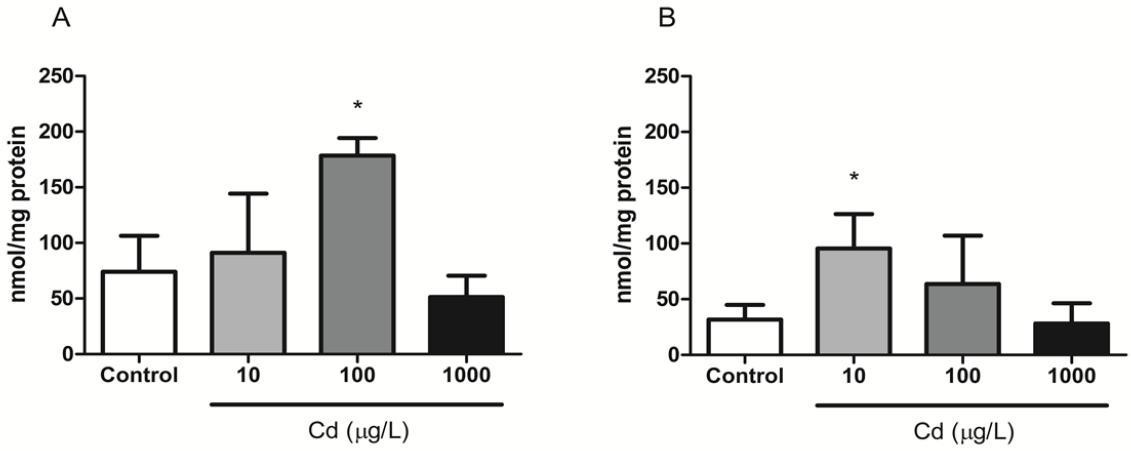

A
nmol/mg protein
250
200
150
100
50
0
*
Control
10
100
1000
Cd (μg/L)
B
nmol/mg protein
250
200
150
100
50
0
*
Control
10
100
1000
Cd (μg/L)

