UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE EDUCAÇÃO SUPERIOR DO OESTE – CEO
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

DISSERTAÇÃO DE MESTRADO

EFEITOS TÓXICOS E COMPORTAMENTAIS DA EPRINOMECTINA SOBRE *Folsomia candida* E *Rhamdia quelen*

SUÉLEN SERAFINI

CHAPECÓ, 2019

SUÉLEN SERAFINI

EFEITOS TÓXICOS E COMPORTAMENTAIS DA EPRINOMECTINA SOBRE *Folsomia candida* E *Rhamdia quelen*

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, Área de Concentração Ciência e Produção Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de **Mestre em Zootecnia**

Orientador: Dr. Dilmar Baretta

Co-orientador(es): Dr. Aleksandro Schafer da Silva
Dra. Julia Corá Segat

Chapecó, SC, Brasil

2019

Ficha catalográfica elaborada pelo programa de geração automática da

Biblioteca Setorial do CEO/UDESC,

com os dados fornecidos pelo(a) autor(a)

Serafini, Suélen
Efeitos tóxicos e comportamentais da eprinomectina sobre Folsomia candida e Rhamdia quelen / Suélen Serafini. -- 2019.
100 p.

Orientador: Dilmar Baretta
Coorientador: Aleksandro Schafer da Silva
Coorientadora: Julia Corá Segat
Dissertação (mestrado) -- Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia, Chapecó, 2019.

1. Antiparasitário. 2. Avermectina. 3. Colêmbolos. 4. Contaminante ambiental. 5. Peixes. I. Baretta, Dilmar . II. Schafer da Silva, Aleksandro . Corá Segat, Julia . III. Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia. IV. Titulo.

Universidade do Estado de Santa Catarina
UDESC Oeste
Programa de Pós-Graduação em Zootecnia

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

EFEITOS TÓXICOS E COMPORTAMENTAIS DA EPRINOMECTINA SOBRE *Folsomia candida* E *Rhamdia quelen*

Elaborada por
Suélen Serafini

como requisito parcial para obtenção do grau de
Mestre em Zootecnia

Comissão Examinadora:

Dr. Dilmar Baretta
Presidente/Orientador (UDESC Oeste)

Dr. Diogo Luiz de Alcantara Lopes
Membro interno PPGZOO (UDESC Oeste)

Dr. Paulo Roger Lopes Alves
Membro externo PPGZOO (UFFS)

Chapecó, 22 de Fevereiro de 2019

DEDICATÓRIA

Ao meu esposo Junior por seu amor e companheirismo em todos os momentos.
Ao nosso filho Álvaro, razão maior de todo o meu esforço.
Aos meus pais Claudenir e Teresinha e ao meu irmão Sandro por todo apoio e motivação.
À minha sogra Janete por todo o cuidado que teve para com o Álvaro em minhas ausências.
À minha avó Santina (*in memoriam*), tia Claudete e prima Amanda por suas presenças confortantes na distância de casa.
Às minhas amigas Kaine e Fernanda pelos laços de amizade que renovaram minhas forças.
Dedico.

AGRADECIMENTOS

Ao meu orientador Dr. Dilmar Baretta pelo auxílio na construção deste estudo e insistência pela compreensão máxima dos interesses científicos e das brechas do conhecimento mundial.

Ao meu coorientador Dr. Aleksandro Schafer da Silva pela inspiração no desenvolvimento da ideia principal do estudo, sua presença e auxílio constantes. Não haveria este trabalho sem sua colaboração e confiança.

À minha coorientadora Drª. Julia Corá Segat pelos conhecimentos técnicos e científicos repassados.

Ao Zootecnista Junior Gonçalves Soares por seus auxílios em cada uma das etapas deste estudo.

Às Zootecnistas Kaine Cristine Cubas da Silva e Fernanda Picoli por suas contribuições técnicas e científicas.

Aos meus colegas e amigos do Laboratório de Solos e Sustentabilidade pela ajuda na execução prática do experimento terrestre.

À Universidade do Federal de Santa Maria (UFSM) pela contribuição estrutural e profissional e na execução prática do experimento aquático.

À Universidade do Estado de Santa Catarina (UDESC) por garantir ensino de qualidade e estrutura para a pesquisa científica.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de mestrado.

Enfim, aos demais que de alguma maneira colaboraram nesta minha etapa profissional.

Muito Obrigada!

RESUMO
Dissertação de Mestrado
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

EFEITOS TÓXICOS E COMPORTAMENTAIS DA EPRINOMECTINA SOBRE *Folsomia candida* E *Rhamdia quelen*

AUTOR: Suélen Serafini
ORIENTADOR: Dr. Dilmar Baretta
Chapecó, 22 de Fevereiro de 2019

O objetivo deste estudo foi reconhecer efeitos ecotoxicológicos da eprinomectina sobre toxicidade e comportamento de organismos da fauna edáfica e aquáticos. Para tanto, foram realizados dois experimentos, um terrestre e outro aquático. Para o experimento terrestre, doses de eprinomectina (0; 0,5; 1; 2; 4; 8; 12; 16 e 20 mg kg^{-1} de solo seco) combinadas com os solos Neossolo Quartzarênico e Latossolo Vermelho foram utilizadas para verificar efeitos sobre sobrevivência, reprodução e comportamento de fuga de colêmbolos (*Folsomia candida*). Para o experimento aquático, peixes jundiás (*Rhamdia quelen*) foram expostos por 24 e 48 h às concentrações de eprinomectina na água (0,0; 1,124; 1,809 e 3,976 µg L^{-1}), seguido de 48 h de recuperação em água livre de eprinomectina, a fim de verificar efeitos sobre o comportamento e variáveis bioquímicas no cérebro e fígado, bem como a capacidade de homeostase dos peixes pós-exposição. Nos testes terrestres, efeitos da eprinomectina sobre os colêmbolos foram observados em ambos os solos. A letalidade ocorreu a partir da dose de 8 e 20 mg kg^{-1} de solo seco em Neossolo e em Latossolo, respectivamente, com efeitos inferiores a 50% nas doses letais. Reduções da taxa de reprodução foram observadas a partir de 8 mg kg^{-1} de solo seco em Neossolo e 0,5 mg kg^{-1} de solo seco em Latossolo. O valor de EC_{50} calculado para Neossolo foi de 4,38 $\pm$ 0,62 mg kg^{-1} de solo seco e para Latossolo foi acima da maior dose testada. A fuga ocorreu a partir de 0,5 mg kg^{-1} de solo seco para ambos os solos. Em Neossolo, todas as doses apresentaram fuga superior a 95% e em Latossolo o valor de AC_{50} foi < 0,5 mg kg^{-1} de solo seco. Nos testes aquáticos, nas duas maiores concentrações de eprinomectina (1,809 e 3,976 µg L^{-1}) os jundiás apresentaram alterações no comportamento; hiperlocomoção e o maior tempo na superfície. Nestas mesmas concentrações, os níveis de espécies reativas ao oxigênio aumentaram no cérebro e a atividade enzimática da acetilcolinesterase diminuiu. Na concentração mais alta a atividade da bomba de sódio-potássio ATPase foi reduzida. No fígado houve aumento dos níveis de espécies reativas ao oxigênio em todas as concentrações de eprinomectina (1,124; 1,809 e 3,976 µg L^{-1}). Níveis da capacidade antioxidante agonística para radicais peroxil e das atividades das enzimas antioxidantes glutationas peroxidase e S-transferase reduziram na maior concentração. Redução da atividade da enzima antioxidante superóxido dismutase e o aumento dos níveis de lipoperoxição foram observados na maior concentração. A atividade da enzima adenilato quinase foi reduzida nas duas maiores concentrações e da piruvato quinase na maior concentração. Efeitos residuais nos jundiás foram observados mesmo após 48 h pós-exposição. Porém, mais estudos deverão ser realizados quanto aos efeitos dos metabólitos da eprinomectina após administração em animais de produção, impactos sobre a biodiversidade do solo e serviços ecossistêmicos, além do comprometimento fisiológico em peixes nos sistemas produtivos e naturais, a fim de esclarecerem-se mais os aspectos da capacidade tóxica da eprinomectina no ambiente.

Palavras-chave: Antiparasitário, avermectina, colêmbolos, contaminante ambiental, peixes

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

TOXIC AND BEHAVIORAL EFFECTS OF EPRINOMECTIN ON *Folsomia candida* AND *Rhamdia quelen*

AUTHOR: Suélen Serafini
ADVISER: Dr. Dilmar Baretta
Chapecó, February, 22nd, 2019

The objective of this study was to recognize eprinomectin ecotoxicological effects on the toxicity and behavior of edaphic fauna and aquatic organisms. For that, two experiments were carried out, one terrestrial and other aquatic. For the terrestrial experiment, doses of eprinomectin (0; 0.5; 1; 2; 4; 8; 12; 16 and 20 mg kg^{-1} of dry soil) combined with Entisol and Oxisol soils were used to verify effects on survival, reproduction and avoidance behavior of springtails (*Folsomia candida*). For the aquatic experiment, fish jundiás (*Rhamdia quelen*) were exposed for 24 and 48 h at the concentrations of eprinomectin in water (0.0; 1.124; 1.809 and 3.976 μg L^{-1}), followed by 48 h of recovery in eprinomectin-free water, in order to verify effects on behavior and biochemical variables in the brain and liver, as well as the post-exposure capacity of homeostasis of fish. In terrestrial tests, effects of eprinomectin on springtails were observed in both soils. The lethality occurred from the dose of 8 and 20 mg kg^{-1} of dry soil in Entisol and Oxisol, respectively, with effects lower than 50% in lethal doses. Reproduction rate reductions were observed from 8 mg kg^{-1} of dry soil in Entisol and 0.5 mg kg^{-1} of dry soil in Oxisol. The EC_{50} value calculated for Entisol was 4.38 + 0.62 mg kg^{-1} of dry soil and for Oxisol was above the highest dose tested. The average occurred from 0.5 mg kg^{-1} dry soil for both soils. In Entisol, all doses had average of more than 95% and in Oxisol the value of AC_{50} was < 0.5 mg kg^{-1} of dry soil. In the aquatic tests, at the two largest concentrations of eprinomectin (1.809 and 3.976 μg L^{-1}) the jundiás showed changes in behavior; hyperlocomotion and the longer time on the surface. At these same concentrations, levels of reactive oxygen species increased in the brain and the enzymatic activity of acetylcholinesterase decreased. At the highest concentration the activity of the sodium-potassium ATPase pump was reduced. In the liver, there was an increase in the levels of oxygen reactive species at all concentrations of eprinomectin (1.124; 1.809 and 3.976 μg L^{-1}). Levels of the antioxidant capacity against peroxyl radicals and the activities of the antioxidant enzymes glutathione peroxidase and S-transferase reduced at the highest concentration. Reduction of the activity of the antioxidant enzyme superoxide dismutase and increased levels of lipoperoxidation were observed at the highest concentration. The activity of the adenylate kinase enzyme was reduced at the two highest concentrations and the pyruvate kinase at the highest concentration. Residual effects on jundiás were observed even after 48 h post-exposure. However, further studies should be carried out on the effects of eprinomectin metabolites after administration to production animals, impacts on soil biodiversity and ecosystem services, as well as the physiological involvement of fish in the productive and natural systems, in order to clarify more aspects of the toxic capacity of eprinomectin in the environment.

Keywords: Antiparasitic, avermectin, environmental contaminant, fish, springtails

SUMÁRIO

CAPÍTULO I **10**

1. REVISÃO DE LITERATURA **10**

1.1 PARASITOSES E ANTIPARASITÁRIOS **10**

1.2 EPRINOMECTINA **13**

1.3 ANTIPARASITÁRIOS E O MEIO AMBIENTE **15**

1.4 ECOTOXICOLOGIA COMO FERRAMENTA DE MONITORAMENTO AMBIENTAL: TERRESTRE E AQUÁTICO **16**

1.5 EPRINOMECTINA E O MEIO AMBIENTE **17**

1.6 OBJETIVOS **19**

1.6.1 OBJETIVO GERAL 19

1.6.2 OBJETIVOS ESPECÍFICOS 20

CAPÍTULO II **21**

2. ARTIGO E MANUSCRITOS **21**

2.1 MANUSCRITO I **22**

2.2 ARTIGO I **43**

2.3 MANUSCRITO II **68**

3. CONSIDERAÇÕES FINAIS **92**

REFERÊNCIAS **93**

ANEXOS **99**

CAPÍTULO I

1. REVISÃO DE LITERATURA

1.1 PARASITOSES E ANTIPARASITÁRIOS

Os problemas sanitários ocasionados por endo e ectoparasitos são fatores limitantes para as produções animais (BORGES et al., 2013; OLIVEIRA et al., 2017; PERRY e RANDOLPH, 1999), uma vez que refletem na queda dos índices zootécnicos e, consequentemente, no aumento dos custos de produção (GRISI et al., 2014; STOTZER, 2014). A ocorrência dos parasitos depende, por exemplo, de elementos como: temperatura, precipitação pluviométrica, tipo de solo, topografia, tipo e manejo da pastagem, espécie, raça, idade, estado fisiológico e nutricional e manejo dos animais (BORGES et al., 2013; GRISI et al., 2014; OLIVEIRA et al., 2017). No Brasil, os ruminantes têm sido os principais afetados pelas parasitoses (BORGES et al., 2013; GRISI et al., 2014; OLIVEIRA et al., 2017; STOTZER et al., 2014), devido à forma como são criados e/ou produzidos, em que se destaca, principalmente, os sistemas produtivos mais extensivos, nos quais há acesso por pastoreio aos estágios infecciosos dos parasitos, associado aos ciclos de vida diretos destes (BORGES et al., 2013; COOP e KYRIAZAKIS, 2001; MCKELLAR e JACKSON, 2004; RUAS e BERNE, 2007). Assim como a predominância de climas tropicais e subtropicais favorece o desenvolvimento desses agentes (ABBAS et al., 2014; GRISI et al., 2014).

Os bovinos, especialmente, representam uma das classes zootécnica mais afetada pelo parasitismo e com maior participação, destacada há anos, no mercado farmacêutico mundial (BNDES SETORIAL, 2007). Em relação às perdas de rendimento em produção de leite e ganho de peso, estimou-se no Brasil perdas anuais totais de US$ 13,96 bilhões, considerando-se os parasitismos por nematódeos gastrointestinais, carrapato *Rhipicephalus microplus* e moscas das espécies *Haematobia irritans*, *Dermatobia hominis*, *Cochliomyia hominivorax* e *Stomoxys calcitrans* (GRISI et al., 2014). Deste valor total, as principais perdas econômicas são representadas pelo parasitismo por nematódeos gastrointestinais (US$ 7,11 bilhões) e pelo *Rhipicephalus microplus* (US$ 3,24 bilhões) (GRISI et al., 2014).

A administração profilática e terapêutica de antiparasitários químico-farmacológicos aos animais, ainda é o método mais disseminado de promover a profilaxia e o tratamento de

parasitoses (MCKELLAR e JACKSON, 2004; MOLENTO, 2005; SUAREZ et al., 2016). Dados apresentados pela Confederação Nacional do Ramo Químico (CNQ CUT) (2015) demonstram que os maiores produtores e os maiores mercados farmacêuticos veterinários do mundo são Estados Unidos e Brasil, com participações de 40% e 8%, respectivamente. Pesquisas do panorama da indústria farmacêutica veterinária mundial e nacional demonstram que os produtos com maior participação no mercado são os antiparasitários (BNDES SETORIAL, 2007; SINDAN, 2017). No Brasil, no ano de 2017, o faturamento líquido estimado com produtos farmacêuticos veterinários foi de R$5,3 bilhões, com participação de 27,2% deste faturamento representado pelos antiparasitários (SINDAN, 2017). A nível mundial, os anti-helmínticos utilizados para profilaxia e tratamento de helmintoses compreendem o maior setor da indústria farmacêutica animal por volume e valor (MCKELLAR e JACKSON, 2004). A pesquisa e o desenvolvimento de anti-helmínticos exigem um grande esforço da indústria farmacêutica veterinária, sendo, provavelmente, a única área onde esforços e sucesso em saúde animal excedem os da saúde humana (MCKELLAR e JACKSON, 2004).

Os antiparasitários químico-farmacológicos são produtos que matam ou paralisam parasitas, sejam eles endo ou ectoparasitos, reduzindo a infecção parasitária a nível tolerável (MONTEIRO, 2011). Formulações comerciais consistem em um ou mais ingredientes ativos que têm eficácia específica contra parasitos, juntamente com uma série de ingredientes inertes ou não ativos. Os ingredientes inertes não ativos típicos são: solventes orgânicos ou inorgânicos, estabilizadores, conservantes, emulgentes, etc. O termo antiparasitário pode ser usado tanto para produtos comerciais acabados quanto para os ingredientes que são efetivos contra os parasitos (ANDRADE, 2008). A profiláxia ou tratamento das parasitoses com antiparasitários consistem na administração do produto químico-farmacêutico, associado ao manejo integrado de fornecimento considerando-se aspectos relacionados ao hospedeiro, ao meio, aos parasitos e à infecção (SHALABY, 2013). Formulações de antiparasitários comerciais apresentam variedades de formas de administração, de tal forma que podem ser aplicados oralmente, topicamente por aspersões ou *pour-on*, via bolus intra-ruminal ou injetável (ZORTÉA et al., 2017).

Há inúmeras marcas e formulações comerciais de antiparasitários, contudo, há um número limitado de moléculas químicas que caracterizam os princípios ativos destes, apesar das variedades zootécnicas, dos diferentes gêneros parasitários, assim como das diferenças regionais, culturais, sociais e econômicas a nível nacional e também mundial. Esta limitação é

ainda maior quando consideramos grupos químicos de moléculas que unem semelhantes modos de ação frente aos parasitos (KÖHLER, 2001; MCKELLAR e JACKSON, 2004; SHALABY, 2013). Outro grande problema no setor farmacológico de antiparasitários é que certas moléculas, denominadas endectocidas, devido à ação eficiente em endo e ectoparasitos (SHOOP et al., 1995; SHOOP et al., 1996), apresentam uso intensificado pela dupla função. Esta característica é comum em moléculas do grupo químico das lactonas macrocíclicas (KÖHLER, 2001; SHOOP et al., 1995) e caracteriza uma das motivações para o uso maciço da ivermectina mundialmente (GEARY, 2005).

No Brasil, dentre os principais grupos químicos utilizados na atualidade em formulações de antiparasitários farmacológicos veterinários, especialmente para ruminantes (Quadro 1), destacamos as lactonas macrocíclicas, os piretróides, as salicilanilidas, os imidazotiazóis e os fenilpirazóis (NETTO et al., 2005; ZORTÉA et al., 2017). Dentre estes, destacam-se as lactonas macrocíclicas, por serem moléculas amplamente utilizadas na agropecuária e na indústria em escala global (LUMARET et al., 2012; VASSILIS et al., 2016).

Quadro 1. Principais grupos químicos e moléculas presentes em antiparasitários farmacológicos veterinários utilizados amplamente no Brasil para ruminantes.

Grupos	Moléculas	Mecanismos de Ação	Utilização
Lactonas Macrocíclicas	Abamectina Ivermectina Moxidectina Eprinomectina	Abertura dos canais de Cl dos neutrotransmissores GABA	Ectoparasitária Endoparasitária
Piretróides	Cipermetrina Fluazuron	Abertura dos canais de Na dos neurotransmissores GABA	Ectoparasitária
Salicilanilidas	Closantel	Inibidor da produção de ATP	Endoparasitária
Imidazotiazóis	Levamisol	Inibidor seletivo de enzimas na musculatura; impede a conversão do fumarato em succinato	Endoparasitária
Fenilpirazóis	Fipronil	Bloqueio da passagem dos íons Cl pelos neurotransmissores GABA	Ectoparasitária

Fonte: KÖHLER (2001); MCKELLAR (1997); NETTO et al. (2005); ZORTÉA et al. (2017).

Associado a este número limitado de moléculas antiparasiátias, o uso indiscriminado dos antiparasitários químico-farmacológicos, o uso de formulações mistas com mais de uma molécula ou grupo químico, a não adoção de programas estratégicos de controle, a ausência da alternância adequada dos grupos químicos das moléculas bioativas ao longo do tempo, devido à negligência ou falta de conhecimento por parte dos produtores rurais e técnicos,

contribuiu para o aparecimento da resistência (ABBAS et al., 2014; BORGES et al., 2013; KÖHLER, 2001; SANGSTER, 2001; SHALABY, 2013; WOLSTENHOLME et al., 2004). Com a resistência parasitária, uma gama de antiparasitários tornou-se ineficiente na profilaxia e tratamento das parasitoses, o que contribui para perdas produtivas e econômicas ainda maiores (BORGES et al., 2013; GRISI et al., 2014; STOTZER et al., 2014).

Para superar este problema, a indústria farmacêutica tem buscado alternativas por meio da pesquisa moléculas antiparasitárias e sua inclusão em novos medicamentos veterinários. Contudo, o desenvolvimento de novas drogas antiparasitárias é dificultado por uma série de fatores de ordem técnica, econômica e também ética (HOPKINS et al., 2007; MCKELLAR e JACKSON, 2004; WOODS e WILLIAMS, 2007). Neste sentido, a indústria farmacêutica não desempenhou esforços na criação de moléculas diferenciadas com novos modos de ação para a superação da resistência parasitária, mas sim, a extração de moléculas novas derivadas de moléculas ou grupos químicos tradicionais (MCKELLAR e JACKSON, 2004). Neste contexto, destaca-se a mais recente lactona macrocíclica, a eprinomectina, cuja síntese conjunta com as demais avermeticnas, entre as décadas de 1970-1990, permitiu um aumento de 1.500 vezes na potência antiparasitária contra nematódeos e artrópodes (CVETOVICH et al., 1994; EMEA, 1996; GEARY, 2005), assim como uma nova alternativa para o tratamento de parasitoses em ruminantes (SHOOP et al., 1996).

1.2 EPRINOMECTINA

Dentre o grupo das lactonas macrocíclicas, a família das avermectinas contempla compostos quimicamente relacionados aos antibióticos macrocíclicos, contudo sem atividade antimicrobiana e antifúngica, porém com destaque à atividade anti-helmíntica extraordinariamente potente, juntamente com uma atividade acaricida e inseticida. As avermectinas foram descobertas a partir de 1975, provenientes de culturas de fungos de solo da espécie *Streptomyces avermitilis* (BURG et al., 1979; SHOOP et al., 1995). A eprinomectina (MK-397 ou 4"-epi-acetylamino-4"-deoxy-avermectin B1) é a mais recente avermectina descoberta e utilizada em tratamentos antiparasitários (SHOOP et al., 1996). Também obtida da fermentação de fungos do gênero *Streptomyces* sp. (EMEA, 1996; MERCK, 1996; SHOOP et al., 1995). É uma molécula de base farmacológica semi-sintética derivada da avermectina B1ou abamectina, constituída por dois homólogos, B1a e B1b, que diferem por um grupo metileno, sendo definida como não inferior a 90% B1a e não superior a 10% B1b, com concentração de até 95% e estabilização por vitamina E que compõem o

restante da composição da molécula comercial (EMEA, 1996; MERCK, 1996). Esta molécula é classificada como de amplo espectro, originalmente selecionada como nematicida, inseticida e acaricida para ser administrada por via tópica (EMEA, 1996; SHOOP e SOLL, 2002; SHOOP et al., 1996).

A eprinomectina mantém as mesmas atividades antiparasitárias endectocidas das demais avermectinas (CVETOVICH et al., 1994; GERENUTTI e SPINOSA, 1997; SHOOP e SOLL, 2002). Contudo, foi desenvolvida para ser uma molécula com maior impacto antiparasitário comparado às demais moléculas tradicionais de seu grupo, particularmente para a profilaxia e tratamento de nematódeos (WOLSTENHOLME e ROGERS, 2005). Embora o mecanismo exato de ação da eprinomectina não seja conhecido, é considerado semelhante ao das demais lactonas macrocíclicas. Estas atuam como agonistas do neurotransmissor ácido γ-aminobutírico (GABA) em células nervosas ou também se ligam aos canais de cloro com glutamato, o que causa a hiperpolarização das células nervosas e musculares de invertebrados e, em ambos os casos, bloqueiam a transmissão de sinais neurais (DOURMISHEV et al., 2005; GEARY e MORENO, 2012; TRETTER e STEPHEN, 2008; WOLSTENHOLME e ROGERS, 2005), provocando perda de coordenação motora, paralisia e morte dos organismos (GEARY e MORENO, 2012; GEARY, 2005).

Apesar de descoberta no século passado, a eprinomectina vem se destacando na atualidade como um princípio ativo eficiente de uso recente em produtos comerciais antiparasitários, especialmente anti-helmínticos, disponíveis tanto para uso tópico quanto injetável. O número de produtos farmacológicos comerciais que a utilizam em suas formulações ainda é consideravelmente baixo e o custo destes é relativamente elevado, comparado às demais avermectinas. Porém, este valor agregado ao uso da eprinomectina pode ser justificado em função de seu amplo espectro de ação, longo período residual de proteção (AKSIT et al., 2016; COUMENDOUROS et al., 2003; CRAMER et al., 2000; EPE et al., 1999), efetividade dos resultados obtidos e, principalmente, ausência de um período de carência dos produtos farmacológicos, recomendado para produtos como carne e leite (DORNY et al., 2000; EPE et al., 1999; GAWOR et al., 2000; MERCK, 1996). A isto se deve também à sua característica molecular mais hidrofílica (MERCK, 1996), o que a difere das demais lactonas que se caracterizam por níveis residuais mais prolongados de eliminação, especialmente no leite (SCHENCK e LAGMAN, 1999).

Após administração de eprinomectina, sendo recomendado para bovinos 0,5 mg kg^{-1} de peso corporal em formulações *pour-on* ou 0,36 mg kg^{-1} de peso corporal para formulações

injetáveis, a metabolização ocorre principalmente no fígado. Cerca de 85,9% da dose é excretada nas fezes dos bovinos de forma inalterada. Nas fezes de bovinos, a eprinomectina pode ser excretada entre 0,80 e 13,6 dias após a administração subcutânea e entre 1 e 20 dias após a administração tópica. A administração subcutânea resulta em maior excreção fecal do que com administração tópica, 1188,9 e 311,5 ηg dia^{-1} g^{-1}, respectivamente (AKSIT et al., 2016). Sua concentração nas fezes em uso tópico varia de aproximadamente 0,0036 a 1,80 mg kg^{-1} de peso úmido, considerando a detecção da fração B1a da avermectina e sua recuperação de 79,50% (HALLEY et al., 2005).

A metabolização da eprinomectina envolve alguns metabólitos, porém com base em resultados cromatográficos, há apenas um metabólito importante nas fezes, este representa 7,4% do total de resíduos de molécula e é mais polar. Valores de solubilidade em água (pH 7,26) e log [(octanol)/(tampão; pH 6,8)] coeficiente de partição foram estimados em 3,5 ± 0,2 mg L^{-1} e 5,4 ± 0,3, respectivamente. Nenhum valor de pKa foi relatado para eprinomectina na faixa de pH de 3 a 10 (MERCK, 1996).

1.3 ANTIPARASITÁRIOS E O MEIO AMBIENTE

Quanto à entrada de novas moléculas antiparasitárias no mercado farmacológico, a comunidade científica também passou a pressionar as autoridades reguladoras para a problemática da contaminação ambiental por medicamentos veterinários ou seus metabólitos (BOXALL et al., 2002; MATAMOROS e BAYONA, 2006; SARMAH et al., 2006; ZORTÉA et al., 2017). Uma gama de moléculas de base farmacológica tradicional e usadas para produção de medicamentos veterinários, não são completamente metabolizadas pelo organismo e apresentam a excreção de resíduos bioativos nas fezes e urinas dos animais (HALLING-SØRENSEN et al., 1998; LUMARET et al., 2012; MCKELLAR, 1997; SARMAH et al., 2006; ZORTÉA et al., 2018). Ao considerarmos a busca dentro da zootecnia pela sustentabilidade dos sistemas de produção animal, estes resíduos podem ser potencialmente transferidos para os compartimentos ambientais solo e/ou água. Dentre as formas de contaminação destacam-se: descarga inadequada de efluentes agropecuários; uso de efluentes das produções animais como fertilizantes orgânicos ou por defecação direta dos animais em áreas de pastagem; escoamento agrícola ou descarte inadequado de medicamentos não utilizados, por aplicação direta ou por descarte inadequado de embalagens, entre outros (CHRISTIAN et al., 2003; HALLING-SØRENSEN et al., 1998; JENSEN e SCOTT-FORDSMAND, 2012; LUMARET et al., 2012; VASSILIS et al., 2016).

Quando em contato com o solo ou a água, moléculas ou metabólitos podem causar impacto sobre os organismos não-alvo responsáveis pela decomposição do material orgânico e ciclagem de nutrientes no solo (DIAO et al., 2007; FLOATE et al., 2005; IWASA et al., 2005; JENSEN e SCOTT-FORDSMAND, 2012; JENSEN et al., 2009; VERDÚ et al., 2015; ZORTÉA et al., 2015; ZORTÉA et al., 2017; ZORTÉA et al., 2018) ou também para organismos aquáticos (ALAK et al., 2017; OLIVEIRA et al., 2016; POLAT et al., 2002; TREMBLAY e WRATTEN, 2002), tanto em nível de toxicidade aguda, quanto crônica ou comportamental, culminando em desequilíbrios na cadeia trófica que podem vir a comprometer severamente o ecossistema.

A toxicidade ambiental das moléculas e dos seus resíduos depende da composição total do medicamento farmacológico, da concentração destes nos efluentes ou resíduos animais, o que abrange a dosagem e o processo de metabolização no organismo ao qual é administrado, da frequência de aplicação, do mecanismo de ação destas e, principalmente, da persistência das moléculas e/ou seus resíduos no ambiente (HALLEY et al., 1993; LITSAKS et al., 2013; LUMARET et al., 2012; MANDAL e SINGH, 2013; ZHANG et al., 2016). Neste contexto de periculosidade e de proteção ambiental, busca-se a identificação e a inclusão em fármacos veterinários de moléculas antiparasitárias de menor toxicidade ambiental ou mesmo o reconhecimento de seus potenciais tóxicos, por meio da identificação de limites de segurança ambiental para uso e descarte destas (TEREKHOVA, 2011), além de seus mecanismos de ação sobre organismos não-alvo, terrestres e aquáticos (ALAK et al., 2017; ZORTÉA et al., 2017).

1.4 ECOTOXICOLOGIA COMO FERRAMENTA DE MONITORAMENTO AMBIENTAL: TERRESTRE E AQUÁTICO

Uma avaliação de risco ambiental e de qual o risco das moléculas antiparasitárias farmacológicas para os organismos não-alvo, deve ser realizada por meio de dados experimentais sobre o destino e os efeitos destas ou dos fármacos que as utilizam, considerando as particularidades dos meios de exposição e as diferentes sensibilidades dos organismos (ALAK et al., 2017; HOKE et al., 2016; ZORTÉA et al., 2017; ZORTÉA et al., 2018). A avaliação de risco ambiental pode ser mediada pela avaliação ecotoxicológica, pois o conhecimento ecotoxicológico permite prever ou avaliar a extensão do risco ambiental, estipular metodologias de vigilância e rastreamento da presença de tóxicos no ambiente e orientar medidas de remediação (AZEVEDO e CHASIN, 2004). Neste contexto, a

ecotoxicologia é uma ferramenta complementar de monitoramento ambiental para a avaliação de interações sinérgicas e antagônicas de substâncias (naturais ou sintéticas) em ambientes receptores (terrestres, aquáticos ou aéreos) e sobre organismos, populações ou comunidades animais e vegetais que os habitam (BIANCHINI et al., 2009).

A ecotoxicologia contempla estudos no solo, água e ar. Para tanto, utiliza testes ecotoxicológicos de caráter laboratorial e de campo, nos quais, espécies bioindicadoras não-alvo (animais ou vegetais) são expostas a um substrato ou meio (solos naturais ou artificiais, água ou ar) contaminado com a substância teste de interesse, com o objetivo de qualificar e quantificar nestas espécies os efeitos tóxicos de caráter fisiológico, morfológico e/ou comportamental, a curto e a longo prazo, com fins de proteger os ecossistemas (ANDRÉA, 2012; LIMA, 2009). Nos estudos ecotoxocicológicos, são utilizados diferentes níveis de contaminação para que seja possível inferir sobre a toxicidade da substância e contribuir para o estabelecimento de limites de uso ou de descarte das substâncias no meio específico, ou mesmo, gerar informações que permitam predizer e controlar seus efeitos em situações de impactos reais, especialmente no solo e na água (ALAK et al., 2017; ZORTÉA et al., 2017).

Os protocolos ecotoxicológicos padronizados pela *International Organization for Standardization* (ISO) e pela *Organization for Economic Co-operation and Development* (OECD) são comumente adotados para a avaliação de moléculas farmacológicas no solo e na água e, utilizam modelos biológicos considerados organismos bioindicadores de qualidade. Estes organismos são selecionados devido à sensibilidade às substâncias poluentes (GREENSLADE e VAUGHAN, 2003; HAVLICEK, 2012; OLIVEIRA et al., 2016), aliada a outras características relacionadas às funções ecossistêmicas que desempenham, especialmente no que se refere ao uso de invertebrados terrestres nos protocolos, pois são organismos não-alvo que têm papel importante em uma série de serviço ecossistêmicos (BOTTINELLI et al., 2015). Contudo, atualmente busca-se incluir na ecotoxicologia, especialmente nos protocolos aquáticos, novas metodologias e organismos de produção comercial, pois a exposição aos contaminantes é direta sobre o meio de cultivo (ALAK et al., 2017).

1.5 EPRINOMECTINA E O MEIO AMBIENTE

Atualmente, pouco se sabe sobre a dinâmica no ambiente das novas moléculas farmacológicas, especialmente da eprinomectina. Além de como as variações existentes em sua estrutura podem afetar o meio ambiente. Referente à eprinomectina há alta variabilidade e

discrepância entre os valores encontrados no solo e nas fezes de animais tratados e, dos valores ditos como tóxicos ou não para diferentes espécies terrestres e aquáticas (ALAK et al., 2017; LUMARET et al., 2012; NIEMAN et al., 2018). Estudos com eprinomectina na água, realizados por Alak et al. (2017), demonstram que exposições de trutas arco-íris (*Oncorhynchus mykiss*) a concentrações sub-letais (abaixo de 1,2 µg L^{-1}) são capazes de ocasionar estresse oxidativo no fígado destes peixes. Alguns estudos realizados com eprinomectina no solo consideram-na ambientalmente segura em condições usais como endectocida (HALLEY et al., 2005; NIEMAN et al, 2018; PALMER e BEAVERS, 1995). Estudos de Palmer e Beavers (1995) apresentam valores de LC_{50} para 28 dias superior a 951 mg kg^{-1} de solo seco para minhocas e, valores nas fezes de bovinos de até 3,34 mg kg^{-1}seco não afetam a alimentação ou ganho de peso das minhocas (HALLEY et al., 2005). Outro estudo mais recente de Nieman et al. (2018) relata que a eprinomectina, mesmo em proveniente de fármacos antiparasitários de longa ação, quando em fezes bovinas em situações de campo não afeta insetos do solo, como os colêmbolos.

Contudo, estes resultados não são suficientes para garantir o aprofundamento científico necessário para confirmar variações de impacto quanto as características de diferentes compartimentos ambientais, substratos e graus de sensibilidade de diversas espécies não-alvo (ALAK et al., 2017). Ademais, também é comum a classificação do comportamento ambiental da eprinomectina como semelhante às demais moléculas do mesmo grupo químico ou família, como ivermectina e abamectina, as quais apresentam grande potencial tóxico sobre a fauna do solo mesmo em baixas concentrações **(**BUENO e FREITAS, 2004; DIAO et al., 2007; JENSEN et al., 2009; JENSEN e SCOTT-FORDSMAND, 2012; SVENDSEN et al., 2003; ZORTÉA et al., 2017), assim como para os organismos aquáticos (DOMINGUES et al., 2016; OLIVEIRA et al., 2016).

No solo, também se sabe que a eprinomectina pode ser caracterizada como de dissipação moderada e altamente persistente, podendo permanecer no solo por mais de 120 dias (LITSKAS et al., 2013). Quanto à absorção, adsorção e, consequentemente a mobilidade, estas podem variar de acordo com as características físico-químicas do solo, contudo características de sorção e adsorção são menos pronunciadas em solos com menor quantidade de argila e matéria orgânica. Portanto, a mobilidade pode ser maior em solos arenosos e quando associada ao seu caráter mais hidrofílico, esta molécula pode ser lixiviada ou percolada e atingir mais facilmente camadas mais profundas do solo, atingir corpos de água e

lençóis freáticos (LITSKAS et al., 2011; LITSKAS et al., 2013; VASSILIS et al., 2016) e apresentar riscos tanto para espécies terrestres quanto aquáticas.

Em ambientes aquático, considerando-se o caráter hidrofóbico dos fármacos que utilizam avermectinas como princípios ativos, estas moléculas tendem a ser absorvidas e acumuladas especialmente no tecido adiposo ou em órgãos e glândulas anexas do sistema hepático, onde podem causar dano celular por estresse oxidativo (ISLAM, 2013), efeito já relatado para eprinomectina em peixes (ALAK et al., 2017). Ao considerarmos o maior potencial antiparasitário da eprinomectina, consequentemente, este mesmo fator pode ser responsável por maior impacto ambiental desta molécula sobre o meio ambiente, aspecto que atenta para a necessidade de maiores conhecimentos ecotoxicológicos sobre esta molécula específica, tanto no compartimento solo como no compartimento água.

O desenvolvimento de estudos com eprinomectina, por meio da aplicação de testes ecotoxicológicos com organismos não-alvo da fauna terrestre e/ou aquática, ou mesmo espécies de produção zootécnica, serve como parte inicial de uma avaliação de risco ambiental que permitirá conhecer e entender o efeito desta molécula farmacológica no ambiente. Além de gerar dados para futuros estudos aprofundados sobre a presença de produtos da degradação desta molécula nas excretas de animais de interesse zootécnico que são utilizadas na adubação orgânica do solo, principalmente em regiões tropicais. Isso poderá contribuir para o estabelecimento de novas recomendações de uso de fármacos à base de eprinomectina no controle de parasitos, de forma eficiente e sem danos ambientais.

1.6 OBJETIVOS

1.6.1 OBJETIVO GERAL

Reconhecer os efeitos ecotoxicológicos do antiparasitário eprinomectina sobre sobrevivência, reprodução e comportamento de organismos da fauna edáfica, além de toxicidade e comportamento em peixes, ocasionados pela contaminação direta de solos subtropicais e água.

1.6.2 OBJETIVOS ESPECÍFICOS

- Estudar os efeitos do antiparasitário eprinomectina em Neossolo Quartzarênico órtico típico e Latossolo Vermelho distrófico, sobre sobrevivência, reprodução e comportamento de fuga de colêmbolos (*Folsomia candida*).
- Estudar os efeitos no cérebro de peixes jundiá (*Rhamdia quelen*), expostos à água contaminada com o antiparasitário eprinomectina, sobre variáveis bioquímicas oxidativas e neurais, além de alterações no comportamento.
- Estudar os efeitos no fígado de peixes jundiá (*Rhamdia quelen*), expostos à água contaminada com o antiparasitário eprinomectina, sobre variáveis bioquímicas oxidativas, antioxidantes e energéticas.

CAPÍTULO II

2. ARTIGO E MANUSCRITOS

Os resultados desta dissertação são apresentados na forma de um artigo e dois manuscritos, organizados em conteúdos terrestre e aquático, respectivamente. O artigo e os manuscritos foram formatados de acordo com as orientações das revistas às quais foram submetidos. A parte do estudo que contempla o uso de peixes foi aprovada pela Comissão de Ética no Uso de Animais (CEUA) da Universidade do Estado de Santa Catarina (UDESC) (Anexo I).