FIGURE 4

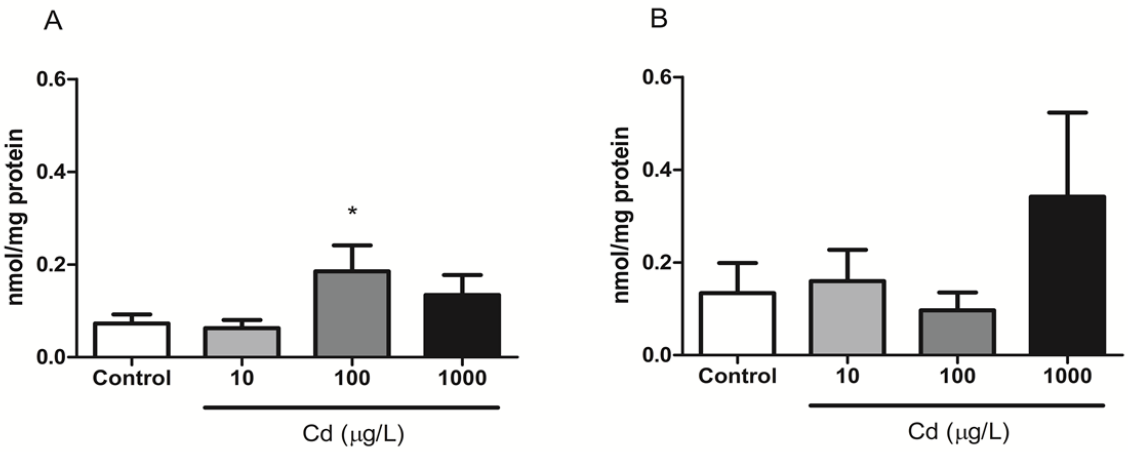
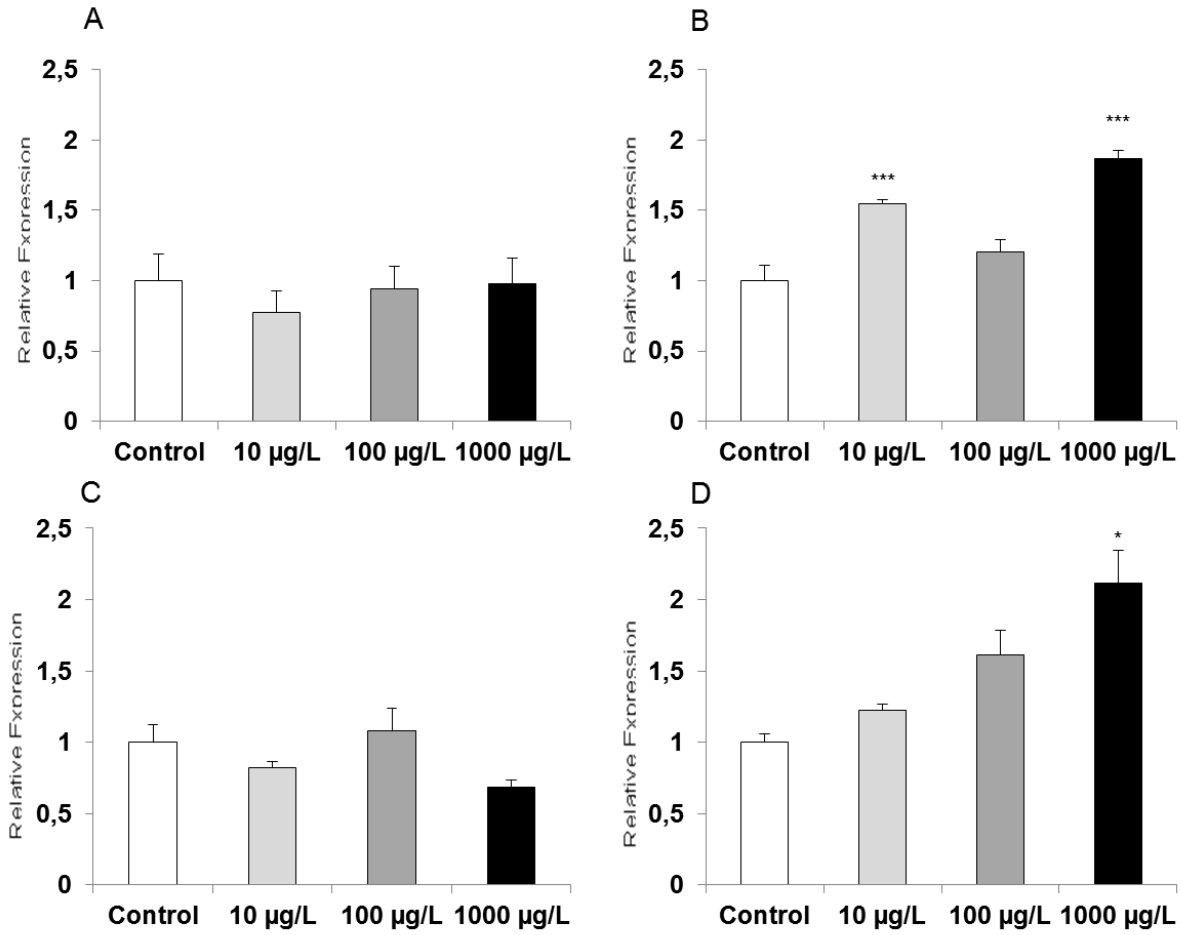


FIGURE 5


A
B
C
D
Relative Expression
2,5
2
1,5
1
0,5
0
***
*
Control
10 µg/L
100 µg/L
1000 µg/L

CAPÍTULO 3

CONSIDERAÇÕES FINAIS

Cádmio representa um risco à saúde humana, bem como de ecossistemas em geral. Desde a descoberta de sua toxicidade na década de 60, os níveis de emissão do metal no ambiente, seja aquático, aéreo ou terrestre, têm diminuído, devido a melhorias no manejo de resíduos e surgimento de novas tecnologias que poluem menos (Cardoso e Chasin, 2001). Atualmente, o metal é encontrado principalmente em baterias de níquel-cádmio e em aparelhos eletrônicos, sendo que o descarte irregular destes itens e a utilização de fertilizantes fosfatados são as principais fontes de contaminação do ambiente (revisado por Chen *et al*, 2011). Uma vez no ambiente, o cádmio contamina diversos organismos, desde plantas e invertebrados até vertebrados, inclusive o ser humano, participando em complexas cadeias alimentares.

Peixes são considerados excelentes bioindicadores de toxicidade aquática. Muitos estudos já foram feitos expondo-se peixes a diferentes concentrações de cádmio e de tempo, a fim de avaliar comportamentos simples e complexos (revisado por Scott e Sloman, 2004), dano oxidativo (Singhal *et al*, 1987; Jihen *et al*, 2011) e apoptose (Krumschnabel, 2010). Todavia, estudos que integrem grandes áreas de estudo como comportamento, biologia celular e molecular são ainda escassos, o que ajudaria a compreender de forma mais específica os efeitos toxicológicos do cádmio sobre os animais, principalmente sobre o sistema nervoso central, cuja compreensão é ainda menor.