Manuscrito I (Terrestre) - **Eprinomectin affects survival, reproduction and behavior of *Folsomia candida*, and its toxicity depends on the type of soil**
Autores: Suélen Serafini, Junior Gonçalves Soares, Camila Felicetti Perosa, Julia Corá Segat, Aleksandro Schafer da Silva, Dilmar Baretta
Submetido: **Environmental Toxicology and Pharmacology**

Artigo I (Aquático) - **Fish exposed to water contaminated with eprinomectin show inhibition of the activities of AChE and Na^+/K^+-ATPase in the brain, and changes in natural behavior**
Autores: Suélen Serafini, Carine de Freitas Souza, Matheus Dellaméa Baldissera, Bernardo Baldisserotto, Julia Corá Segat, Dilmar Baretta, Renato Zanella, Aleksandro Schafer da Silva
Publicado: **Chemosphere** (Anexo II)

Manuscrito II (Aquático) - **Fish exposed to eprinomectin show hepatic oxidative stress and impairment in enzymes of the phosphotransfer network**
Autores: Suélen Serafini, Carine de Freitas Souza, Matheus Dellaméa Baldissera, Bernardo Baldisserotto, Fernanda Picoli, Julia Corá Segat, Dilmar Baretta, Aleksandro Schafer da Silva
Submetido: **Aquaculture**

2.1 MANUSCRITO I

Eprinomectin affects survival, reproduction and behavior of *Folsomia candida*, and its toxicity depends on the type of soil

Suélen Serafini [a], Junior Gonçalves Soares [a], Camila Felicetti Perosa [a], Julia Corá Segat [a, b], Aleksandro Schafer da Silva [a, b, **], Dilmar Baretta[a, b, *]

[a] Program of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[b] Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[*] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Belloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

[**] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Beloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

E-mail addresses: dilmar.baretta@udesc.br (D. Baretta), aleksandro.silva@udesc.br (A. Schafer da Silva).

De acordo com normas para publicação em:

Environmental Toxicology and Pharmacology

Situação do Manuscrito: Submetido

Eprinomectin affects survival, reproduction and behavior of *Folsomia candida*, and its toxicity depends on the type of soil

Abstract

The objective of this study was to evaluate the toxicity of the antiparasitic agent eprinomectin in two subtropical soils, using ecotoxicological lethality, reproduction and avoidance behavior tests with springtails (*Folsomia candida*). The eprinomectin treatments doses were 0, 0.5, 1, 2, 4, 8, 12, 16 and 20 mg kg^{-1} of dry soil combined with Entisol and Oxisol soils, individually. Statistically significant toxic effects of eprinomectin on springtails were observed in both soils. Eprinomectin was lethal starting at 8 mg kg^{-1} of dry soil in Entisol and 20 mg kg^{-1} of dry soil in Oxisol, with effects less than 50% at lethal doses. Reductions in the reproduction rate of the springtails were also observed from 8 mg kg^{-1} of dry soil in Entisol and 0.5 mg kg^{-1} in Oxisol. EC_{50} (effective concentration that reduces reproduction rate by 50%) value calculated for Entisol was 4.38 $\pm$ 0.62 mg kg^{-1} of dry soil; for Oxisol the EC_{50} was above the highest tested dose. For avoidance behavior the effect occurred from 0.5 mg kg^{-1} of dry soil for both soils. In Entisol, all doses showed avoidance of more than 95% and in Oxisol the AC_{50} (concentration that causes 50% avoidance) value was < 0.5 mg kg^{-1} of dry soil. In both soils, eprinomectin affected survival, reproduction and caused avoidance behavior of *F. candida*. The toxic effects were higher as the dose in the soils increased. Effects on Oxisol were less intense considering the affected springtails, compared to the effects on Entisol. This may be associated with the different physicochemical characteristics between the soils that determine the retention capacity for eprinomectin, especially the greater values of cation exchange capacity, and content of clay and organic matter of the Oxisol.

Keywords: Antiparasitic, avermectin, Collembola, edaphic fauna, pharmacological molecule, subtropical soils

1. Introduction

Soil is one of the most important bases for the maintenance of life on Earth, including for humans. Life depends on food production; therefore, all the webs of the trophic chain are dependent on the balance between the chemical, physical and biological properties of the soil

(Hatfield et al., 2017). Among these properties, biological activity is important in terms of the provision of ecosystem services (Lavelle et al., 1996; Doran and Zeiss, 2000; Bottinelli et al., 2015; Brevik et al., 2015). Springtails, Collembola order, are soil microarthropods with significant influence on microbial ecology, nutrient cycling and soil fertility (Oliveira Filho and Baretta, 2016).

The springtails *Folsomia candida* are described as one of the bioindicator species that best represents the effects of soil contamination (Smit and Van Gestel, 1996; Fountain and Hopkin, 2005; Buch et al., 2016; Zortéa et al., 2018), particularly with respect to their sensitivity to the non-target effects of veterinary pharmaceuticals, including antiparasitics (Zortéa et al., 2017). Ecotoxicological studies have shown that antiparasitics of the most widely used pharmacological groups in the world that may have a negative impact on *F. candida*. These groups include pyrethroids (Zortéa et al., 2015; Zortéa et al., 2017), salicylanilides (Zortéa et al., 2017), phenylpyrazoles (Cortet et al., 2002; San Miguel et al., 2008; Zortéa et al., 2018) and macrocyclic lactones (Diao et al., 2007; Jensen et al., 2009; Zortéa et al., 2017). Among the forms of soil contamination by antiparasitics are the inappropriate disposal of drugs or agricultural and livestock effluents, as well as the use of effluents as organic fertilizers (Boxall et al., 2002; Christian et al., 2003; Floate et al., 2005; Jensen and Scott-Fordsmand, 2012; Vassilis et al., 2016).

Among the group of macrocyclic lactones is the family of avermectins; in particular, eprinomectin is a molecule whose effects on *F. candida* still need to be better studied. This is a broad-spectrum semisynthetic molecule, similar to other avermectins, that is recommended for the control and endectocidal treatment of nematodes and arthropod parasites (Cvetovich et al., 1994; Shoop and Soll, 2002). After being absorbed by the body, its metabolism occurs mainly via bile, with almost total elimination through the feces (Wells, 1998; Shoop and Soll, 2002; Aksit et al., 2016). In bovine feces, eprinomectin can be excreted between 0.80 and 13.6 days after subcutaneous administration and between 1 and 20 days after topical administration. Subcutaneous administration results in greater fecal excretion than with topical administration, 1188.9 and 311.5 ηg day^{-1} g^{-1}, respectively (Aksit et al., 2016). Its concentration in the feces in topical use varies from approximately 0.0036 to 1.80 mg kg^{-1} of wet weight, considering the detection of the B1a fraction of avermectin and its recovery of 79.50% (Halley et al., 2005). The B1a fraction makes up about 90% of the eprinomectin molecule and is one of the most important metabolites in terms of concentration in feces (Wells, 1998).

Eprinomectin is more hydrophilic than ivermectin or abamectin (Holste et al., 1997; Wells, 1998). In the soil, it is characterized by moderate dissipation and high persistence, remaining in soil for more than 120 days (Litskas et al., 2013). Its absorption and adsorption to soil particles and its mobility profile vary according to the physicochemical characteristics of the soil, primarily depending on the contents of the organic matter and clay that determine the soil retention capacity for the antiparasitic (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016). According to some studies, eprinomectin has a high-margin of soil environmental safety for earthworms (Palmer and Beavers, 1995; Halley et al., 2005), as well as for organisms of the Collembola order (Nieman et al., 2018), when typically used as an endectocide in animals. Nevertheless, these studies are restricted to soils characteristic of temperate regions; varying retention capacities of each soil can directly influence toxicity to non-target organisms (Zortéa et al., 2018).

According to the literature, ecotoxicological tests can be used to rapidly measure effective responses for the identification of veterinary antiparasitics toxicity (Zortéa et al., 2015; Zortéa et al., 2017). These tests also help identify toxicological variations of the effects of pharmacological molecules in different soils (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016; Zortéa et al., 2018). Therefore, the present study was carried out to evaluate the toxicity of the antiparasitic agent eprinomectin in two subtropical soils, using ecotoxicological lethality, reproduction and avoidance behavior tests with *F. candida*. The hypotheses of our study are: (I) eprinomectin causes lethality, reduction of reproduction and avoidance behavior in *F. candida*; (II) these effects are less intense in soils with greater values of cation exchange capacity, and content of clay and organic matter.

2. Materials and methods

2.1. Collection, preparation and variables of soils

Two Brazilian subtropical soils were used in the ecotoxicological tests. The first was Entisol with sandy texture (USDA, 2014), collected in the municipality of Araranguá, SC, Brazil [29°00′19.98″ S and 49°31′02.84″ W]. The second was Oxisol with clay-like texture (USDA, 2014), collected in the municipality of Chapecó, SC, Brazil [27°05′274″S and 052°38′085″W]. Both were collected at depths of 0.00-0.20 m in forested areas, with no history of pesticide application or livestock activity. Subsequently, the samples were dried in

an oven (65 °C) and sieved at 2 mm. The natural pH of soils was maintained for ecotoxicological tests.

In addition to subtropical soils, a Tropical Artificial Soil (TAS) was used as a control soil for validation of the tests. The TAS, proposed by Garcia (2004), consists of a homogeneous mixture of 75% fine sand, 20% kaolin and 5% coconut fiber, with pH corrected with $CaCO_3$ between 5.5 and 6.5 (ISO 10390, 2005). The physical and chemical variables of the soils and the TAS used are presented in Table 1.

2.2. Substance testing and treatments

The treatments consisted of doses of a veterinary antiparasitic drug containing only eprinomectin (Eprinex® Injectable 3.6%), combined with the two soils, individually. The tested doses were 0, 0.5, 1, 2, 4, 8, 12, 16 and 20 mg kg^{-1} of dry soil, corrected for eprinomectin concentration and estimated from the recommended therapeutic dose in topical formulations for beef cattle and milk, i.e., 0.5 mg kg^{-1} of body weight (Wells, 1998). In addition to previous studies on the behavior of eprinomectin in different soils (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016), ecotoxicological tests of *F. candida* with ivermectin soil contamination (Zortéa et al., 2017) and field trials of soil contaminations with cattle feces that received commercial long-acting eprinomectin formulas on insects of the Collembola order (Nieman et al., 2018).

Because the commercial drug used was insoluble in water, it was necessary to dilute it in acetone (50 mL per treatment) prior to soil contamination. For each dose of eprinomectin, a solvent solution was prepared according to validated methodologies (ISO 11267, 1999; Jensen et al., 2003) and applied to the soils. After the homogenization of the solutions (acetone + eprinomectin) in the soils, they were conditioned in an exhaust chamber, where they remained for 12 h for the total evaporation of the acetone. For each soil, ‘solvent control’ (soil + acetone) was performed to ensure that there was no acetone effect. This control of solvent effect was included in the study as a treatment, called ‘Solvent’. Subsequently, the humidity of each soil and the TAS was adjusted to 65% of the maximum water retention capacity (WRC) of each soil (ISO 11465, 1993) and the ecotoxicological tests were conducted.

2.3. Organisms and ecotoxicological tests

For the ecotoxicological tests springtails of the species *F. candida* were used. Springtails were cultured according to methodology ISO 11267 (1999) for the specie. To carry out the study, the springtails had their ages synchronized between 10 and 12 days of age post-hatching of the eggs for the lethality and reproduction tests (ISO 11267, 1999) and 20 to 22 days post-hatching for avoidance behavior tests (ISO 17512-2, 2008).

The lethality and reproduction tests were conducted according to ISO 11267 (1999) methodologies, in a completely randomized experimental design with five replicates for lethality and six replicates for reproduction. The avoidance behavior tests were conducted according to ISO 17512-2 (2008) in a completely randomized design with six replicates. The lethality, reproduction and avoidance behavior tests lasted 14 days, 28 days and 48 hours, respectively. The methodologies for assembling and disassembling the lethality, reproduction and avoidance behavior tests are described in detail in Zortéa et al. (2015).

2.4. Statistical analysis

Lethality and reproduction data were evaluated for outliers, as well as normality and homoscedasticity using the Kolmogorov-Smirnov and Levene's tests, respectively. When the hypotheses were met, the data were subjected to analysis of variance (one-way ANOVA), followed by the Dunnett's test ($p < 0.05$) using the software Statistica 7.0 (Statsoft, 2004). When the normality and homoscedasticity hypotheses were not met (which occurred with the reproduction data in Entisol), data were analyzed using the Kruskal-Wallis non-parametric test ($p < 0.05$) using Statistica 7.0 software (Statsoft, 2004).

Avoidance behavior data were evaluated for the outliers and the responses to the tests were calculated according to the determination of ISO 17512-2 (2008), using the formula: $A = ((C-T)/N).100$, where A represents percent avoidance, C is the number of organisms in untreated soil (control), T is the number of organisms in treated soil, and N is the total number of organisms. The values obtained in each of the combinations were evaluated using the Fisher's test ($p < 0.05$) (Zar, 1996).

Concentrations of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) were determined from the statistical analyses described using Statistica 7.0 software (Statsoft, 2004) and Fisher's test (Zar, 1996). LC_{50} values (lethal

concentration that reduces survival by 50%) for the lethality tests were not calculated because the effects were less than 50%. EC_{50} values (effective concentration that reduces reproduction rate by 50%) for reproduction tests were calculated using mathematical models that best fit the regression analysis using Statistica 7.0 software (Statsoft, 2004). AC_{50} values (concentration that causes 50% avoidance) for avoidance behavior tests were calculated using PriProbit® 1.63 (Sakuma, 1998).

3. Results

3.1. Lethality and reproduction

The lethality and reproduction tests fulfilled the validity criteria according to ISO 11267 (1999). In the lethality tests there was no effect of the solvent acetone ($p > 0.05$) and the average survival of *F. candida* was 100% for TAS (data not shown), 98% for Entisol and 92% for Oxisol. In the reproduction tests there was also no effect of acetone ($p > 0.05$) and the average number of juveniles was 288 for TAS with coefficient of variation (CV) of 3.63% (data not shown), 179 organisms for Entisol with a CV of 5.72% and 203 organisms with a CV of 6.89% for Oxisol (Fig. 1 and Fig. 2 – A, B; CV not shown).

The application of the eprinomectin treatments resulted in the lethality of the springtails at doses 8, 12 and 20 mg kg^{-1} of dry soil in Entisol ($p < 0.05$). The absence of effect at the dose of 16 mg kg^{-1} of dry soil ($p = 0.16$) can be attributed to its higher standard deviation (Fig. 1 - A). Oxisol had an effect at the highest dose evaluated ($p < 0.05$) (Fig. 1 - B). The effects on survival were less than 50% at lethal doses in both soils. NOEC and LOEC values for lethality are presented in Table 2.

As for reproduction there was an effect on the Entisol on springtails starting at 8 mg kg^{-1} dose of dry soil ($p < 0.05$) (Fig. 2 - A), with an estimated EC_{50} of 4.38 ± 0.62 mg kg^{-1} of dry soil according to the mathematical model of Gompertz ($R = 0.98$) (Table 2). Oxisol had an effect starting at the lowest dose tested ($p < 0.05$) (Fig. 2 - B). The estimated value of EC_{50} for Oxisol occurred above the highest tested dose of eprinomectin. NOEC and LOEC values for reproduction are presented in Table 2.

3.2. Avoidance behavior

The ecotoxicological tests of avoidance behavior also met the validation criteria regarding double controls, proposed for *F. candida* by ISO 17512-2 (2008). The average distributions of the organisms between the sections in the double controls with TAS were 44.54% and 55.46% (data not shown). For Entisol they were 48.33% and 51.67% and for Oxisol they were 54.78% and 45.22% (Fig. 3 - A, B). For both soils there was no effect of acetone and lethality in the combinations tested ($p > 0.05$). The solvent *vs.* solvent controls distribution between the sections were 49.58% and 50.42% in Entisol and 50.83% and 49.17% in Oxisol (data not shown).

The application of eprinomectin at different doses caused the springtails avoidance behavior starting at the lowest dose tested for both soils ($p < 0.05$), Entisol (Fig. 3 - A) and Oxisol (Fig. 3 - B). For Entisol it was not possible to calculate the AC_{50}, because all doses had avoidance of over 95% and for Oxisol the AC_{50} calculated for eprinomectin was < 0.5 mg kg^{-1} of dry soil (Table 2). NOEC and LOEC values for avoidance behavior are presented in Table 2.

4. Discussion

In general, the behavior or mode of action of avermectins varies depending on the biological model used for the study or how the compound comes into contact with the organism (Turner and Schaeffer, 1989). Springtails lethality caused by avermectins, including eprinomectin, is a known effect and associated in the literature on the mechanism of action of this family on invertebrates in general (Jensen et al., 2009; Zortéa et al., 2017). These molecules act as GABA (Gamma-Aminobutyric Acid) neurotransmitter agonists in nerves and muscle cells by irreversibly binding to chloride ion channels controlled by these neurotransmitters, resulting in hyperpolarization of these cells and blocking the transmission of neural signals (Dourmishev et al., 2005; Geary and Moreno, 2012; Wolstenholme, 2012). This blockage in neurotransmission causes loss of motor coordination and paralysis, with consequent death due to direct or indirect effects on feeding and hydration (Turner and Schaeffer, 1989; Geary, 2005).

Although there are no previous ecotoxicological studies with eprinomectin on *F. candida*, a study by Zortéa et al. (2017) evaluating the effects of ivermectin, avermectin analogous to eprinomectin, in TAS, observed that it did not cause significant effects on the

survival of *F. candida*, even at the highest tested dose of 10 mg kg^{-1} of dry soil, a concentration within the range of lethal toxic effects we obtained when applying eprinomectin in Entisol. This difference in toxicity between molecules of the same family may be associated with the different physicochemical characteristics of the soils used between the studies, because TAS has a greater retention capacity than does Entisol, primarily due to the higher values of cation exchange capacity, and content of clay and organic matter (Litskas et al., 2011; Zortéa et al., 2018). Furthermore, the lethal effects of eprinomectin on Oxisol, which also has a greater retention capacity due to its content of clay and organic matter (Litskas et al., 2011), were significant only at the highest tested dose of 20 mg kg^{-1} of dry soil, a value higher than that of the study by Zortéa et al. (2017).

Macrocyclic lactones are also considered potent inhibitors of reproduction in several species of invertebrates (Lumaret et al., 2012; Zortéa et al., 2017). Glancey et al. (1982) found that ant queens (*Solenopsis invicta*), when fed at concentrations of 0.0025 to 1.0% of the compound avermectin B1a, became completely sterile or reduced the number and size of eggs laid due to cell and tissue irreversible changes in the ovaries. Another reported effect for ivermectin is the agonist action on GABA neurotransmitter receptors, especially $GABA_B$ receptors, observed in olfactory sensory neurons in antennae of males of *Heliothis virescens*. In insects, these receptors mediate mechanisms of gain control mediated by GABA, playing a fundamental role in the processing of pheromone signals linked to the reproductive process (Pregitzer et al., 2013). These changes and interferences may help to explain the effects of eprinomectin on the reproduction of springtails.

Zortéa et al. (2017) also evaluated the adverse effects of ivermectin in TAS on *F. candida* reproduction and obtained in NOEC below 0.2 mg kg^{-1} of dry soil and an EC_{50} of 0.43 mg kg^{-1} of dry soil. In a multispecies study, Jensen et al. (2009) exposing two species of springtails, *F. fimetaria* and *F. candida*, to a temperate agricultural soil (67% sand, 21% clay and total carbon 2.22%) contaminated with ivermectin, obtained EC_{10} values of 0.19 mg kg^{-1} of dry soil in the test system for the single species, *F. fimetaria* and 0.02 mg kg^{-1} of dry soil in the test system for both species, *F. fimetaria* and *F. candida*. Diao et al. (2007) evaluating the effect of the other avermectin, abamectin, on a sandy European soil (21% clay and 67% sand) in two species of springtails, found an EC_{50} of 0.33 mg kg^{-1} of dry soil for *F. fimetaria* and an EC_{50} of 0.68 mg kg^{-1} of dry soil for *F. candida.* Results obtained with the antiparasitics ivermectin and abamectin differ from those with eprinomectin. Outstanding reproductive effect values in all these studies presented were below the values in Entisol and below the

doses tested for Oxisol. This may reinforce the possibility that eprinomectin may have a lower toxicity to springtails than do other avermectins.

Avoidance behaviors can be attributed to the characteristics of eprinomectin, common to avermectins, that at first can cause a repellent effect for insects (Floate, 2007; Nieman et al., 2018). According to Floate (2007) the avoidance or repellency responses or even attraction to insects, caused by avermectins in feces, are related to its volatile components or metabolites. Several studies suggest that these molecules need to undergo metabolism in the animal such that there is release and effect of the volatile components (Mahon and Wardhaugh, 1991; Lumaret et al., 1993; Bernal et al., 1994). Despite the fact that we did not measure the volatilization of compounds in our tests, our results with the application of eprinomectin directly to the soil open the door to questioning whether it is possible that the metabolism of the molecule is not necessary for the release of these repellency components, even for short periods of exposure, such as the 48 hours of the test. A study by Sollai et al. (2007) with *Culicoides imicola* mosquitoes demonstrated that, with longer exposures to avermectins, there was reduced sensitivity in the olfactory responses of insects. Considering the mode of action of these antiparasitics on chloride ion channels controlled by GABA, these channels are present in abundance in interneurons and antennal lobes of insects and in these organs are essential for the olfactory processing (Hoskins et al., 1986; Berg et al., 2007). It is also important to note that endectocidal molecules, including avermectins, have high polarity and molecular weight, favoring the binding with particles of organic matter or particles of the soil itself, such as clay (Floate et al., 2005; Litskas et al., 2011), and this can avoid the volatilization of compounds that remain in the environment and cause repellency (Floate et al., 2005), explaining the difference in the intensity of the effects on the behavior of the springtails in the different soils.

The toxicity of eprinomectin to *F. candida* for all evaluated parameters, although occurring in both soils, was less intense in Oxisol soil, possibly associated with its greater capacity to retain substances, related to higher values of cation exchange capacity, organic matter and clay, characteristics that condition the lower availability of substances and compounds toxic to soil organisms (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016; Zortéa et al., 2018). According to the literature, springtails are more sensitive to the toxicity of contaminants when they are dissolved in the soil solution, because the main route of contamination of these organisms is water absorption present in specialized organs (Peijnenburg et al., 2012). This factor also helps to explain the more pronounced toxic effects

of eprinomectin contamination in Entisol (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016; Zortéa et al., 2018), associated with the more hydrophilic characteristics of the molecule (Holste et al., 1997; Wells, 1998). It is important to note that, in soils with less retention capacity such as Entisol, there may be a greater mobility of eprinomectin due to leaching or percolation, possibly reaching water bodies and groundwater more easily (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016) and presenting risks to aquatic species (Alak et al., 2017).

Lethal or non-lethal effects on reproduction and behavior are extremely important and harmful; if there is a reduction in the number of organisms, whether by lethality, reproductive reduction or avoidance, there may be an ecosystem imbalance. The molecule or its metabolites may have longer life in the soil, because detoxification and xenobiosis functions may be reduced, because there is a direct balance between Collembola and microorganisms (Filser et al., 2002). The eprinomectin molecule is also almost not degraded without the action of the microbial population (Litskas et al., 2013), which may favor larger environmental impacts, such as the increase in the area of contamination caused by percolation or leaching of the contaminant (Litskas et al., 2011; Litskas et al., 2013; Vassilis et al., 2016). According to researchers, this may cause imbalance in the webs of the trophic chain of a given ecosystem, because contaminants can biomagnify in the food chain (Scheffczyk et al., 2016), culminating in a reduction of other ecosystem services (Lavelle et al., 1996; Bottinelli et al., 2015). Therefore, there is a need for further laboratory and field studies of the antiparasitic agent eprinomectin, as well as studies of concentrations of this molecule in manure after metabolism in animals. Studies are needed evaluating the various non-target species of the soil as well as the use of soils with other physicochemical characteristics, considering the toxic potential of eprinomectin or even the confirmation of its possible lower environmental toxicity compared to those of other avermectins in soil.

5. Conclusions

We conclude that eprinomectin affected survival, as well as the reproductive and caused avoidance behavior of *F. candida* in both soils. The toxic effects were higher as the dose in the soils increased. Effects on Oxisol were less intense considering the affected springtails, compared to the effects on Entisol. This may be associated with the different physicochemical characteristics between the soils that determine the retention capacity for

eprinomectin, especially thegreater values of cation exchange capacity, and content of clay and organic matter of the Oxisol.

References

Aksit, D., Korkut, O., Aksoz, E., Gokbulut, C., 2016. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. N. Z. Vet. J. 64, 207-211.

Alak, G., Yeltekin, A.Ç., Tas, I.H., Ucar, A., Parlak, V., Topal, A., Kocaman, E.M., Atamanalp, M., 2017. Investigation of 8-OHdG, CYP1A, HSP70 and transcriptional analyses of antioxidant defence system in liver tissues of rainbow trout exposed to eprinomectin. Fish Shellfish Immunol. 65, 136-144.

Berg, B.G., Schachtner, J., Utz, S., Homberg, U., 2007. Distribution of neuropeptides in the primary olfactory center of the heliothine month *Heliothis virescens*. Cell Tissue Res. 327, 385-398.

Bernal, J.L., Del Nozal, M.J., Salas, M., Galante, E., Lumaret, J.P., 1994. HPLC determination of residual ivermectin in cattle dung following subcutaneous injection. J. Liq. Chromatogr. 17, 2429-2444.

Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., Peng, X., 2015. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil Tillage Res. 146, 118-124.

Boxall, A.B.A., Fogg, L., Blackwell, P.A., Kay, P., Pemberton, E.J., 2002. Review of veterinary medicines in the environment. Technical Report European Environment Agency, United Kingdom. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/290328/sp6-012-8-tr-e-e.pdf. (accessed 08 November 2018).

Brevik, E.C., Cerdà, A., Mataix-Solera, J., Pereg, L., Quinton, J.N., Six, J., Van Oost, K., 2015. The interdisciplinary nature of SOIL. Soil 1, 117-129.

Buch, A.C., Niemeyer, J.C., Correia, M.E.F., Silva-Filho, E.V., 2016. Ecotoxicity of mercury to *Folsomia candida* and *Proisotoma minuta* (Collembola: Isotomidae) in tropical soils: Baseline for ecological risk assessment. Ecotoxicol. Environ. Saf. 127, 22-29.

Christian, T., Schneider, R.J., Farber, H.A., Skutlarek, D., Meyer, M.T., Goldbach H.E., 2003. Determination of antibiotic residues in manure, soil, and surface waters. Acta Hydroch.

Hydrob. 31, 36-44.

Cortet, J., Gillon, D., Joffre, R., Ourcival, J.M., Poinsot-Balaguer, N., 2002. Effects of pesticides on organic matter recycling and microarthropods in a maize field: Use and discussion of the litterbag methodology. Eur. J. Soil Biol. 38, 261-265.

Cvetovich, R.J., Kelly, D.H., DiMichele, L.M., Shuman, R.F., Grabowski, E.J.J., 1994. Synthesis of 400-epi-amino-400-deoxyavermectins B1. J. Org. Chem. 59, 7704-7708.

Diao, X., Jensen, J., Duus Hansen, A., 2007. Toxicity of the anthelmintic abamectin to four species of soil invertebrates. Environ. Pollut. 148, 514-519.

Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl. Soil Ecol. 15, 3-11.

Dourmishev, A.L., Dourmishev, L.A., Schwartz, R.A., 2005. Ivermectin: Pharmacology and application in dermatology. Int. J. Dermatol. 44, 981-988.

Filser, J., Mebes, K.-H., Winter, K., Lang, A., Kampichler, C., 2002. Long-term dynamics and interrelationships of soil Collembola and microorganisms in an arable landscape following land use change. Geoderma 105, 201-221.

Floate, K.D., 2007. Endectocide residues affect insect attraction to dung from treated cattle: Implications for toxicity tests. Med. Vet. Entomol. 21, 312-322.

Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A., Sherratt, T.N., 2005. Fecal residues of veterinary pharmaceuticals: Nontarget effects in the pasture environment. Annu. Rev. Entomol. 50, 153-179.

Fountain, M.T., Hopkin, S.P., 2005. *Folsomia candida* (Collembola): A “standard” soil arthropod. Annu. Rev. Entomol. 5, 20-22.

Garcia, M. 2004. Effects of pesticides on soil fauna: Development of ecotoxicological test methods for tropical regions. Doctoral thesis. Hohen Landwirtschaftlichen Fakultät: Universidade de Bonn.

Geary, T.G., 2005. Ivermectin 20 years on: Maturation of a wonder drug. Parasitology 21, 530-532.

Geary, T.G., Moreno, Y., 2012. Macrocyclic lactone anthelmintics: Spectrum of activity and mechanism of action. Curr. Pharm. Biotechnol. 13, 866-872.

Glancey, B.M., Lofgren, C.S., Williams, D.F., 1982. Avermectin B1a: Effects on the ovaries of red imported fire ant queens (Hymenoptera: Formicidae). J. Med. Entomol. 19, 743-747.

Halley, B.A., Winter, R., Yoon, S., Marley, S.E., Rehbein, S., 2005. The environmental safety

of eprinomectin to earthworms. Vet. Parasitol. 128, 109-114.

Hatfield, J.L., Sauer, T.J., Cruse, R.M., 2017. Chapter one - soil: The forgotten piece of the water, food, energy nexus. Adv. Agron. 143, 1-46.

Holste, J.E., Smith, L.L., Hair, J.A., Lancaster, J.L., Lloyd, J.E., Langholff, W.K., Barrick, R.A., Eagleson, J.S., 1997. Eprinomectin: A novel avermectin for control of lice in all classes of cattle. Vet. Parasitol. 73, 153-16l.

Hoskins, S.G., Homberg, U., Kingan, T.G., Christensen, T.A., Hildebrand, J.G., 1986. Immunocytochemistry of GABA in the antennal lobes of the sphinx moth *Manduca sexta*. Cell Tissue Res. 244, 243-252.

International Organization for Standardization – ISO, 1999. ISO 11267. Soil quality - Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. Genève, Switzerland.

International Organization for Standardization – ISO, 2005. ISO 10390. Soil quality - Determination of pH. Beuth-Verlag, Berlin.

International Organization for Standardization – ISO, 2008. ISO 17512-2. Soil quality - Avoidance test for determining the quality of soils and effects of chemicals on behavior. Part 2: Test with collembolans (*Folsomia candida*). Genève, Switzerland.

International Organization for Standardization – ISO, 1993. ISO 11465. Soil quality - Determination of dry matter and water content on a mass basis - Gravimetric method. Genève, Switzerland.

Jensen, J., Diao, X., Hansen, A.D., 2009. Single- and two-species tests to study effects of the anthelmintics ivermectin and morantel and the coccidiostatic monensin on soil invertebrates. Environ. Toxicol. Chem. 8, 316-323.

Jensen, J., Krogh, P.H., Sverdrup, L.E., 2003. Effects of the antibacterial agent tiamulin, olaquindox and metronidazole and the anthelmintic ivermectin on the soil invertebrate species *Folsomia fimetaria* (Collembola) and *Enchytraeus crypticus* (Enchytraeidae). Chemosphere 50, 437-443.

Jensen, J., Scott-Fordsmand, J.J., 2012. Ecotoxicity of the veterinary pharmaceutical ivermectin tested in a soil multi-species (SMS) system. Environ. Pollut. 171, 133-139.

Lavelle, P., 1996. Diversity of soil fauna and ecosystem function. Biology International 33, 3-16.

Litskas, V.D., Karamanlis, X.N., Batzias, G.C., Kamarianos, A.P., 2011. Sorption of the antiparasitic drug eprinomectin in three soils. Chemosphere 82, 193-198.

Litskas, V.D., Karamanlis, X.N., Batzias, G.C., Tsiouris, S.E., 2013. Are the parasiticidal avermectins resistant to dissipation in the environment? The case of eprinomectin. Environ. Int. 60, 48-55.

Lumaret, J.P., Galante, E., Lumbreras, C., Crowe, D., 1993. Field effects of ivermectin residues on dung beetles. J. Appl. Ecol. 30, 428-436.

Lumaret, J.P., Errouissi, F., Floate, K., Römbke, J., Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Curr. Pharm. Biotechnol. 13, 1004-1060.

Mahon, R.J., Wardhaugh, K.G., 1992. Impact of dung from ivermectin-treated sheep on oogenesis and survival of adult *Lucilia cuprina*. Aust. Vet. J. 68, 173-177.

Nieman, C.C., Floate, K.D., Düring, R-A., Heinrich, A.P., Young, D.K., Schaefer, D.M., 2018. Eprinomectin from a sustained release formulation adversely affected dung breeding insects. Plos One 13, 1-11.

Oliveira Filho, L.C.I., Baretta, D., 2016. Why should we care about edaphic springtails? Sci. Agrar. 17, 21-40.

Palmer, S.J., Beavers, J.B., 1995. Reported (Appendix D-9) in Ivomec® Eprinex® (eprinomectin) pour on for beef and dairy cattle, Environmental Assessment, United States. https//:www.fda.gov/CVM/efoi/EA_Files/141-079EA.PDF. (accessed 08 November 2018).

Peijnenburg, W., Capri, E., Kula, C., Liess, M., Luttik, R., Montforts, M., Nienstedt, K., Römbke, J., Sousa, J.P., Jensen, J., 2012. Evaluation of exposure metrics for effect assessment of soil invertebrates. Crit. Rev. Environ. Sci. Technol. 42, 1862-1893.

Pregitzer, P., Schultze, A., Raming, K., Breer, H, Krieger, J., 2013. Expression of a $GABA_B$ receptor in olfactory sensory neurons of Sensilla trichodea on the male antenna of the moth *Heliothis virescens*. Int. J. Biol. Sci. 9, 707-715.

Sakuma, M., 1998. Probit analysis of preference data. Applied Entom. Zool. 33, 339-347.

San Miguel, A., Raveton, M., Lempérière, G., Ravanel, P., 2008. Phenylpyrazoles impact on *Folsomia candida* (Collembola). Soil Biol. Biochem. 40, 2351-2357.

Scheffczyk, A., Floate, K.D., Blanckenhorn, W.U., Düring, R.A., Klockner, A., Lahr, J., Lumaret, J.P., Salamon, J.A., Tixier, T., Wohde, M., Römbke, J., 2016. Non-target effects of ivermectin residues on earthworms and springtails dwelling beneath dung of treated cattle in four countries. Environ. Toxicol. Chem. 35, 1959-1969.

Shoop, W., Soll, M., 2002. Chemistry, pharmacology and safety of the macrocyclic lactones -

ivermectin, abamectin and eprinomectin, in: Vercruysse, J., Rew, R.S. (Eds.), Macrocyclic lactones in antiparasitic therapy. CABI Publishing, Wallingford, pp. 1-29.

Smit, C.E., Van Gestel, C.A.M., 1996. Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils. Appl. Soil Ecol. 3, 127-136.

Statsoft, 2004. Statistica 7.0 (Data Analysus Software System). https//:www.statsoft.com. (accessed 08 November 2018).

Turner, M.J., Schaeffer, J. M., 1989. Mode of action of ivermectin, in: Campbell, W.C. (Eds.), Ivermectin and abamectin. Springer-Verlag, New York, pp. 73-88.

United States Department of Agriculture - USDA, 2014. Soil survey staff: Keys to soil taxonomy, twelfth ed. USDA - Natural Resources Conservation Service, Washington.

Vassilis, L.D., George, B.C., Charalampos, P.G., Athina, P-V., Xanthipposa, K.N., 2016. Mobility of pharmaceutical compounds in the terrestrial environment: Adsorption kinetics of the macrocyclic lactone eprinomectin in soils. Chemosphere 144, 1201-1206.

Wells, R.J., 1998. Eprinomectin, in: FAO food and nutrition paper (Eds), Residues of some veterinary drugs in animals and foods. Joint FAO/WHO Expert Committee on Food Additives. Meeting, FAO., Food and Agriculture Organization of the United Nations, World Health Organization, Rome, pp. 31-47.

Wolstenholme, A.J., 2012. Glutamate-gated chloride channels. J. Biol. Chem. 287, 40232-40238.

Zar, J, 1996. Biostatistical analysis, third ed. Prentice-Hall, London.

Zortéa, T., Baretta, D., Maccari, A.P., Segat, J.C., Boiago, E.S., Sousa, J.P., Da Silva A.S., 2015. Influence of cypermethrin on avoidance behavior, survival and reproduction of *Folsomia candida* in soil. Chemosphere 122, 94-98.

Zortéa, T., Dos Reis, T.R., Serafini, S., Sousa, J.P., Da Silva, A.S., Baretta, D., 2018. Ecotoxicological effect of fipronil and its metabolites on *Folsomia candida* in tropical soils. Environ. Toxicol. Pharmacol. 62, 203-209.

Zortéa, T., Segat, J.C., Maccari, A.P., Sousa, J.P., Da Silva, A.S., Baretta, D., 2017. Toxicity of four veterinary pharmaceuticals on the survival and reproduction of *Folsomia candida* in tropical soils. Chemosphere 173, 460-465.

Table 1 - Physical and chemical variables of subtropical soils, Entisol and Oxisol, and Tropical Artificial Soil (TAS).

Variables	Entisol	Oxisol	TAS
[1]OM (%)	0.90	2.70	1.5
pH (H_2O)	5.80	4.60	6.30
[2]CEC ($cmol_c\ dm^{-3}$)	4.92	15.60	14.24
Clay (%)	4.00	49.00	11.00
Sand (%)	37.00	12.00	75.00
Silt (%)	59.00	39.00	0.00
P ($mg\ kg^{-1}$)	1.90	6.70	8.10
K ($cmol_c\ dm^{-3}$)	0.09	0.23	0.07
Ca ($cmol_c\ dm^{-3}$)	2.00	2.69	9.80
Mg ($cmol_c\ dm^{-3}$)	0.83	1.80	1.11
Al ($cmol_c\ dm^{-3}$)	0.00	0.83	0.00
H + Al ($cmol_c\ dm^{-3}$)	2.00	10.90	2.32
Cu ($mg\ kg^{-1}$)	1.50	1.40	-
Zn ($mg\ kg^{-1}$)	1.00	0.80	-
Fe ($mg\ kg^{-1}$)	72.50	79.70	-
Mn ($mg\ kg^{-1}$)	2.10	5.40	-

[1]OM = Organic matter.

[2]CEC = Cation exchange capacity at pH 7.0.

Table 2 - NOEC[1], LOEC[2], EC_{50}[3] and AC_{50}[4] values calculated on the basis of the ecotoxicological tests for lethality, reproduction and avoidance behavior of springtails *Folsomia candida* exposed to Entisol and Oxisol soils treated with the antiparasitic agent eprinomectin (values in mg kg^{-1} of dry soil).

Ecotoxicological Tests	Parameters	Subtropical soils treated with eprinomectin (mg kg^{-1} of dry soil)	
		Entisol	Oxisol
Lethality	NOEC	16	16
	LOEC	8	20
Reproduction	NOEC	4	< 0.5
	LOEC	8	0.5
	EC_{50}	4.38	> 20
	Mathematical Model	Gompertz (R = 0.98)	-
	Inferior Limit	3.76	-
	Upper Limit	5.00	-
Avoidance Behavior	NOEC	< 0.5	< 0.5
	LOEC	0.5	0.5
	AC_{50}	-	< 0.5
	Inferior Limit	-	-
	Upper limit	-	-

[1]NOEC = No observed effect concentration.

[2]LOEC = Lowest observed effect concentration.

[3]EC_{50} = Effective concentration that reduces reproduction rate by 50%.

[4]AC_{50} = Concentration that causes 50% avoidance.