A fim de contribuir para um melhor entendimento, nós avaliamos o comportamento de *zebrafish* adultos tratados com cádmio por 1 dia e 7 dias. Os animais apresentaram distância percorrida e velocidade média elevadas em 1 dia, exceto aqueles tratados com a dose de 100 µg/L. Ademais, os animais permaneceram mais tempo em movimento bem como realizaram mais rotações em torno do corpo. Após 7 dias de exposição ao cádmio, os animais apresentaram uma crescente resposta dose-dependente em relação a distância percorrida (r=0,63) e velocidade média (r=0,64), assim como tempo móvel (r=0,69) e número rotações (r=0,55). Atividade locomotora elevada tem sido avaliada em peixes (Giusi *et al*, 2005) bem como em roedores (Rastogi *et al,* 1976; Beauvais *et a,l* 2001; Fernández-Pérez *et al,* 2010). Muitos estudos em humanos têm mostrado os efeitos deletérios do cádmio sobre o sistema nervoso central, tais como déficits de aprendizagem e

hiperatividade (Pihl and Parkes, 1977; revisado por Schoeters *et al*, 2006) em crianças, bem como perturbações neuropsicológicas em adultos (Hart *et* al, 1989; Viaene *et al*, 2000). Portanto, exposição cádmio induz hiperlocomoção em animais tratados por curtos e longos períodos.

Animais tratados com cádmio por 7 dias com 10 e 1000 µg/L permaneceram menos tempo na porção inferior do aquário-teste, sugerindo que os animais estavam menos ansiosos, elevando a atividade exploratória dos mesmos no novo ambiente. Em seu ambiente natural, *zebrafish* passa mais tempo em locais profundos, típico comportamento para evitar predadores. Além disto, sabe-se que cádmio prejudica o olfato de peixes (revisado por Scott & Sloman, 2004). Assim, a hiperlocomoção, que poderia ser causada por menor ansiedade, e o olfato prejudicado podem culminar na mortalidade dos animais devido à predação.

O Conselho Nacional do Meio Ambiente (CONAMA) estabelece o limite de 10 µg/L de cádmio em água doce. Inesperadamente, esta dose apresentou efeitos comportamentais deletérios a partir do primeiro dia de exposição. Desta forma, a dose mais baixa pode implicar na sobrevivência dos animais através de alterações na locomoção e ansiedade, que culminam em perturbações comportamentais como forrageio, interação social (Sloman *et al*, 2003) e reprodução (Baker and Montgomery, 2001), bem como comportamentos antipredatórios (Scott *et al*, 2003), e consequentemente, desestruturando ecossistemas.

Com base nos resultados obtidos, decidimos verificar o possível dano oxidativo em amostras de encéfalo. A exposição ao cádmio por 1 dia elevou os níveis de TBARS e carbonil em amostras de encéfalo. Exposição a curto prazo ao metal tende a diminuir a atividade das enzimas antioxidantes, culminando no aumento de espécies reativas de oxigênio (revisado por Thévenod, 2009). A exposição ao cádmio por 7 dias diminuiu os níveis de carbonil quando comparados com 1 de tratamento. Exposições a longo prazo e doses elevadas de cádmio tendem a diminuir os níveis de espécies reativas de oxigênio, ou por um mecanismo de adaptação, induzindo maior expressão das enzimas antioxidantes (revisado por Thévenod, 2009), ou pela inibição da cadeia transportadora de elétrons, reduzindo o consumo de oxigênio e, consequentemente, de espécies reativas de oxigênio (revisado por Cannino *et al*, 2009). Por fim, os mecanismos protetores e desintoxicantes no encéfalo são menos eficientes do que em outros tecidos, como o

músculo (Gonzalez *et al*, 2006), sendo assim, considerado um órgão-alvo pelo cádmio.

Os níveis de expressão gênica de p53 e bax no encéfalo, medidos por Real-Time PCR, não foram alterados após 1 dia de tratamento com cádmio. Células possuem um *pool* de p53 e, caso elas sofram algum dano, este *pool* é recrutado até que ocorra transcrição gênica, sendo assim, um mecanismo de proteção agudo. Exposição ao cádmio por 7 dias aumentou os níveis de p53 e bax significativamente. O esgotamento de enzimas antioxidantes e excessivo dano causado ao DNA podem ter ativado a expressão de p53, de maneira a proteger o encéfalo contra mutagênese. Tokumoto *et al* (2011) demonstraram que células NRK-52E tratadas com cádmio induzem a ativação de p53 após 3h de tratamento mas os níveis de RNAm estavam diminuídos até 12h de tratamento. Ademais, cádmio não apenas induz morte celular por apoptose, mas também por necrose, pois ele é capaz de perturbar os níveis intracelulares de ATP e inibir caspase-3 em doses elevadas (López *et al*, 2003). Finalmente, evidências sugerem uma ligação entre apoptose induzida por cádmio no sistema nervosa central e doenças neurodegenerativas, tais como Parkinson (Okuda *et al*, 1997), acumulando-se principalmente no estriado, em roedores, (Fernández-Pérez *et al*, 2010) e regiões responsáveis pela atividade motora em peixes (Giusi *et al*, 2005), que poderia explicar a hiperlocomoção observada neste estudo.