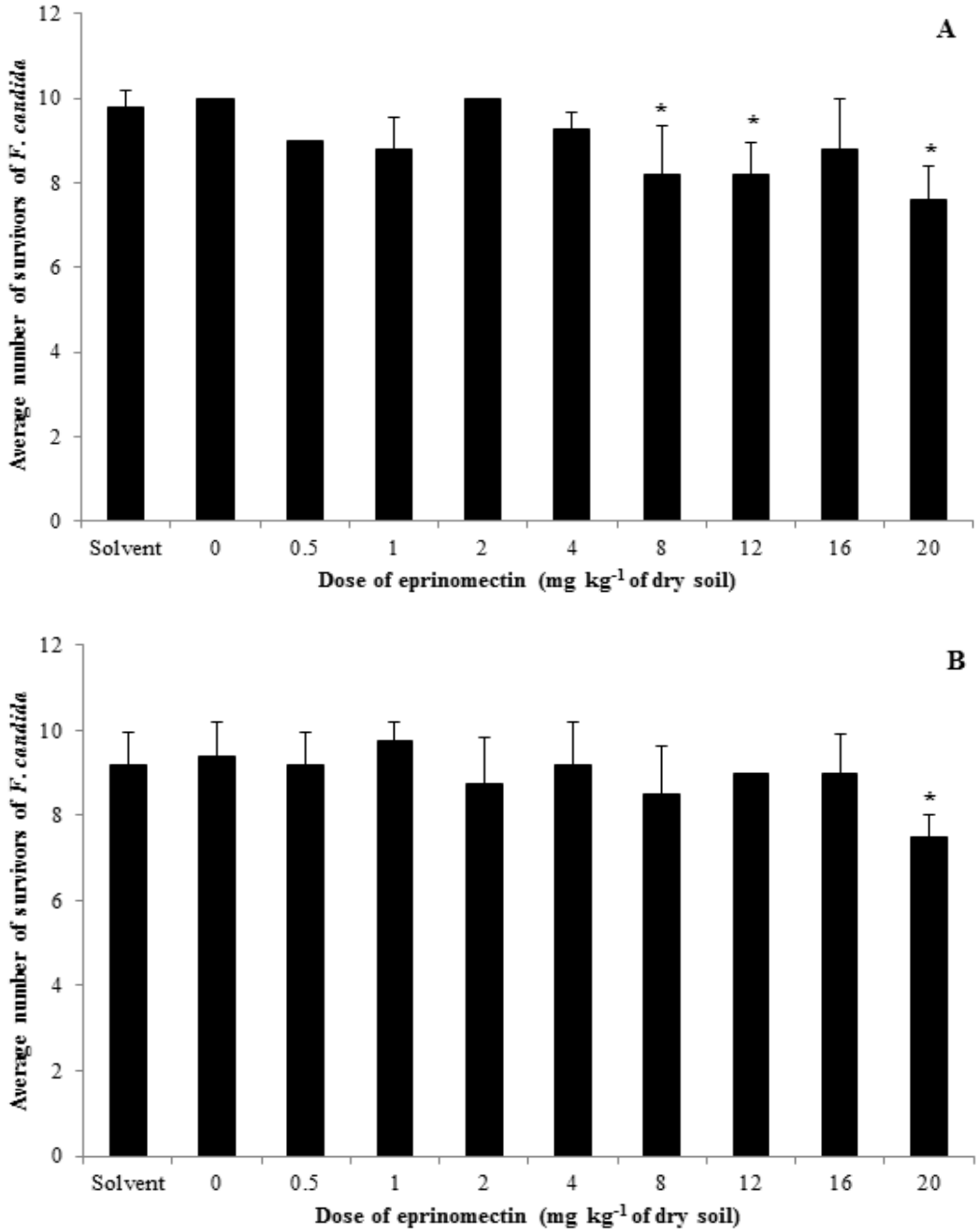


Fig. 1 - Average number of springtails *Folsomia candida* survivors in ecotoxicological lethality tests when exposed to subtropical soils untreated (control) and treated with the antiparasitic agent eprinomectin. (A) Entisol and (B) Oxisol. (┬) Standard deviation (n = 5). Asterisk (*) indicates a significant difference for the average number of springtails survivors between untreated soils and treated soils ($p < 0.05$, one-way ANOVA followed by the Dunnett's test).

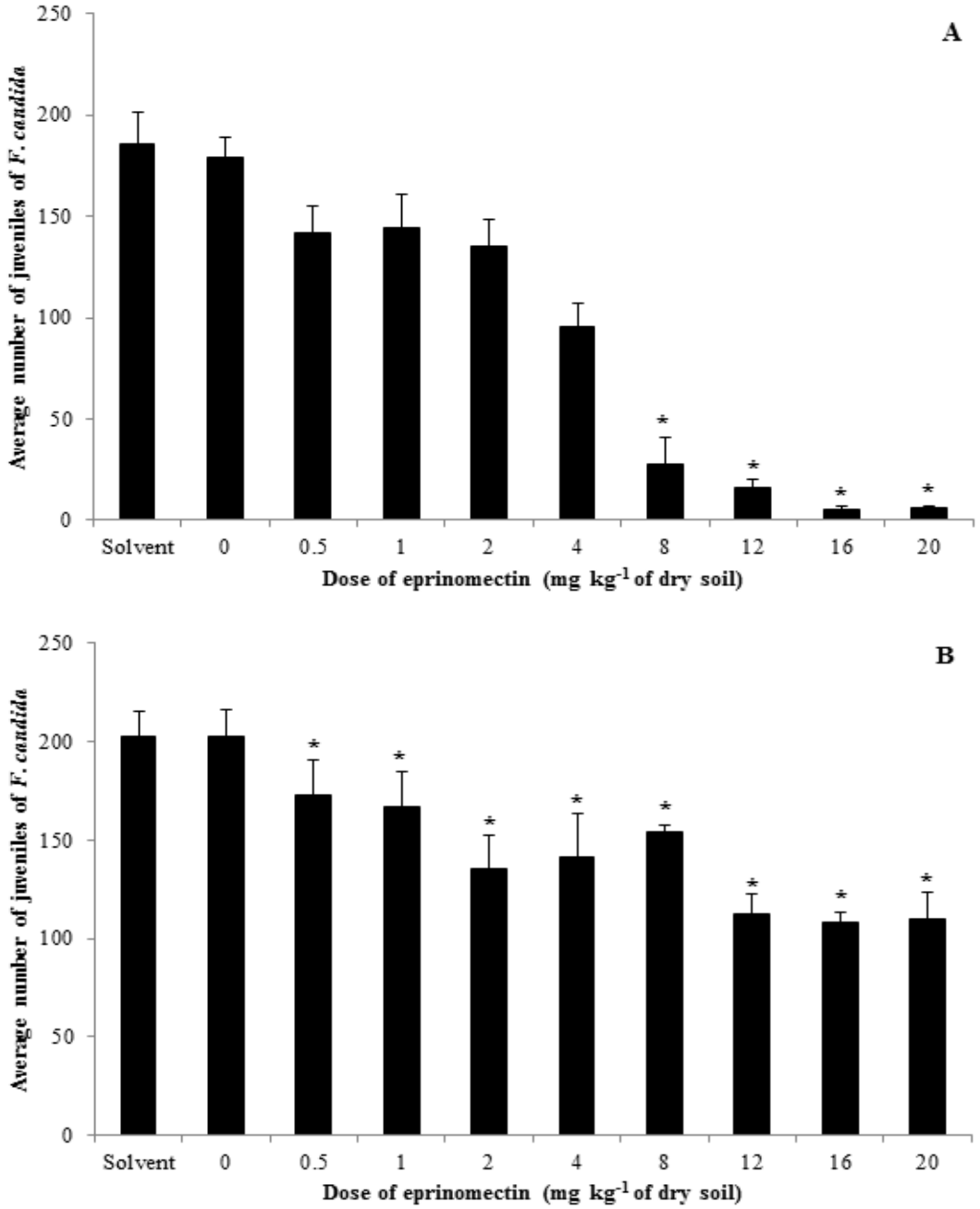


Fig. 2 - Average number of springtails *Folsomia candida* juveniles in ecotoxicological reproduction tests when exposed to subtropical soils untreated (control) and treated with the antiparasitic agent eprinomectin. (A) Entisol and (B) Oxisol. (┬) Standard deviation (n = 6). Asterisk (*) indicates a significant difference for the average number of springtails juveniles between untreated soils and treated soils ($p < 0.05$, Kruskal-Wallis non-parametric test (A) and one-way ANOVA followed by the Dunnett's test (B)).

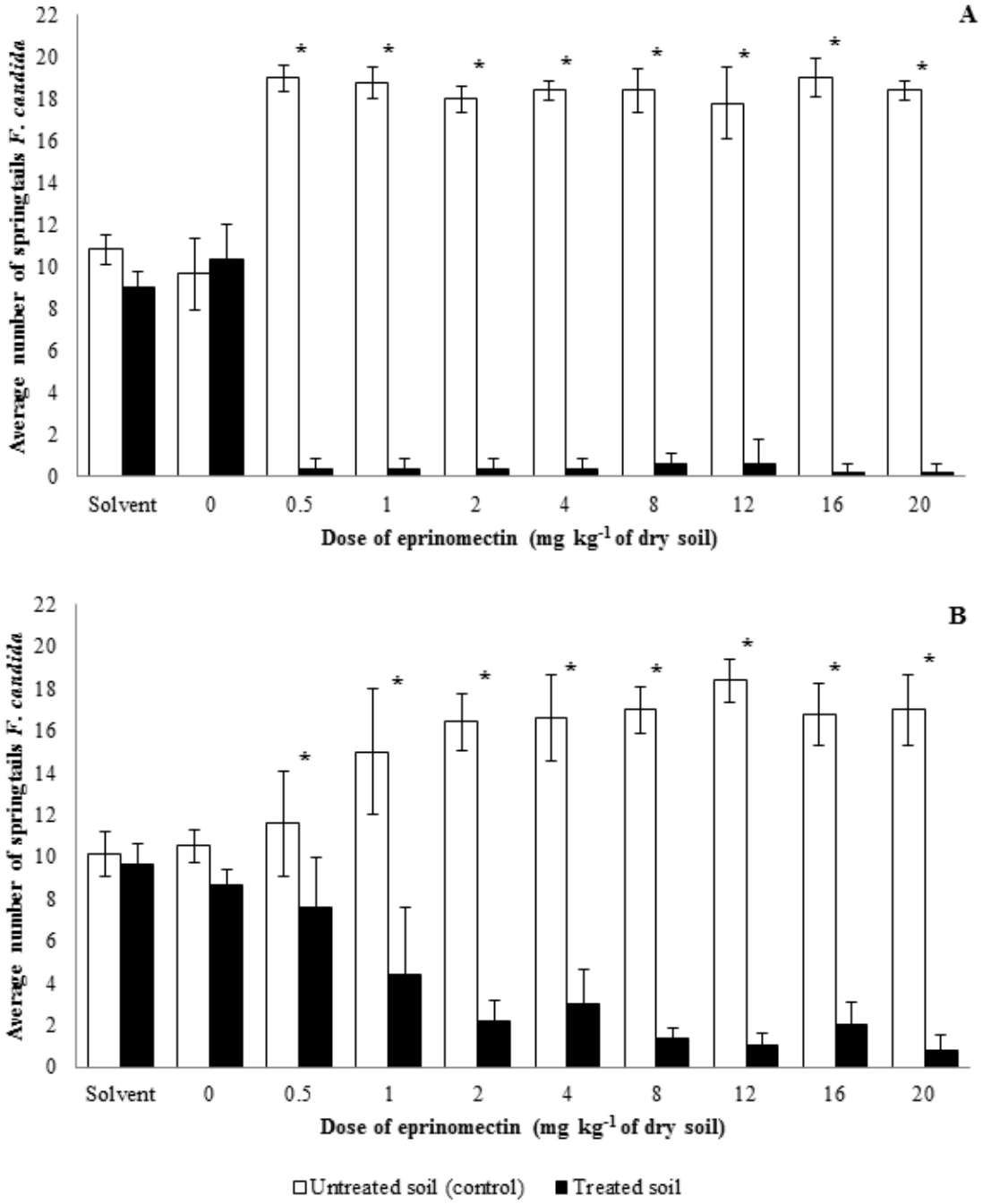


Fig. 3 - Average number of springtails *Folsomia candida* in ecotoxicological avoidance behavior tests when exposed to subtropical soils untreated (control) and treated with the antiparasitic agent eprinomectin. (A) Entisol and (B) Oxisol. (┬) Standard deviation (n = 6). Asterisk (*) indicates a significant difference for the average number of springtails between untreated soils and treated soils ($p < 0.05$, Fisher's test).

2.2 ARTIGO I

Fish exposed to water contaminated with eprinomectin show inhibition of the activities of AChE and Na^+/K^+-ATPase in the brain, and changes in natural behavior

Suélen Serafini [a], Carine de Freitas Souza [b], Matheus Dellaméa Baldissera [b], Bernardo Baldisserotto [b], Julia Corá Segat [a], Dilmar Baretta [a, c, *], Renato Zanella [d], Aleksandro Schafer da Silva [a, c, **]

[a] Graduate Program of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[b] Graduate Program of Pharmacology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

[c] Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[d] Department of Chemistry, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

[*] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Beloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

[**] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Beloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

E-mail addresses: dilmar.baretta@udesc.br (D. Baretta), aleksandro.silva@udesc.br (A. Schafer da Silva).

De acordo com normas para publicação em:

Chemosphere

Situação do Artigo: Publicado

Fish exposed to water contaminated with eprinomectin show inhibition of the activities of AChE and Na^+/K^+-ATPase in the brain, and changes in natural behavior

Abstract

The objective of this study was to evaluate whether antiparasitic eprinomectin may be an environmental contaminant in water compartment in low concentrations, negatively affecting neurotransmission and, consequently, the natural behavior of the jundiá (*Rhamdia quelen*). Fish were randomly allocated in tanks and exposed for 24 and 48 h to eprinomectin concentrations in water [0.0 (Control), 1.124 (T1), 1.809 (T2) and 3.976 (T3) µg L^{-1}], followed by 48 h of recovery in eprinomectin-free water, in order to evaluate the behavioral parameters, levels of reactive oxygen species (ROS) in the brain, as well as cerebral enzymatic activities of acetylcholinesterase (AChE) and of the sodium-potassium ATPase pump (Na^+/K^+-ATPase). Especially at the two highest concentrations of eprinomectin (T2 and T3), the fish showed alterations in natural behavior, particularly hyperlocomotion and spending longer time on the surface. Furthermore, at these same concentrations, cerebral ROS levels increased and cerebral AChE activity decreased. At the highest concentration (T3) cerebral Na^+/K^+-ATPase activity was reduced. Increased ROS and impairment of AChE and Na^+/K^+-ATPase enzymes in the brain may have contributed directly to behavioral changes, due to neuronal damage and synapse impairment. Even after 48 h in water without eprinomectin, behavioral changes and neurotoxic effects were observed in fish, suggesting residual effects of the antiparasitic. In conclusion, eprinomectin even in low concentrations may be a hazardous environmental contaminant for aquatic organisms, as it causes brain damage and affects the natural behavior of fish.

Keywords: Antiparasitic, aquatic organisms, brain damage, contaminant, locomotor alterations

1. Introduction

Recent ecotoxicological studies have revived environmental concerns regarding the effects of avermectins on the environment, especially those of ivermectin and abamectin in soil or animal feces, as well as their effects on non-target organisms (Bai and Ogbourne,

2016; Jochmann and Blanckenhorn, 2016; Scheffczyk et al., 2016; Verdú et al., 2015; Zortéa et al., 2017). Eprinomectin is an avermectin whose behavior has been studied in various soils (Litskas et al., 2011, 2013; Vassilis et al., 2016) and in incipient studies on its impact on non-target soil organisms (Halley et al., 2005; Nieman et al., 2018), and is considered environmentally safe for soil and its fauna under typical conditions when used as an endectocide. It is important to emphasize that one of the main entry routes of antiparasitics and their metabolites in the environment is through the use of animal waste as soil fertilizers (Vassilis et al., 2016). However, eprinomectin exhibits a higher degree of hydrophilicity than abamectin or ivermectin (Holste et al., 1997; Merck, 1996) and one of its major metabolites excreted via feces is polar in character (Merck, 1996). In soil contamination, this molecule shows moderate dissipation and high persistence, remaining in the soil for more than 120 days (Litskas et al., 2013). As for their absorption and adsorption, these vary according to the physicochemical characteristics of the soil, factors that may facility their mobility and, consequently, water contamination (Litskas et al., 2011, 2013; Vassilis et al., 2016).

Inadequate disposal of veterinary drugs in water, as well as the use in incorrect quantities and forms in fish production systems for the prophylaxis and treatment for parasites can also contribute to environmental contamination. Avermectins, especially ivermectin, are used for the control of parasitoses in aquaculture (Athanassopoulou et al., 2001; Davies and Rodger, 2000; Grant and Briggs, 1998; Hemaprasanth et al., 2012; Roth et al., 1993). There are no reports of the use of eprinomectin as an antiparasitic for fish, but $LC50_{96}$ values for the species *Onchorhynchus mykiss* (rainbow trout) and *Lepomis macrochirus* (bluegill) were determined to be 1.2 mg L^{-1} and 0.37 mg L^{-1}, respectively (Merck, 1996). The mode of action of eprinomectin is similar to that of other avermectins. Among the most known mechanisms, in invertebrates, ivermectin acts as a positive allosteric regulator of several ligand-gated channels including, gamma-aminobutyric acid (GABA)-gated chloride channels and glutamate-gated chloride channel (GluCl). As these channels are inhibitory, the effect of ivermectin is potentiation of the inhibitory effect of neurotransmission produced by GABA and glutamate in invertebrates, culminating in a paralysis of the somatic muscles (Duce and Scott, 1985; Geary and Moreno, 2012; Verdú et al., 2015). In vertebrates, ivermectin also interacts with neuronal GABA receptors, however, to a lesser extent than in invertebrates (Paredes and Âgmo, 1992).

Studies have shown that expositions in the water for up to 96 h to the sublethal concentrations of eprinomectin induced hepatic oxidative stress in *O. mykiss* (Alak et al.,

2017); however, there are no reports of its effect on neurotransmission, and consequently on behavior in fish. Neurotoxic effects of ivermectin were reported at relatively low doses administered orally in *Salmo salar* (Atlantic salmon), because of the distribution of the molecule in the central nervous system (CNS) and the accumulation in the brain associated with its possible ability to cross the blood-brain barrier in fish (Høy et al., 1990). Darkening reactions, loss of appetite and lethargy associated with possible neurotoxic effects have also been reported in *Sparus aurata* (sea bream) with intraperitoneal injections of ivermectin in single doses of 0.4 and 0.8 mg kg^{-1} of body weight (Katharios et al., 2001). The crossing of the blood-brain barrier by ivermectin and the substantial accumulation of this compound in brain tissues was confirmed in *S. aurata* (Katharios et al., 2004). Ivermectin doses of 100-200 µg kg^{-1} were considered safe for use in aquaculture (Katharios et al., 2004).

More studies are needed regarding CNS and behavioral effects of avermectins in fish, especially eprinomectin, because of their differentiated molecular characteristics, as well as the use of lower concentrations than those described as safe in aquaculture in the case of ivermectin use. Measurement of reactive oxygen species (ROS) levels and activity of neuroregulatory enzymes in brain tissues may indicate the effects of eprinomectin on neurotransmission in fish, associated with behavioral monitoring. This is because ROS are biomarkers linked to oxidative stress and neuronal damage (Liew et al., 2015), and its increase in fish brain tissues may trigger behavioral changes (Baldissera et al., 2017). According to the literature, changes in the activity of acetylcholinesterase (AChE) and the sodium-potassium ATPase pump (Na^+/K^+-ATPase) in cerebral synaptosomes may compromise synaptic activities (Abdalla et al., 2013; Jaques et al., 2011). AChE is an essential enzyme of the cholinergic pathway that hydrolyzes the neurotransmitter acetylcholine (ACh) and regulates its levels in synaptic clefts (Čolović et al., 2013; Ren et al., 2015). On the other hand, Na^+/K^+-ATPase is responsible for the active transport of sodium and potassium ions in the nervous system and for the maintenance and reestablishment of the electrochemical gradient necessary for the mechanism of neuronal excitability after each depolarization (Souza et al., 2014).

According to previous studies, the cerebral activities of AChE and Na^+/K^+-ATPase play essential roles in locomotion (Drever et al., 2011; Sahu et al., 2013) and are associated with locomotor changes in fish (Baldissera et al., 2017, 2018). Our hypothesis was that possible behavioral changes caused by eprinomectin, especially in terms of locomotion, may be associated with limitations in neurotransmission. Therefore, the objective of this study

were to evaluate whether antiparasitic eprinomectin could be an environmental contaminant in water in low concentrations, and whether it could negatively affect neurotransmission and, consequently, the natural behavior of the jundiá (*Rhamdia quelen*).

2. Materials and methods

2.1. Ethics committee

This study was approved by the Ethical and Animal Welfare Committee of the Universidade do Estado de Santa Catarina (protocol number 4679260518).

2.2. Chemical and water eprinomectin concentration

Eprinomectin (3.6%) was purchased in a Brazilian market with the commercial name of Eprinex® (Merial, Brazil). The antiparasitic was added to the test water at the concentrations (treatments): 0.0 (Control), 0.0025 (T1), 0.01 (T2) and 0.05 (T3) µg L^{-1} prior to the beginning of the experiment (calculated concentration), based on the protocol established by Alak et al. (2017). Eprinomectin was diluted in ethanol (1:10 v v^{-1}) prior to the experiment. The dilution in ethanol was also used as control, named treatment T4.

The concentration of eprinomectin was determined in tanks water at the beginning (0 h), and at 24 and 48 h of exposure, as well as at 48 h, during the eprinomectin-free water recovery span, using ultra-high-performance liquid chromatography coupled to mass spectrophotometry (UHPLC-MS).

2.3. Fish maintenance and experimental study

Juvenile *R. quelen* (popular name: jundiá or silver catfish) (males; seven months old; 60.99 ± 4.14 g; 14.10 ± 1.11 cm) were collected for experimental purposes from a fish farm located in Southern Brazil. The *R. quelen* were transported alive and were acclimatized in 250 L fiberglass tanks with continuous aeration and controlled water variables (temperature: 21 ± 0.2 ºC; pH: 6.41 ± 0.1; ammonia: 0.84 ± 0.06 mg L^{-1}; non-ionized ammonia: 0.0046 ± 0.00031 mg L^{-1}; dissolved oxygen: 6.71 ± 0.59 mg L^{-1}) for ten days. The fish were fed to apparent satiation with commercial pellets once a day and with continuous feeding during the

experimental period. Any uneaten food, feces, and other residues were removed daily 30 min after feeding.

The jundiá were randomly allocated inside tanks of 250 L with continuous aeration and were exposed for 24 and 48 h to eprinomectin in the water, followed by 48 h of recovery in eprinomectin-free water (three replicates per concentration/treatment; six fish per replicate; total of 90 fish). Water quality variables (temperature, pH, ammonia, non-ionized ammonia, and dissolved oxygen) remained constant throughout all experimental periods.

2.4. Behavioral tests – novel tank test

Before sample collection (24 and 48 h of eprinomectin exposure in the water, and after 48 h of recovery in eprinomectin-free water) fish were transferred individually to a test tank (50 × 20 × 50 cm) and recorded for 6 min, according the protocol established by Baldissera et al. (2017). The following behavioral parameters were analyzed: distance travelled (m), mean speed (m s^{-1}) and absolute turn angle (^{o}), for to evaluate the locomotor activity; as well as number of entries in the zones (upper and bottom), time in the zones (upper and bottom) (s), latency to the first (1st) entry in the upper zone and total time of freezing (s), for to evaluate the movement displaying in the two tank zones. Behavioral tests were recorded using a Logitech HD Webcam C525 camera (Logitech, Romanel-sur-Morges, Switzerland), and the videos were analyzed using ANY-Maze® tracking software (Stoelting CO, USA).

2.5. Sample collection

After 24 and 48 h of exposure, and 48 h after recovery in eprinomectin-free water, two fish from each tank (six fish per treatment at each given time; total of 90 fish) were anesthetized with 50 mg L^{-1} of eugenol (Da Cunha et al., 2010), followed by spinal cord transection according to ethics committee recommendations. Thereafter, brains were removed and dissected in glass dishes over ice to evaluate the parameters described below.

2.6. Cerebral ROS levels

Cerebral ROS levels were determined by the DCFH oxidation method described by LeBel et al. (1992), recently published in detail by Souza et al. (2018) using excitation and emission wavelengths of 485 and 538 ηm, respectively. Results were expressed as U DCF mg of protein^{-1}.

2.7. Cerebral AChE and Na^+/K^+-ATPase activities

For the determination of cerebral AChE activity, brains were homogenized (1:10 w v^{-1}) in a buffer (10 mM Tris-HCl, pH 7.2) and centrifuged at 800 *x g* for 10 min. The supernatants were collected and used to determine activity based on the spectrophotometric method proposed by Ellman et al. (1961), as described by Rocha et al. (1993). Enzymatic activity was expressed in µmol AcSCh h^{-1} mg of protein^{-1}.

For the determination of cerebral Na^+/K^+-ATPase activity, brains were homogenized (1:10 w v^{-1}) in a buffer (50 mM Tris-HCl, pH 7.5) and centrifuged at 4.000 *x g* for 10 min at 4 ºC. The supernatants were immediately collected and used to determine activity based on the spectrophotometric (405 ηm) method proposed by Muszbek et al. (1977), published in detail by Baldissera et al. (2017). Enzymatic activity was expressed as ηmol pyruvate (Pi) released min^{-1} mg of protein^{-1}.

2.8. Cerebral protein content

Cerebral protein content was evaluated by the Coomassie blue G dye method (Read and Northcote, 1981), using serum bovine albumin as the standard.

2.9. Statistical analysis

The data met the assumption of parametric testing (normality and homoscedasticity) using the Kolmogorov-Smirnov and Levene's tests, respectively. Subsequently, statistical analysis was performed using bilateral two-way analysis of variance (ANOVA) followed by the Tukey post-hoc test considering significance at $p < 0.05$.

3. Results

3.1. Eprinomectin concentration in water

At 0 h, eprinomectin concentrations in water were 1.124, 1.809 and 3.976 µg L^{-1} in treatments 0.0025 (T1), 0.01 (T2) and 0.05 (T3) µg L^{-1}, respectively. At 24 h, eprinomectin concentrations in water were 0.850, 1.149 and 1.152 µg L^{-1} in treatments T1, T2 and T3, respectively. At 48 h, eprinomectin concentrations in water were 0.394, 0.699 and 1.286 µg L^{-1} in treatments T1, T2 and T3, respectively.

3.2. Behavioral tests

In the novel tank test, exposure to treatments T2 and T3 of eprinomectin increased ($p < 0.001$) the distance travelled and mean speed compared to the Control at 48 h of exposure, and remained higher ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 1 - A, B). No significant differences ($p > 0.05$) were observed for all treatments regarding absolute turn angle (Fig. 1 - C).

The number of entries in the upper zone was higher ($p < 0.001$) in fish exposed to treatment T3 compared to the Control at 48 h of exposure, and remained higher ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - A). While the time in the upper zone was higher ($p < 0.001$) in fish exposed to treatments T2 and T3 compared to the Control at 48 h of exposure, and remained higher ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - B). Furthermore, latency to the 1st entry in the upper zone was lower ($p < 0.001$) in fish of treatment T3 than in the Control at 48 h of exposure, and remained lower ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - C).

The number of entries in the upper zone was higher ($p < 0.001$) in fish exposed to treatment T3 than in the Control at 48 h of exposure, and remained higher ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - D). While the time in the bottom zone was lower ($p < 0.001$) in fish of treatments T2 and T3 than in the Control at 48 h of exposure, and remained lower ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - E). Finally, total time of freezing was lower ($p < 0.001$) in fish exposed to treatments T2 and T3 compared to the Control at 48 h of exposure, and remained lower ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 2 - F).

3.3. Cerebral ROS levels

Cerebral ROS levels were higher ($p < 0.001$) in fish exposed to eprinomectin of treatments T2 and T3 than in the Control at 48 h of exposure, and remained higher ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 3).

3.4. Cerebral AChE and Na^+/K^+-ATPase activities

Cerebral AChE activity was lower ($p < 0.001$) in fish exposed to eprinomectin of treatment T3 than in the Control at 24 h of exposure, and was lower ($p < 0.001$) in fish exposed to treatments T2 and T3 than in the Control at 48 h of exposure, and remained lower ($p < 0.001$) after 48 h of recovery in eprinomectin-free water. Over time, AChE was lower ($p < 0.001$) in fish exposed to eprinomectin of treatment T2 at 48 h compared to 24 h of exposure (Fig. 4 - A).

Cerebral Na^+/K^+-ATPase activity was lower ($p < 0.001$) in fish exposed to eprinomectin of treatment T3 than in the Control at 48 h of exposure, and remained lower ($p < 0.001$) after 48 h of recovery in eprinomectin-free water (Fig. 4 - B).

4. Discussion

In this study, changes in behavior of jundiá (*R. quelen*) coincided with changes in the activities of enzymes involved in neurotransmission. Therefore, it is possible that eprinomectin (as with ivermectin) are able to cross the blood-brain barrier and cause neurotoxicity in fish, corroborating with the results of other studies (Høy et al., 1990; Katharios et al., 2001, 2004). Our results also showed that, as with ivermectin, eprinomectin is able to alter the fish's natural behavior (Katharios et al., 2004). However, the effects of eprinomectin on water occurred at concentrations well below those considered safe for ivermectin in aquaculture (100-200 µg kg^{-1}), as reported by Katharios et al. (2004).

Hyperlocomotion and longer time on the surface, observed in fish exposed to eprinomectin, are atypical behaviors for *R. quelen*. According to the literature, this species prefers to live near the bottom, between vegetation or rocky protections, and shows little mobility (Gomes et al., 2000). Hyperlocomotion seen in jundiá differs from the effects described for other fish such as *S. aurata*, where lethargy was observed when exposed to ivermectin (Katharios et al., 2001), although the behavior of this species is very similar to that

of *R. quelen* (Bauchot and Hureau, 1990). Varied behavioral effects may occur because the behavior or mode of action of avermectins is distinct, depending on the biological model used for the study or how it contacts the organism (Turner and Schaeffer, 1989).

In fish, behavioral changes in response to environmental and physiological stressors, such as hyperlocomotion, can cause energy expenditure (Barcellos et al., 2007). Studies with *R. quelen* also reported that exposure to sublethal concentrations of agrichemicals may have deleterious effects on the fish and might impair its ability to respond to environmental stressors (Cericato et al., 2008, 2009). In nature, changes in the natural behavior of the species, associated with energy expenditure related to hyperlocomotion, may have negative effects on activities necessary for survival of the individual or species, interfering with feeding, reproduction, as well as increased susceptibility to predation (Domingues et al., 2016). These impacts can extend to changes in the networks of the food chain and may cause subsequent environmental imbalances. In commercial fish production, hyperlocomotion hypothetically can affect fish appetite, associated with energy expenditure, and may have implications for protein deposition in particular tissues of commercial value (Domingues et al., 2016). Consequently, there may be effects on productivity and profitability in aquaculture.

Studies of the effects of agrichemicals on *R. quelen* reported that the toxic effects on behavior occur in the hypothalamus-pituitary-inter-renal axis (HPI) (Cericato et al., 2008). Agrichemicals affects the elevation of plasma cortisol levels and the primary tissue affected in the HPI axis is the head kidney (interrenal tissue), with behavioral effects in terms of the reduction of the ability of fish to respond to stressors (Cericato et al., 2009); as observed with 96 h of exposure to the compounds (Cericato et al., 2008, 2009). We believe that eprinomectin is capable of inducing neurotoxicity and, consequently, behavioral changes at up to 24 h. Furthermore, exposure to eprinomectin caused an increase in cerebral ROS levels; it is known that there is a strong relationship between cortisol elevation and increased ROS in brain tissues (Baldissera et al., 2017).

The elevation of ROS in the brain suggests that eprinomectin may be a threat to homeostasis, because the overproduction of ROS may interact with many cellular components, including structural carbohydrates and lipids, enzymes, proteins and nucleic acids, leading to pathological and damage to these structures (Dröge, 2002). It also reinforces the hypothesis that eprinomectin causes neuronal damage (Liew et al., 2015), possibly explaining the behavioral changes. According to the literature, the increase of cerebral ROS also interferes with the activity of the enzymes involved in the neurotransmission processes

(Baldissera et al., 2017), that may have occurred in the present study. Therefore, we believe that the behavioral changes caused by eprinomectin have a direct relationship with neuronal impairment and synaptic mechanisms, because the activity of regulatory enzymes, AChE and Na^+/K^+-ATPase in brain tissues were altered.

Exposure to eprinomectin caused inhibition of cerebral AChE activity. As observed in this present study, Baldissera et al. (2019) observed a significant inhibition on cerebral AChE activity of *R. quelen* exposed to 11 and 22 mg L^{-1} of organophosphate pesticide trichlorfon, which contribute to its neurotoxic effects. The reduction of AChE activity implies a lower hydrolysis of ACh, an important neurotransmitter in the synaptic cleft, associated with functions related to learning, memory, anxiety and locomotion (Baldissera et al., 2017). The imbalance in ACh hydrolysis interferes with cholinergic neurotransmission, causing hyperstimulation of nicotinic and muscarinic receptors and consequent interruption of neurotransmission (Čolović et al., 2013). According to the literature, the lethal action of insecticidal compounds is related to the reversible or irreversible inactivation of AChE, causing cholinergic poisoning (Čolović et al., 2013; Silva et al., 2013). Friedman et al. (1996) in a study of pyridostigmine, an AChE inhibitory drug, reported that stressful conditions may favor the diffusion of AChE inhibitor compounds into the CNS by facilitation of blood-brain barrier penetration. Inhibition of AChE activity was related to cerebral electrical excitability (Friedman et al., 1996).

The reduction of cerebral Na^+/K^+-ATPase activity observed in this study was associated with locomotor changes and interference with neurotransmission in fish, because of electrochemical imbalances in synaptic transmissions (Baldissera et al., 2017, 2018). Also, this inhibition may explain the impairment on fish behavior elicited by eprinomectin, since its downregulation causes neuron depolarization and reduce its neural activity, provoking negative consequences on behavioral function, including locomotor change (Pereira et al., 2002). According to Moseley et al. (2007), Na^+/K^+-ATPase activity plays a crucial role in ionic homeostasis and synaptic transmission. According to the literature, the primary physiological response to ivermectin is an increase in membrane permeability due to an agonistic action on chloride channels in nerve and muscle cells (Albert et al., 1986), an effect that causes decreases in the membrane input resistance and therefore in a reduced probability of action potential generation (Verdú et al., 2015); this may also have occurred when *R. quelen* were exposed to eprinomectin. Recently, study conducted by Adefegha et al. (2016) revealed that reduction on Na^+/K^+-ATPase activity in the brain is associated with excessive

ROS production. In this sense, the excessive ROS production elicited by exposure to eprinomectin can impairs of Na^+/K^+-ATPase activity, that may contribute to behavioral alterations observed in this study, as observed by Baldissera et al. (2019) in *R. quelen* exposed to trichlorfon. According to these authors, the excessive cerebral ROS production cause alteration in lipid component of brain cells, which may impair the structure of Na^+/K^+-ATPase, which explain its inhibition.

In summary, we believe that these behavioral changes were due to the association of deleterious effects between cerebral ROS and neurotransmission impairment. Even after 48 h in water without eprinomectin, behavioral changes and neurotoxic effects were observed in fish, suggesting residual effects of the antiparasitic. Future studies are important to verify the consequences of long-term eprinomectin exposure.

5. Conclusions

We conclude that jundiá (*R. quelen*) exposed to water experimentally contaminated with the antiparasitic eprinomectin shows behavioral changes, particularly hyperlocomotion and the longer time on the surface. Also, the increases in cerebral ROS levels and inhibition of cerebral AChE and Na^+/K^+-ATPase activities can be a pathway associated to impairment on fish behavior. Therefore, eprinomectin even in low concentrations may be a hazardous environmental contaminant for aquatic organisms, as it causes brain damage and affects the natural behavior of fish.

Conflict of interest

Authors declare they have no conflict of interest.

Acknowledgements

The authors would like to thank the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 2017TR754, Brazil). A.S. Da Silva (Process No. 304328/2015-4) and D. Baretta (Process No. 307162/2015-0) acknowledges research grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

References

Abdalla, F.H., Cardoso, A.M., Pereira, L.B., Schmatz, R., Gonçalves, J.F., Stefanello, N., Fiorenza, A.M., Gutierres, J.M., Serres, J.D.S., Zanini, D., Pimentel, V.C., Vieira, J.M., Schetinger, M.R.C., Morsch, V.M., Mazzanti, C.M., 2013. Neuroprotective effect of quercetin in ectoenzymes and acetylcholinesterase activities in cerebral cortex synaptosomes of cadmium-exposed rats. Mol. Cell. Biochem. 381, 1-8. http://doi.org/10.1007/s11010-013-1659-x

Adefegha, S.A., Oboh, G., Omojokun, O.S., Adefegha, O.M., 2016. Alterations of Na^+/K^+-ATPase, cholinergic and antioxidant enzymes activity by protocatechuic acid in cadmium-induced neurotoxicity and oxidative stress in Wistar rats. Biomed. Pharmacother. 83, 559-568. http://doi.org/10.1016/j.biopha.2016.07.017

Alak, G., Yeltekin, A.Ç., Tas, I.H., Ucar, A., Parlak, V., Topal, A., Kocaman, E.M., Atamanalp, M., 2017. Investigation of 8-OHdG, CYP1A, HSP70 and transcriptional analyses of antioxidant defence system in liver tissues of rainbow trout exposed to eprinomectin. Fish Shellfish Immunol. 65, 136-144. http://doi.org/10.1016/j.fsi.2017.04.004

Albert, J., Lingle, C.J, Marder, E., O'Neil, M.B., 1986. A GABA-activated chloride-conductance not blocked by picrotoxin on spiny lobster neuromuscular preparations. Br. J. Pharmacol. 87, 771-779. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1916806/

Athanassopoulou, F., Bouboulis, D., Martinsen, B., 2001. *In vitro* treatments of deltamethrin against the isopod parasite *Cerathoa oestroides*, a pathogen of seabass *Dicentrarchus labrax*. L. B. Eur. Assoc. Fish Pat. 21, 26-29.

Bai, S.H., Ogbourne, S., 2016. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. Chemosphere 154, 204-214. http://doi.org/10.1016/j.chemosphere.2016.03.113

Baldissera, M.D., Souza, C.F., Descovi, S.N., Zanella, R., Prestes, O.D., Da Silva, A.S., Baldisserotto, B., 2019. Organophosphate pesticide trichlorfon induced neurotoxic effects in freshwater silver catfish *Rhamdia quelen* via disruption of blood-brain barrier: implications on oxidative status, cell viability and brain neurotransmitters. Comp. Biochem. Physiol. C. 218, 8-13. http://doi.org/10.1016/j.cbpc.2018.12.006

Baldissera, M.D., Souza, C.F., Santos, R.C.V., Da Rosa, L.V.C., Rosemberg, D.B., Baldisserotto, B., 2017. *Pseudomonas aeruginosa* strain PAO1 infection impairs

locomotor activity in experimentally infected *Rhamdia quelen*: interplay between a stress response and brain neurotransmitters. Aquaculture 473, 74-79. http://doi.org/10.1016/j.aquaculture.2017.02.004

Baldissera, M.D., Souza, C.F., Zeppenfeld, C.C., Descovi, S.N., Moreira, K.L.S., Da Rocha, M.I.U.M, Da Veiga, M.L., Da Silva, A.S., Baldisserotto, B., 2018. Aflatoxin B_1-contaminated diet disrupts the blood-brain barrier and affects fish behavior: involvement of neurotransmitters in brain synaptosomes. Environ. Toxicol. Pharmacol. 60, 45-51. http://doi.org/10.1016/j.etap.2018.04.003

Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., Da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L., 2007. Whole-body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture 272, 774-778. http://doi.org/10.1016/j.aquaculture.2007.09.002

Bauchot, M.-L., Hureau, J.-C., 1990. Sparidae. In: Quero, J.C., Hureau, J.C., Karrer, C., Post, A., Saldanha, L. (Eds.), Check-list of the Fishes the Eastern Tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; UNESCO, Paris, pp. 790-812.