A grande variedade de efeitos deletérios provocados pelo cádmio ressalta a complexa interação entre este metal com vias intracelulares, culminando em alterações comportamentais. A compreensão destes efeitos nos permitiria desenvolver estratégias preventivas e terapêuticas. Por exemplo, uma alternativa seria o tratamento com quelantes, que diminuem tais efeitos, inclusive no encéfalo (Gonçalves *et al*, 2010). Contudo, mais estudos são necessários para a compreensão das vias, pois pontos mais básicos, como a permeabilidade da barreira hematoencefálica e as próprias características do metal, ainda permanecem obscuros.

REFERÊNCIAS

ARORA, M., WEUVE, J., SCHWARTZ, J., WRIGHT, R. O. Association of environmental cadmium exposure with pediatric dental caries. Environmental Health Perspectives. v. 116, p. 821–825, 2008.

[ATSDR] Agency for Toxic Substance and Disease Registry. Toxicological Profile for cadmium. Atlanta: ATSDR, p. 347, 1997.

_____. 2007 CERCLA Priority List of Hazardous Substances. U.S, 2007.

AZZOUQIEL, B.; TSANGARIS, G.T.; PELLEGRINI, O.; MANUEL, Y.; BIENVIENISTE, J.; THOMAS, Y. Cadmium induces apoptosis in a human T cell line. Toxicology. 88, 127-39, 1994.

BAKER, C.F., MONTGOMERY, J.C. Sensory deficits induced by cadmium in banded kokopu, Galaxias fasciatus, juveniles. Environ. Biol. Fish. 62, 455–464, 2001.

BAR-SELA, S., REINGOLD, S., RICHTER, E.D. Amyotrophic lateral sclerosis in a battery-factory worker exposed to cadmium. Int. J. Occup. Environ. Health. 7, 109–112, 2001.

BEAUVAIS, S.L., JONES, S.B., PARRIS, J.T., BREWER, S.K., LITTLE, E.E., 2001. Cholinergic and behavioral neurotoxicity of carbaryl and cadmium to larval rainbow trout (Oncorhynchus mykiss). Ecotoxicol. Environ. Saf. 49(1), 84-90.

BERGLUND, M., ÅKESSON, A., NERMELL, B., VAHTER, M. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environmental Health Perspectives. v. 102, p. 1058–1066, 1994.

BERNARD, A.; LAUWERYS, R. Cadmium in human population. Experientia. v. 40, p. 143–152, 1984.

BERNARD, A. Cadmium and its adverse effects on human health. Indian Journal of Medical Research. v. 128, p. 557-564, 2008.

BONDIER, J.R.; MICHEL, G.; PROPPER, A.; BADOT, P.M. Harmful effects of cadmium on olfactory system in mice. Inhal Toxicol. 20(13), p. 1169-77, 2008.

BOYD, R. S. Heavy metal pollutants and chemical toxicology: exploring new frontiers. Journal of Chemical Ecology. v. 36, p. 46-58, 2010.

BRASIL. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 357: dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. http://www.mma.gov.br/port/conama/res/res05/res35705.pdf>. Acesso em: 15 de abril de 2010.

CANNINO, G.; FERRUGGIA, E.; LUPARELLO, C.; RINALDI, A. M. Cadmium and Mitochondria. Mitochondrion. v. 9, p. 377-384, 2009, doi: 10.1016/j.mito.2009.08.009.

CALMANO, W.; FÖRSTNER, U. Sediments and toxic substances. Springer-Verlag, Berlin, Germany, 1996.

CARDOSO, L. M. N.; CHASIN, A. A. M. 2001. Ecotoxicologia do cádmio e seus compostos. Série Cadernos de Referência Ambiental. v. 6, 2001.

CHALLIS, G. L. A widely distributed bacterial pathway for siderophore biosynthesis independent of nonribosomal peptide synthetases. ChemBioChem v. 6, p. 601–611, 2005.

CHAN, P. K.; CHENG, S. H. Cadmium-induced ectopic apoptosis in zebrafish embryos. Archives of Toxicology. v. 77, p. 69–79, 2003.