Cericato, L., Machado Neto, J.G., Fagundes, M., Kreutz, L.C, Quevedo, R.M., Finco, J., Da Rosa, J.G.S, Koakoski, G., Centenaro, L., Pottker, E., Anziliero, D., Barcellos, L.J.G., 2008. Cortisol response to acute stress in jundiá *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. Comp. Biochem. Physiol. C. 148, 281-286. http://doi.org/10.1016/j.cbpc.2008.06.008

Cericato, L., Machado Neto, J.G., Kreutz, L.C., Quevedo, R.M., Da Rosa, J.G., Koakoski, G., Centenaro, L., Pottker, E., Marqueze, A., Barcellos, L.J.G., 2009. Responsiveness of the interrenal tissue of Jundiá (*Rhamdia quelen*) to an *in vivo* ACTH test following acute exposure to sublethal concentrations of agrichemicals. Comp. Biochem. Physiol. C. 149, 363-367. http://doi.org/10.1016/j.cbpc.2008.09.002

Colović, M.B., Krstić, D.Z., Lazarević-Pašti, T.D., Bondžić, A.M., Vasić, V.M., 2013. Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr. Neuropharmacol. 11, 315-335. http://doi.org/10.2174/1570159X11311030006

Da Cunha, M.A., Zeppenfeld, C.C., Garcia, L.O., Loro, V.L., Da Fonseca, M.B., Emanuelli, T., Veeck, A.P.L., Copatti, C.E., Baldisserotto, B., 2010. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. Ciência Rural 40, 2107-2114. http://dx.doi.org/10.1590/S0103-84782010005000154

Davies, I.M., Rodger, G.K., 2000. A review of the use of ivermectin as a treatment for sea lice

[*Lepeophtheirus salmonis* (Krøyer) and *Caligus elongatus* Nordmann] infestation in farmed Atlantic salmon (*Salmo salar* L.). Aquacult. Res. 31, 869-883. http://doi.org/10.1046/j.1365-2109.2000.00510.x

Domingues, I., Oliveira, R., Soares, A.M.V.M., Amorim, M.J.B., 2016. Effects of ivermectin on *Danio rerio*: a multiple endpoint approach: behaviour, weight and subcellular markers. Ecotoxicology 25, 491-499. http://doi.org/10.1007/s10646-015-1607-5

Drever, B.D., Riedel, G., Platt, B., 2011. The cholinergic system and hippocampal plasticity. Behav. Brain Res. 221, 505-514. http://doi.org/10.1016/j.bbr.2010.11.037

Dröge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82, 47-95. http://doi.org/10.1152/physrev.00018.2001

Duce, I.R., Scott, R.H., 1985. Actions of dihydroavermectin B1a on insect muscle. Br. J. Pharmacol. 85, 395-401. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1916587/

Ellman, G.L., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-90. http://doi.org/10.1016/0006-2952(61)90145-9

Friedman, A., Kaufer, D., Shemer, J., Hendler, I., Soreq, H., Tur-Kaspa, I., 1996. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nat. Med. 2, 1382-1385. http://doi.org/10.1038/nm1296-1382

Geary, T.G., Moreno, Y., 2012. Macrocyclic lactone anthelmintics: spectrum of activity and mechanism of action. Curr. Pharmaceut. Biotechnol. 13, 866-872. http://doi.org/10.2174/138920112800399077

Gomes, L.C., Golombieski, J.I., Gomes, A.R.C., Baldisserotto, B., 2000. Biology of *Rhamdia quelen* (Teleostei, Pemelodidae). Ciência Rural 30, 179-185. http://dx.doi.org/10.1590/S0103-84782000000100029

Grant, A., Briggs, A.D., 1998. Use of ivermectin in marine fish farms: some concerns. Mar. Pollut. Bull. 36, 566-568. http://doi.org/10.1016/S0025-326X(98)00032-0

Halley, B.A., Winter, R., Yoon, S., Marley, S.E., Rehbein, S., 2005. The environmental safety of eprinomectin to earthworms. Vet. Parasitol. 128, 109-114. http://doi.org/10.1016/j.vetpar.2004.11.007

Hemaprasanth, K.P., Raghavendra, A., Singh, R., Sridhar, N., Raghunath, M.R., 2008. Efficacy of doramectin against natural and experimental infections of *Lernaea cyprinacea* in carps. Vet. Parasitol. 156, 261-269.

http://doi.org/10.1016/j.vetpar.2008.06.005

Holste, J.E., Smith, L.L., Hair, J.A., Lancaster, J.L., Lloyd, J.E., Langholff, W.K., Barrick, R.A., Eagleson, J.S., 1997. Eprinomectin: a novel avermectin for control of lice in all classes of cattle. Vet. Parasitol. 73, 153-16l. http://doi.org/10.1016/S0304-4017(97)00063-0

Høy, T., Horsberg, T.E., Nafstad, I., 1990. The disposition of ivermectin in Atlantic salmon (*Salmo salar*). Pharmacol. Toxicol. 67, 307-312. http://doi.org/10.1111/j.1600-0773.1990.tb00835.x

Jaques, J.A.S., Rezer, J.F.P., Gonçalves, J.F., Spanevello, R.M., Gutierres, J.M., Pimentel, V.C., Thomé, G.R., Morsch, V.M., Schetinger, M.R.C., Leal, D.B.R., 2011. The effect of curcumin in the ectonucleotidases and acetylcholinesterase activities in synaptosomes from the cerebral cortex of cigarette smoke-exposed rats. Cell Biochem. Funct. 29, 703-707. http://doi.org/10.1002/cbf.1804

Jochmann, R., Blanckenhorn, W.U., 2016. Non-target effects of ivermectin on trophic groups of the cow dung insect community replicated across an agricultural landscape. Basic Appl. Ecol. 17, 291-299. http://doi.org/10.1016/j.baae.2016.01.001

Katharios, P., Iliopoulou-Georgudaki, J., Kapata-Zoumbos, K., Spiropoulos, S., 2001. Toxicity of intraperitoneally injected ivermectin in sea bream, *Sparus aurata*. Fish Physiol. Biochem. 25, 99-108. http://doi.org/10.1023/A:1020574810332

Katharios, P., Pavlidis, M., Iliopoulou-Georgudaki, J., 2004. Accumulation of ivermectin in the brain of sea bream, *Sparus aurata* after intraperitoneal administration. Environ. Toxicol. Pharmacol. 17, 9-12. http://doi.org/10.1016/j.etap.2004.01.003

LeBel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227-231. http://doi.org/10.1021/tx00026a012

Liew, H.J., Fazio, A., Faggio, C., Blust, R., De Boeck, G., 2015. Cortisol affects metabolic and ionoregulatory responses to a different extent depending on feeding rations in common carp, *Cyprinus carpio*. Comp. Biochem. Physiol. A. 189, 45-57. http://doi.org/10.1016/j.cbpa.2015.07.011

Litskas, V.D., Karamanlis, X.N., Batzias, G.C., Kamarianos, A.P., 2011. Sorption of the antiparasitic drug eprinomectin in three soils. Chemosphere. 82, 193-198. http://doi.org/10.1016/j.chemosphere.2010.10.024

Litskas, V.D., Karamanlis, X.N., Batzias, G.C., Tsiouris, S.E., 2013. Are the parasiticidal

avermectins resistant to dissipation in the environment? The case of eprinomectin. Environ. Int. 60, 48-55. http://doi.org/10.1016/j.envint.2013.07.017

Merck, 1996. Ivomec Eprinex (Eprinomectin) Pour-On for Beef and Dairy Cattle: Environmental Assessment. Report NADA 141-079EA. Merck and Company, Rahway, NJ, USA.

Moseley, A.E., Williams, M.T., Schaefer, T.L., Bohanan, C.S., Neumann, J.C., Behbehani, M.M., Vorhees, C.V., Lingrel, J.B., 2007. Deficiency in Na, K-ATPase α isoform genes alters spatial learning, motor activity, and anxiety in mice. J. Neurosci. 27, 616-626. http://doi.org/10.1523/JNEUROSCI.4464-06.2007

Muszbek, L., Szabó, T., Fésüs, L., 1977. A highly sensitive method for the measurement of the ATPase activity. Anal. Biochem. 77, 286-288. http://doi.org/10.1016/0003-2697(77)90315-3

Nieman, C.C., Floate, K.D., Düring, R.-A., Heinrich, A.P., Young, D.K., Schaefer, D.M., 2018. Eprinomectin from a sustained release formulation adversely affected dung breeding insects. PLoS One. 13, 1-11. http://doi.org/10.1371/journal.pone.0201074

Paredes, R.G., Âgmo, A., 1992. GABA and behavior: the role of receptor subtypes. Neurosci. Biobehav. Rev. 16, 145-170. http://doi.org/10.1016/S0149-7634(05)80177-0

Pereira, G.S., Mello e Souza, T., Battastini, A.M.O., Izquierdo, I., Sarkis, J.J.F., Bonan, C.D., 2002. Effects of inhibitory avoidance training and/or isolated foot-shock on ectonucleotidase activities in synaptosomes of the anterior and posterior cingulate cortex and the medial precentral area of adult rats. Behav. Brain Res. 22, 121-127. http://doi.org/10.1016/S0166-4328(01)00312-6

Read, S.M., Northcote, D.H., 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal. Biochem. 116, 53-64. http://doi.org/10.1016/0003-2697(81)90321-3

Ren, Z., Zhang, X., Wang, X., Qi, P., Zhang, B., Zeng, Y., Fu, R., Miao, M., 2015. AChE inhibition: one dominant factor for swimming behavior changes of *Daphnia magna* under DDVP exposure. Chemosphere 120, 252-257. http://doi.org/10.1016/j.chemosphere.2014.06.081

Rocha, J.B.T., Emanuelli, T., Pereira, M.E., 1993. Effects of early undernutrition on kinetic parameters of brain acetylcholinesterase from adult rats. Acta Neurobiol. Exp. 53, 431-437. http://www.ane.pl/pdf/5351.pdf

Roth, M., Richards, R.H., Sommerville, C., 1993. Current practices in the chemotherapeutic

control of sea lice infestations in aquaculture: a review. J. Fish Dis. 16, 1-26. http://doi.org/10.1111/j.1365-2761.1993.tb00844.x

Sahu, S.S., Madhyastha, S., Rao, G.M., 2013. Neuroprotective effect of resveratrol against prenatal stress induced cognitive impairment and possible involvement of Na^+, K^+-ATPase activity. Pharmacol. Biochem. Behav. 103, 520-525. http://doi.org/10.1016/j.pbb.2012.09.012

Scheffczyk, A., Floate, K.D., Blanckenhorn, W.U., Düring, R-A., Klockner, A., Lahr, J., Lumaret, J.-P., Salamon, J.-A., Tixier, T., Wohde, M., Römbke, J., 2016. Nontarget effects of ivermectin residues on earthworms and springtails dwelling beneath dung of treated cattle in four countries. Environ. Toxicol. Chem. 35, 1959-1969. http://doi.org/10.1002/etc.3306

Silva, K.C.C., Assis, C.R.D., Oliveira, V.M., Carvalho Jr., L.B., Bezerra, R.S., 2013. Kinetic and physicochemical properties of brain acetylcholinesterase from the peacock bass (*Cichla ocellaris*) and *in vitro* effect of pesticides and metal ions. Aquat. Toxicol. 126, 191-197. http://doi.org/10.1016/j.aquatox.2012.11.001

Souza, C.F., Baldissera, M.D., Bianchini, A.E., Da Silva, E.G., Mourão, R.H.V., Da Silva, L.V.F., Schmidt, D., Heinzmann, B.M., Baldisserotto, B., 2018. Citral and linalool chemotypes of *Lippia alba* essential oil as anesthetics for fish: a detailed physiological analysis of side effects during anesthetic recovery in silver catfish (*Rhamdia quelen*). Fish Physiol. Biochem. 44, 21-34. http://doi.org/10.1007/s10695-017-0410-z

Souza, L.C., Wilhelm, E.A., Bortolatto, C.F., Nogueira, C.W., Boeira, S.P., Jesse, C.R., 2014. The protective role of melatonin against brain in oxidative stress and hyperlocomotion in a rat model of mania induced by ouabain. Behav. Brain Res. 271, 316-324. http://doi.org/10.1016/j.bbr.2014.06.030

Turner, M.J., Schaeffer, J. M., 1989. Mode of action of ivermectin. In: Campbell, W.C. (Ed.), Ivermectin and abamectin. Springer-Verlag, New York, pp. 73-88.

Vassilis, L.D., George, B.C., Charalampos, P.G., Athina, P.-V., Xanthippos, K.N., 2016. Mobility of pharmaceutical compounds in the terrestrial environment: adsorption kinetics of the macrocyclic lactone eprinomectin in soils. Chemosphere 144, 1201-1206. http://doi.org/10.1016/j.chemosphere.2015.09.100

Verdú, J.R., Cortez, V., Ortiz, A.J., González-Rodríguez, E., Martinez-Pinna, J., Lumaret, J.-P., Lobo, J.M., Numa, C., Sánchez-Piñero, F., 2015. Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. Sci. Rep-UK. 5, 1-10.

http://doi.org/10.1038/srep13912

Zortéa, T., Segat, J.C., Maccari, A.P., Sousa, J.P., Da Silva, A.S., Baretta, D., 2017. Toxicity of four veterinary pharmaceuticals on the survival and reproduction of *Folsomia candida* in tropical soils. Chemosphere 173, 460-465. http://doi.org/10.1016/j.chemosphere.2017.01.069

Figures legends

Fig. 1 - Novel test tank for the locomotor activity for jundiá (*Rhamdia quelen*): distance travelled [A], mean speed [B] and absolute turn angle [C], after 24 and 48 h to eprinomectin exposure in the water, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) of six fish for each treatment. Capital letters indicate significant differences between times for the same treatment. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by the Tukey's test ($p < 0.05$; $n = 6$ per treatment). Treatments: 0.0 µg L^{-1} (Control), 1.124 µg L^{-1} (T1), 1.809 µg L^{-1} (T2), 3.976 µg L^{-1} (T3) and dilution in ethanol used as control (T4).

Fig. 2 - Novel test tank for the movement displaying for jundiá (*Rhamdia quelen*) in the two tank zones: number of entries in the upper zone [A], time in the upper zone [B], latency to the 1st entry in the upper zone [C], number of entries in the bottom zone [D], time in the bottom zone [E] and total time of freezing [F], after 24 and 48 h to eprinomectin exposure in the water, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) of six fish for each treatment. Capital letters indicate significant differences between times for the same treatment. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by the Tukey's test ($p < 0.05$; $n = 6$ per treatment). Treatments: 0.0 µg L^{-1} (Control), 1.124 µg L^{-1} (T1), 1.809 µg L^{-1} (T2), 3.976 µg L^{-1} (T3) and dilution in ethanol used as control (T4).

Fig. 3 - Cerebral reactive oxygen species (ROS) levels in jundiá (*Rhamdia quelen*) after 24 and 48 h to eprinomectin exposure in the water, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) of six fish for each treatment. Capital letters indicate significant differences between times for the same treatment. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by the Tukey's test ($p < 0.05$; $n = 6$ per treatment). Treatments: 0.0 µg L^{-1} (Control), 1.124 µg L^{-1} (T1), 1.809 µg L^{-1} (T2), 3.976 µg L^{-1} (T3) and dilution in ethanol used as control (T4).

Fig. 4 - Cerebral acetylcholinesterase (AChE) [A] and sodium-potassium ATPase pump (Na^+/K^+-ATPase) [B] activities in jundiá (*Rhamdia quelen*), after 24 and 48 h to eprinomectin exposure in the water, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) of six fish for each treatment. Capital letters indicate significant differences between times for the same treatment. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by the Tukey's test ($p < 0.05$; $n = 6$ per treatment). Treatments: 0.0 µg L^{-1} (Control), 1.124 µg L^{-1} (T1), 1.809 µg L^{-1} (T2), 3.976 µg L^{-1} (T3) and dilution in ethanol used as control (T4).

Fig. 1

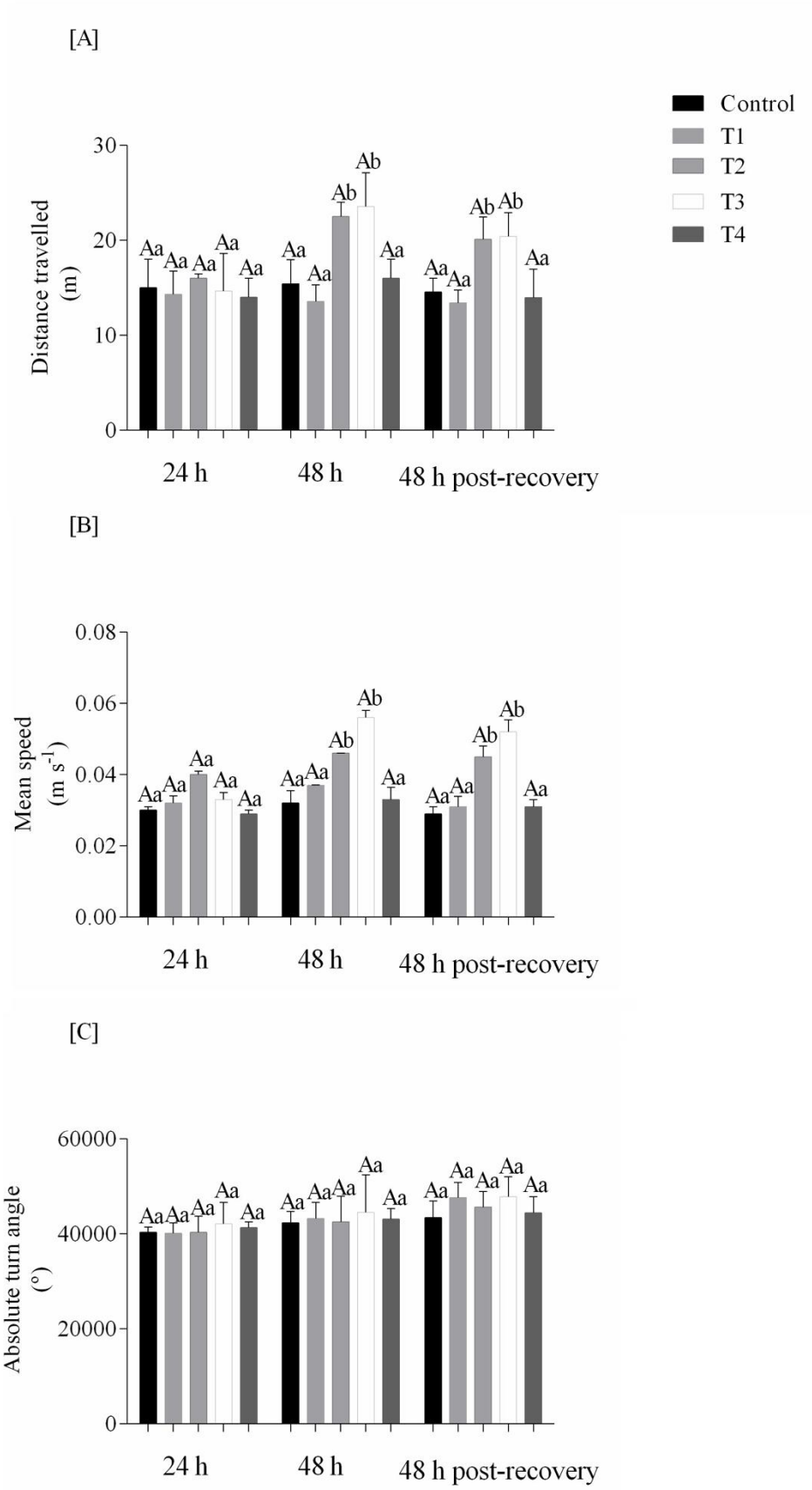

[A]
Control
T1
T2
T3
T4
Distance travelled (m)
30
20
10
0
Aa
Ab
24 h
48 h
48 h post-recovery
[B]
Mean speed (m s-1)
0.08
0.06
0.04
0.02
0.00
[C]
Absolute turn angle (°)
60000
40000
20000
0

Fig. 2

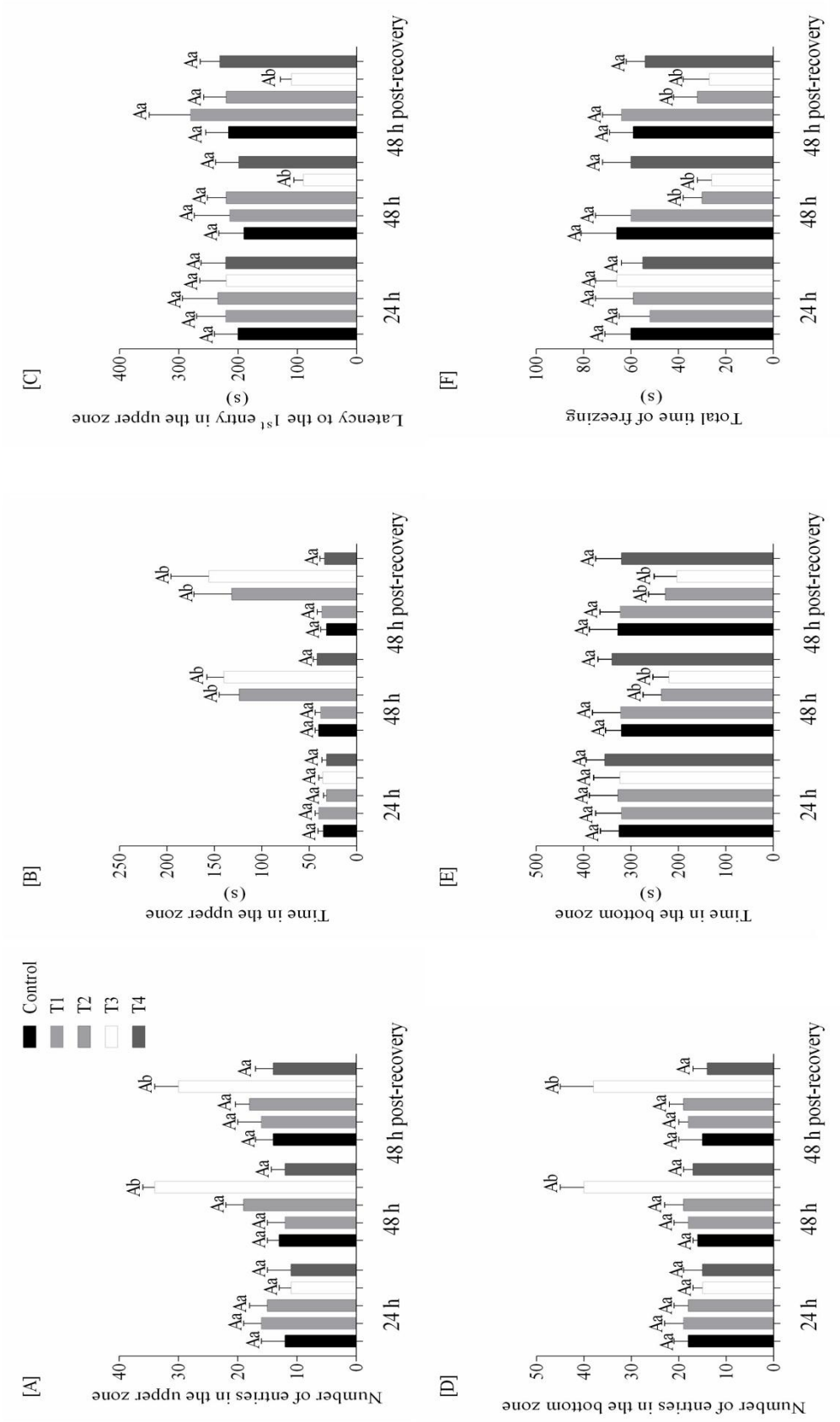

[A]
Number of entries in the upper zone
[B]
Time in the upper zone
(s)
[C]
Latency to the 1st entry in the upper zone
(s)
[D]
Number of entries in the bottom zone
[E]
Time in the bottom zone
(s)
[F]
Total time of freezing
(s)
24 h
48 h
48 h post-recovery
Control
T1
T2
T3
T4