CHAO, S. H.; BU, C. H.; CHEUNG, W. Y. Stimulation ofmyosin light-chain kinase by Cd2 + and Pb2+. Archives of Toxicology. v. 69, p. 197–203, 1995.

CHEN, W.-Y.; JOHN, J. A. C.; LIN, C.-H.; LIN, H.-F.; WU, S.-C.; LIN, C.-H.; CHANG, C.-Y. Expression of metallothionen gene during embryonic and early larval development in zebrafish. Aquatic Toxicology. v. 69, p. 215–227, 2004.

CHOW, E. S. H.; HUI, M. N. Y.; LIN, C. C.; CHENG, S. H. Cadmium inhibits neurogenesis in zebrafish embryonic brain development. Aquatic Toxicology. v. 87, p. 157–169, 2008.

CHOW, E.S.; Hui, M.N.; Cheng, C.W.; Cheng, S.H. Cadmium affects retinogenesis during zebrafish embryonic development. Toxicol Appl Pharmacol. 235(1), p. 68-76, 2009.

DARGUSCH, R.; PIASECKI, D.; TAN, S.; LIU, Y.; SCHUBERT, D.; The role of Bax in glutamate-induced nerve cell death, J. Neurochem. 76, 295–301, 2001.

DE CONTO CINIER, C.; PETIT-RAMEL, R.; FAURE, R.; BORTOLATO, M. Cadmium accumulation and metallothionein biosynthesis in *Cyprinus carpio* tissues. Bulletin of Environmental Contamimantion and Toxicology, v. 61, p. 793-799, 1998.

DONG, S.; SHEN, H.M.; ONG, C.N. Cadmium induced apoptosis and phenotypic changes in mouse thymocytes. Mol. Cell Biochem. 222, 11-20, 2001.

DURUIBE, J. O., OGWOEGBU, M. O. C., and EGWURUGWU, J. N. Heavy metal pollution and human biotoxic effects. International. Journal of Physical Sciences, v. 2, p. 112–118, 2007.

EGAN, S.K., BOLGER, P.M., CARRINGTON, C.D. Update of US FDA's Total Diet Study food list and diets. Journal of Exposure Science and Environmental Epidemiology, 17 (6), p. 573–582, 2007.

EGAN RJ, BERGNER CL, HART PC, CACHAT JM, CANAVELLO PR, ELEGANTE MF, ELKHAYAT SI, BARTELS BK, TIEN AK, TIEN DH, MOHNOT S, BEESON E, GLASGOW E, AMRI H, ZUKOWSKA Z, KALUEFF AV. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioral Brain Research. v. 205 (1), p. 38-44, 2009.

FERNÁNDEZ-PÉREZ, B., CARIDE, A., CABALEIRO, T., LAFUENTE, A. Cadmium effects on 24h changes in glutamate, aspartate, glutamine, GABA and taurine content of rat striatum. J Trace Elem Med Biol. 24 (3), p. 212-8, 2010.

FLEEGER, J. W., CARMAN, K. R., and NISBET, R. M. Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment, v. 317, p. 207–233, 2003.

FOULKES, E. C. Cadmium. Handbook Experimental Pharmacology, v. 80, Springer-Verlag, New York, 1986.

FRIBERG, L.; ELINDER, C.-G.; KJELLSTRÖM, T.; NORDBERG, G. Cadmium and Health: a Toxicological and Epidemiological Appraisal. CRC Press, Inc., Boca Raton, FL. v. 1-2, 1986

FRIDMAN, J.S.; LOWE, S.W. Control of apoptosis by p53. Oncogene. 22, 9030–9040, 2003.

GIUSI, G., FACCIOLO, R.M., AIÒ, R., CARELLI, A., MADEO, M., BRANDMAYR, P., CANONACO, M. Some environmental contaminants influence motor and feeding behaviors in the ornate wrasse (Thalassoma pavo) via distinct cerebral histamine receptor subtypes. Environ. Health. Perspect. 113(11), 1522-9, 2005.

GOERING, P. L.; WAALKES, M. P.; KLAASSEN, C. D. Toxicology of Cadmium. In Toxicology of Metals, Biochemical Aspects, Handbook Experimental Pharmacology, v. 115, p. 189–214, Springer Verlag, New York, 1995.

GONÇALVES, J. F.; FIORENZA, A. M.; SPANEVELLO, R. M.; MAZZANNTI, C. M.; BOCHI, G. V.; ANTES, F. G.; STEFANELLO, N.; RUBIN, M. A.; DRESSLER, V.; MORSCH, V. M.; SCHETINGER, M. R. C. N-acetylcysteine prevents memory deficits, the decrease in acetylcholinesterase activity and oxidative stress in rats exposed to cadmium. Chemico-Biological Interactions. 186, p. 53–60, 2010.

GONZALEZ, P.; BAUDRIMONT, M.; BOUDOU, A.; BOURDINEAUD, J. P. Comparative effects of direct cadmium contamination on gene expression in gills, liver, skeletal muscles and brain of the zebrafish (Danio rerio). BioMetals v. 19, p. 225–235, 2006. DOI 10.1007/s10534-005-5670-x

GREGER, M. Metal availability, uptake, transport and accumulation in plants, pp. 1–27, in M. N. V. Prasad (ed.). Heavy Metal Stress in Plants: From Biomolecules to Ecosystems. Springer, Berlin, 2004.

HABEEBU, S.S.; LIU, J.; KLASSEN, C.D. Cadmium induced apoptosis in mouse liver. Toxicol Appl Pharmacol. 149, 203-9, 1998.

HAN, F. X., BANIN, A., SU, Y., MONTS, D. L., PLODINEC, M. J., KINGERY, W. L., and TRIPLETT, G. E. Industrial age anthropogenic inputs of heavy metals into the pedosphere. Naturwissenschaften, v. 89, p. 497–504, 2002.

HANSEN, J.M., ZHANG, H., JONES, D.P. Differential oxidation of thioredoxin-1,-2, and glutathione by metal ions. Free Radical Biology & Medicine, v. 40, p. 138–145, 2006.

HOFSETH, L.J.; HUSSAIN, S.P.; HARRIS, C.C. p53: 25 years after its discovery. Trends Pharmacol. Sci. 25, 177–181, 2004.

[IARC] International Agency for Research on Cancer. Monographs on cancer risks. Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. IARC Scientific Publications, Lyon, v. 58, p. 1–415, 1994.
JÄRUP, L.; BERGLUND, M.; ELINDER, C. G.; NORDBERG, G.; VAHTER, M. Health effects of cadmium exposure–a review of the literature and a risk estimate. Scandinavian Journal of Work, Environment and Health, v. 24 (Suppl. 1), p. 1–51, 1998.

JÄRUP, L., AKASSON, A. Current status of cadmium as an environmental health problem. Toxicology and Applied Pharmacology. v. 238, p. 201–208, 2009.

JAYANTHI, S.; DENG, X.; NOAILLES, P.A.; LADENHEIM, B.; CADET, J.L. Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades, FASEB J. 18, 238–251, 2004.

JIHEN, E.H., et al., Interrelationships between cadmium, zinc and antioxidants in the liver of the rat exposed orally to relatively high doses of cadmium and zinc. Ecotoxicol. Environ. Saf. (2011), doi:10.1016/j.ecoenv.2011.06.008.

JOERS, A.; JAKS, V.; KASE, J.; TOIVO, M. p53-dependent transcription can exhibit both on/off and graded response after genotoxic stress. Oncogene. 23, 6175–6185, 2004.

KLASCHKA, U. The infochemical effect—a new chapter in ecotoxicology. Environmental Science and Pollution. Research, v. 15, p. 452–462, 2008.

KORICHEVA, J., LARSSON, S., and HAUKIOJA, E. Insect performance on experimentally stressed woody plants: A metaanalysis. Annual Review of Entomology, v. 43, p. 195–216, 1998.

KOSTRZEWSKA, A., SOBIESZEK, A. Diverse actions of cadmium on the smooth muscle myosin phosphorylation system. FEBS Letters, v. 263, p. 381–384, 1990.

KRUMSCHNABEL, G., EBNER, H. L.; HESS, M. W.; VILLUNGER, A.; Apoptosis and necroptosis are induced in rainbow trout cell lines exposed to cadmium. Aquatic Toxicology. 99 (1), p73-85, 2010.

KUSCH, R. C.; KRONE, P. H.; CHIVERS, D. P. Chronic exposure to low concentrations of waterborne cadmium during embryonic and larval development results in the long-term hindrance of antipredator behavior in zebrafish., v. 27(3), p. 705-10, 2008.

LARSEN, E.H., ANDERSEN, N.L., MOLLER, A., PETERSEN, A., MORTENSEN, G.K., PETERSEN, J. Monitoring the content and intake of trace elements from food in Denmark. Food Additives and Contaminants, 19 (1), p. 33–46, 2002.

LASS, S., SPAAK, P. Chemically induced anti-predator defences in plankton: a review. Hydrobiologia. v. 491, p. 221–239, 2003.

LEFCORT, H., AGUON, M. Q., BOND, K. A., CHAPMAN, K. R., CHAQUETTE, R., CLARK, J., KORNACHUK, P., LANG, B. Z., and MARTIN, J. C. Indirect effects of heavy metals on parasites may cause shifts in snail species composition. Archives of Environmental Contamiantion and Toxicology, v. 43, p. 34–41, 2002.

LIU, Y.; TEMPLETON, D.M. Cadmium activates CaMK-II and initiates CaMK-IIdependent apoptosis in mesangial cells. FEBS Letters, v. 581, p. 1481–1486, 2007.

LIU. Y.; WANG, J.; WEI, Y.; ZHANG, H.; XUA, M.; DAI, J. Induction of time-dependent oxidative stress and related transcriptional effects of perfluorododecanoic acid in zebrafish liver. Aquatic Toxicology. v. 89, p. 242–250, 2008.

LIU, F., TANG, Y., DU, R., YANG, H., WU, Q., and RONGLIANG, Q. Root foraging for zinc and cadmium requirement in the Zn/ Cd hyperaccumulator plant Sedum alfredii. Plant Soil. (2009), doi:10.1007/s11104-009-0060-8.

LIU, M.; TEE, C.; ZENG, F.; SHERRY, J.P.; DIXON, B.; BOLS, N.C.; DUNCKER, B.P. Characterization of p53 expression in rainbow trout. Comp. Biochem. Physiol. C., Toxicol Pharmacol. 154(4), 326-32, 2011.

LLOBET, J.M., FALCO, G., CASAS, C., TEIXIDO, A., DOMINGO, J.L. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. Journal of Agricultural and Food Chemistry, 51 (3), p. 838–842, 2003.

LÓPEZ, E.; FIGUEROA, S.; OSET-GASQUE, M.J.; GONZÁLEZ, M.P. Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture. British Journal of Pharmacology. 138, 901-11, 2003.

LÜRLING, M., and SCHEFFER, M. Info-disruption: pollution and the transfer of chemical information between organisms. Trends in Ecology & Evolution. v. 22, p. 374–379, 2007.

MACINTOSH, D.L., SPENGLER, J.D., OZKAYNAK, H., TSAI, L., RYAN, P.B. Dietary exposures to selected metals and pesticides. Environmental Health Perspectives. 104 (2), p. 202–209, 1996.

MATOVIĆ, V., BUHA, A., BULAT, Z., ĐUKIĆ-ĆOSIĆ, D. Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. Arh Hig Rada Toksikol. 62, p. 65-76, 2011.

MAZZEI, G.J., GIRARD, P.R., KUO, J.F. Environmental pollutant Cd2+ biphasically and differentially regulates myosin light chain kinase and phospholipid/Ca2+- dependent protein kinase. FEBS Letters. v. 173, p. 124–128, 1984.

MCPHERSON, T. D., MIRZA, R. S., and PYLE, G. G. Responses of wild fishes to alarm chemicals in pristine and metalcontaminated lakes. Canadian Journal of Zoology, v. 82, p. 694–700, 2004.

MORSELT, A. W. F. Environmental pollutants and diseases: A cell biological approach using chronic cadmium exposure in the animal model as a paradigm case. Toxicology, v. 70, p. 1–84, 1991.

O'CALLAGHAN, J. P., MILLER, D. Diethyldithiocarbamate increases distribution of cadmium to brain but prevents cadmium-induced neurotoxicity. Brain Res. 370, 354–358, 1986.

OKUDA, B., IWAMOTO, Y., TACHIBANA, H., SUGITA, M. Parkinsonism after cadmium poisoning. Clinical Neurology and Neurosurgery. 99, 263-265, 1997.

OLSSON, I.M., BENSRYD, I., LUNDH, T., OTTOSSON, H., SKERFVING, S., OSKARSSON, A. Cadmium in blood and urine–impact of sex, age, dietary intake, iron status, and former smoking–association of renal effects. Environ. Health Perspect. 110, 1185–1190, 2002.

PETERSON, R.T., NASS, R., BOYD, W.A., FREEDAN, J.H., DONG, K., NARAHASHI, T. Use of non-mammalian alternative models for neurotoxicological study. Neurotoxicology. 29(3), p. 546-55, 2008.

POLSTER, B.M.; FISKUM, G. Mitochondrial mechanisms of neural cell apoptosis, J. Neurochem. 90, 1281–1289, 2004.

SÁNCHEZ, M. L. (ed.). Causes and Effects of Heavy Metal Pollution, Nova Science Publishers, Hauppauge, p. 392, 2008

SCHERTZER, J. W., BOULETTE, M. L., and WHITELEY, M. More than a signal: non-signaling properties of quorum sensing molecules. Trends in Microbiology, (2009), doi:10.1016/j.tim.2009.02.001.

SHERR, C.J., MCCORMICK, F. The RB and p53 pathways in cancer. Cancer Cell. 2, 103–112, 2002.

SHUKLA, A., SHUKLA, G.S, SRIMAL, R.C. Cadmium-induced alterations in blood- brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. Human & Experimental Toxicology, v. 15, p. 400-405, 1996. doi: 10.1177/096032719601500507

SINGHAL, R.K., ANDERSON, M.E., MEISTER, A. Glutathione, a first line of defense against cadmium toxicity. FASEB J. 1(3), p. 220-3, 1987.

SLEE, E.A.; O'CONNOR, D.J.; LU, X. To die or not to die: how does p53 decide? Oncogene 23, 2809–2818, 2004.

SLOMAN, K. A.; SCOTT, G. R.; DIAO, Z.; ROULEAU, C.; WOOD, C. M.; MCDONALD, D. G. Cadmium affects the social behaviour of rainbow trout, Oncorhynchus mykiss. Aquatic Toxicology, v. 65, p. 171–185, 2003.

SLOMAN, K. A. Effects of trace metals on salmonid fish: the role of social hierarchies. Applied Animal Behavior Science, v. 104, p. 326–345, 2007.

SPENCE, R.; GERLACH, G.; LAWRENCE, C.; SMITH, C. The behavior and ecology of the zebrafish, Danio rerio. Biological Reviews of the Cambridge Philosophical Society, v. 83 (1), p. 13-34, 2008.

TAKEDA, A.; TAKEFUTA, S.; IJIRO, H.; OKADA, S.; OKU, N. 109Cd transport in rat brain. Brain Res Bull. 49(6), p. 453-7, 1999.

TEVES, M. L. U. Lixo urbano: contaminação por resíduos de tintas e vernizes. São Paulo: Fundacentro, 2001.

THÉVENOD, F. Cadmium and cellular signaling cascades: To be or not to be? Toxicology and Applied Pharmacology. 238, 221–239, 2009.

THOMAS, K.W., PELLIZZARI, E.D., BERRY, M.R. Population-based dietary intakes and tap water concentrations for selected elements in the EPA region V National Human Exposure Assessment Survey (NHEXAS). Journal of Exposure Analysis and Environmental Epidemiology, 9 (5), p. 402–413, 1999.

TOKUMOTO, M., FUJIWARA, Y., SHIMADA, A., HASEGAWA, T., SEKO, Y., NAGASE, H., SATOH, M. Cadmium toxicity Is caused by accumulation of p53 through the down-regulation of Ube2d family genes *in vitro* and *in vivo*. The Journal of Toxicological Sciences, 36 (2), p. 191-200, 2011.

TROPEPE, V.; SIVE, H. L. Can zebrafish be used as a model to study the neurodevelopmental causes of autism? Genes Brain Behavior, v. 2, p. 268– 281, 2003.

WARD, M.W.; KOGEL, D.; PREHN, J.H. Neuronal apoptosis: BH3 only proteins the real killers? J. Bioenerg. Biomembr. 36, 295–298, 2004.

[WHO] World Health Organization. Cadmium. Geneva, 1992. (Environment Health Criteria 134).

WILLERS, S., GERHARDSSON, L., LUNDH, T. Environmental tobacco smoke (ETS) exposure in children with asthma — relation between lead and cadmium, and cotinine concentrations in urine. Respiratory Medicine, v. 99, p. 1521–1527, 2005.

WILSON, A. J. The Living Rock: The Story of Metals Since Earliest Time and Their Impact on Civilization. Whitehurst & Clark, Flemington. p. 272, 1996.

YANNAI, S., BERDICEVSKY, I. Formation of Organic Cadmium by Marine Microorganisms. Ecotoxicology and Environmental Safety. v. 32, p. 209-214, 1994.

YSART, G., MILLER, P., CROASDALE, M., CREWS, H., ROBB, P., BAXTER, M., DE L'ARGY, C., HARRISON, N. 1997 UK Total Diet Study — dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. Food Additives and Contaminants, 17 (9), p. 775–786, 2000.

ANEXOS

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 004/11 – CEUA Porto Alegre, 06 de janeiro de 2011.

Senhora Pesquisadora:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 10/00214, intitulado: **"Efeito da exposição ao cádmio sobre dano oxidativo, atividade da via de sinalização caderina/catenina e comportamento de zebrafish".**

Sua investigação está autorizada a partir da presente data.

Atenciosamente,

Profa. Dra. Anamaria Gonçalves Feijó
Coordenadora do CEUA – PUCRS

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