Fig. 3

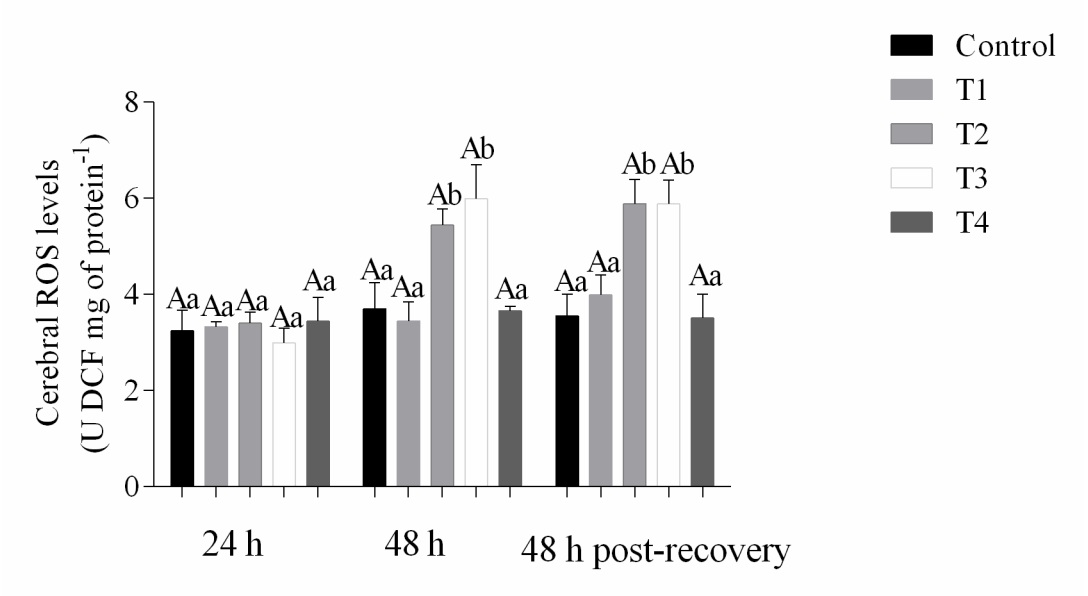
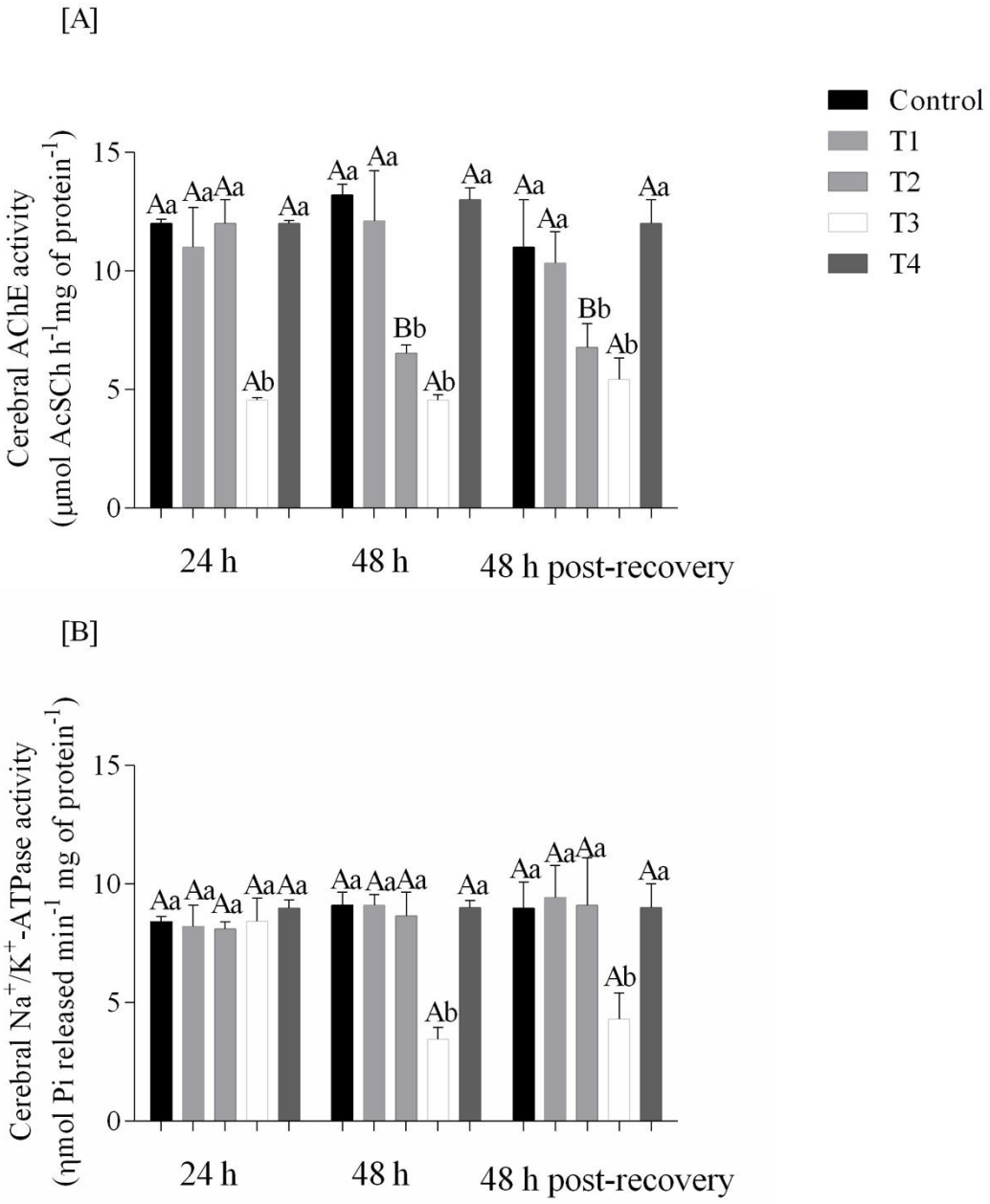

Control
T1
T2
T3
T4
Cerebral ROS levels
(U DCF mg of protein-1)
8
6
4
2
0
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Ab
Ab
Aa
Aa
Aa
Ab
Ab
Aa
24 h
48 h
48 h post-recovery

Fig. 4


[A]
Control
T1
T2
T3
T4
Cerebral AChE activity
(µmol AcSCh h-1mg of protein-1)
15
10
5
0
Aa
Aa
Aa
Ab
Aa
Aa
Aa
Bb
Ab
Aa
Aa
Aa
Bb
Ab
Aa
24 h
48 h
48 h post-recovery
[B]
Cerebral Na+/K+-ATPase activity
(ηmol Pi released min-1 mg of protein-1)
15
10
5
0
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Ab
Aa
Aa
Aa
Aa
Ab
Aa
24 h
48 h
48 h post-recovery

2.3 MANUSCRITO II

Fish exposed to eprinomectin show hepatic oxidative stress and impairment in enzymes of the phosphotransfer network

Suélen Serafini [a], Carine de Freitas Souza [b], Matheus Dellaméa Baldissera [b], Bernardo Baldisserotto [b], Fernanda Picoli [a], Julia Corá Segat [a], Dilmar Baretta [a, c, *], Aleksandro Schafer da Silva [a, c, **]

[a] Graduate Program of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[b] Graduate Program of Pharmacology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

[c] Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

[*] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Beloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

[**] Corresponding author. Department of Animal Science, Centro de Educação Superior do Oeste, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil. 680 E, Beloni Trombeta Zanin Street, Chapecó, SC, 89815-630, Brazil.

E-mail addresses: dilmar.baretta@udesc.br (D. Baretta), aleksandro.silva@udesc.br (A. Schafer da Silva).

De acordo com normas para publicação em:

Aquaculture

Situação do Manuscrito: Submetido

Fish exposed to eprinomectin show hepatic oxidative stress and impairment in enzymes of the phosphotransfer network

Abstract

The aim of this study was to evaluate whether the antiparasitic drug eprinomectin in an experimentally aquatic contamination can cause hepatic oxidative stress, as well as impairment of hepatic energetic metabolism in the silver catfish (*Rhamdia quelen*). The fish were exposed for 24 and 48 h at various concentrations of eprinomectin in the water (0.0, 1.124, 1.809 and 3.976 µg L^{-1}), followed by 48 h recovery in eprinomectin-free water. We measured levels of reactive oxygen species (ROS) in hepatic tissue, lipoperoxidation (LOOH), as well as levels of antioxidant capacity against peroxyl radicals (ACAP) and activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST), in addition to the activities of enzymes involved in energy metabolism, adenylate kinase (AK) and pyruvate kinase (PK). After 24 h, hepatic ROS levels increased in fish exposed to all concentrations of eprinomectin and the same occurred with LOOH at the two highest concentrations of eprinomectin. ACAP levels decreased after 24 h of exposure to the highest concentration, also occurring with SOD activity at the two highest concentrations. Glutathione enzyme activities (GPx and GST) decreased after 48 h of exposure to the highest concentration. Fish transfer to eprinomectin-free water and re-analysis after 48 h post-recovery was unable to reverse ROS and LOOH increases, likewise with reductions of ACAP, SOD, GPx and GST, especially at the two highest concentrations. AK and PK activities decreased after 48 h of exposure, AK at the two highest concentrations and PK at the highest concentration. PK activity returned to normal after 48 h in eprinomectin-free water. In conclusion, silver catfish exposed to the aquatic contamination with the antiparasitic drug eprinomectin showed hepatic oxidative stress, possibly related to the impairment of the phosphotransfer network and energy metabolism.

Keywords: Aquatic contaminant, energetic metabolism, liver damage, oxidative stress, *Rhamdia quelen*

1. Introduction

Avermectins are a family of semisynthetic chemical molecules widely used in veterinary pharmacology for prevention and treatment of endo- and ectoparasitoses (Lumaret et al., 2012; Vassilis et al., 2016; Alak et al., 2017). In Brazil, eprinomectin was relatively recently introduced as an alternative to avermectin in the context of increasing parasitic resistance (Baiak et al., 2017). In the scientific literature, there are numerous reports of routes of environmental contamination by avermectins with more widespread use, as well as their non-target effects on terrestrial organisms (Svendsen et al, 2003; Floate et al., 2005; Diao et al., 2007; Jensen and Scott-Fordsmand, 2012; Scheffczyk et al., 2016; Zortéa et al., 2017), aerial (Li et al., 2013) and aquatic (Høy et al., 1990; Katharios et al., 2001; Varó et al, 2010; Domingues et al., 2016; Oliveira et al., 2016). There are studies that report the effect of eprinomectin in cattle feces on non-target organisms in the soil that conclude there is a high safety margin, with toxic effects in doses not usual in veterinary medicine (Halley et al., 2005; Nieman et al., 2018). However, there is concern about eprinomectin contamination in aquatic environments, as this molecule has hydrophilic characteristics and polar metabolites (Merck, 1996; Holste et al., 1997) that may facilitate its entry and diffusion into water by direct or indirect contamination (Litskas et al., 2013; Vassilis et al., 2016). Even low environmental concentrations of pharmaceuticals such as eprinomectin can biomagnify in the environment by bioaccumulation, reaching pharmacological levels and representing environmental risks (Alak et al., 2017; Baron et al., 2017). Associated with this, lethal toxic values for fish (Lumaret et al., 2012) are within the levels that can be found in bovine feces treated with eprinomectin (Halley et al., 2005; Aksit et al., 2016).

In fish, metabolism/detoxification of pharmaceuticals occurs mainly via the hepatic system (Horsberg, 2012; Alak et al., 2017; Baron et al., 2017). Even low concentrations of pharmaceutical products such as avermectins can induce negative biological effects in fish liver (Varó et al., 2010; Alak et al., 2017). Eprinomectin reduced activity of the hepatic antioxidant defense system in rainbow trout (*Oncorhynchus mykiss*) in sublethal concentrations (< LC_{96} 1.2 µg L^{-1} for *O. mykiss*) at up to 24 h exposure to experimentally-contaminated water (Alak et al., 2017). Aquatic pollutants are physiological stressors that may cause increased generation of ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxide radical (ROO^-), among others (Dröge, 2002; Valavanidis et al., 2006; Slaninova et al., 2009). ROS can interact with cellular components and cause oxidative damage to proteins, nucleic acids and membrane lipids (Dröge, 2002), especially by lipoperoxidation (LOOH), as well as causing changes in the activity and efficiency of the

antioxidant system (Amado et al., 2009; Slaninova et al., 2009; Li et al., 2013; Alak et al., 2017). Oxidative stress is caused when there is an imbalance between ROS levels generated and detoxifying antioxidant mechanisms, including ROS elimination enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST), among others (Davies, 1999). Stress and oxidative damage are extremely important for fish because there may be impairment of the animal's homeostasis and physiological activities, leading to adverse health effects (Valavanidis et al., 2006; Slaninova et al., 2009; Domingues et al., 2016). Therefore, the balance between oxidants and antioxidants (antioxidant capacity), as well as oxidative damage are important bioindicators of fish health (Valavanidis et al., 2006; Amado et al., 2009; Domingues et al., 2016; Alak et al., 2017), especially as a result hepatic toxicity caused by eprinomectin, a subject that requires more study (Alak et al., 2017).

The liver is a key metabolic organ that regulates energetic metabolism, in addition to metabolically connecting various body tissues (Rui, 2014). The maintenance of homeostasis between energy release and absorption is regulated by intracellular processes, and the proper functioning of energy metabolism depends on the production and delivery of energetic compounds, including adenosine triphosphate (ATP) (Baldissera et al., 2017). The cytosolic and mitochondrial enzymes adenylate kinase (AK) and pyruvate kinase (PK) catalyze the transfer of phosphoryl groups between synthesis sites and ATP in the regulation of K_{ATP} channels (Dzeja and Terzic, 2003). These enzymes form the main route for communication between compartments responsible for the production and use of metabolic energy, especially in tissues with high energy demand (Dzeja and Terzic, 2003). AK catalyzes the reversible phosphotransfer between adenosine diphosphate (ADP) and ATP in the presence of adenosine monophosphate (AMP) and is implicated in the processing of cellular signals associated with the use of ATP (Dzeja and Terzic, 1998). PK is a key glycolysis pathway enzyme that catalyzes the irreversible transphosphorylation of phosphoenolpyruvate (PEP) in ADP to form pyruvate and ATP (Wang et al., 2002), which plays a key role in the glycolytic pathway, the main route of energy supply to tissues (Baldissera et al., 2017). Therefore, because of the importance of energetic metabolism of the liver and its interrelation with other body tissues, as well as the implications of the damage that eprinomectin can cause in hepatic tissue (Alak et al., 2017), the activities of the AK and PK enzymes in fish exposed to water contaminated with eprinomectin should be evaluated in order to verify if there is compromise of the phosphotransfer network.

Thus, our hypothesis was that exposure of fish to eprinomectin in water would cause an imbalance between oxidants and antioxidants, lipoperoxidation in hepatic tissue, and that these negative effects would compromise energy metabolism associated with the phosphotransfer network, because enzymes belonging to phosphotransfer network are susceptible to oxidative stress (Grings et al., 2018). Therefore, the objective of this study was to evaluate whether the antiparasitic drug eprinomectin in an experimentally aquatic contamination can cause hepatic oxidative stress, as well as impairing hepatic energetic metabolism in the silver catfish (*Rhamdia quelen*). We also evaluated the physiological homeostatic capacity of fish in the absence of the contaminant in the water.

2. Materials and methods

2.1. Chemical and water eprinomectin concentration

Eprinomectin (3.6%) was purchased in Brazil under the commercial name Eprinex® (Merial, Brazil). The antiparasitic drug was added to the test water in the following nominal concentrations: 0.0 (control), 0.0025, 0.01 and 0.05 µg L^{-1} prior to the beginning of the experiment (calculated concentration), based on the protocol established by Alak et al. (2017). Eprinomectin was diluted in ethanol (1:10 v v^{-1}) prior to the experiment. The dilution in ethanol was also used as control, denominate 'Ethanol'.

The actual concentrations of eprinomectin were determined in the tanks water at the beginning (0 h), and at 24 and 48 h of exposure, as well as at 48 h, during the eprinomectin-free water recovery span (Table 1), using ultra-high-performance liquid chromatography coupled to mass spectrophotometry (UHPLC-MS).

2.2. Fish maintenance and experimental design

Juvenile *R. quelen* (popularly known as silver catfish or jundiá) (males; seven months old; 60.99 ± 4.14 g; 14.10 ± 1.11 cm) were collected from a fish farm located in Southern Brazil. The animals were transported alive and maintained for acclimation in 250 L fiberglass tanks with continuous aeration and controlled water variables (temperature: 21 ± 0.2 ºC; pH: 6.41 ± 0.1; ammonia: 0.84 ± 0.06 mg L^{-1}; non-ionized ammonia: 0.0046 ± 0.00031 mg L^{-1}; dissolved oxygen: 6.71 ± 0.59 mg L^{-1}) for ten days. The fish were fed to apparent satiation

with commercial pellets of granulometry 2.5 mm (Supra, Alisul, RS, Brazil) once a day, in the proportion of 10% of the biomass of the group and continuous feeding during the experimental period. Any uneaten food, feces and other residues were removed daily 30 min after feeding.

The fish were randomly allocated inside tanks of 250 L with continuous aeration, with three replicates per concentration and six fish per replicate, a total of 90 animals. Water quality variables remained unaltered throughout the entire experimental period. Five treatments were applied, two of them being controls: 0.0 µg L^{-1} of eprinomectin and Ethanol (diluent used to adjust the concentration of eprinomectin). Three increasing actual concentrations of eprinomectin in water accounted for the other three treatments: 1.124, 1.809 and 3.976 µg L^{-1}.

2.3. Sample collection

After 24 and 48 h of exposure, and after 48 h of recovery in eprinomectin-free water, two animals from each tank (six fish per treatment at each given time; total of 90 animals) were anesthetized with 50 mg L^{-1} eugenol (Da Cunha et al., 2010), followed by spinal cord section according to ethics committee recommendations. Subsequently, livers were removed and dissected in glass dishes over ice to evaluate the parameters detailed below.

To evaluate hepatic oxidative stress-related parameters, hepatic tissue was homogenized (1:10 w v^{-1}) in a glass Potter tube with Tris-HCl buffer (10 mM, pH 7.4) and centrifuged at 2.000 *x g* for 10 min. The supernatants were used to evaluate ROS, LOOH and antioxidant capacity against peroxyl radicals (ACAP) levels, as well as SOD, GPx and GST activities.

To evaluate enzymes belonging to phosphotransfer network, hepatic tissue was washed in SET buffer (0.32 M sucrose, 1 mM EGTA, 10 mM Tris-HCl, pH 7.4) and homogenized (1:10 w v^{-1}) in the same SET buffer using a Potter-Elvehjem glass homogenizer. The homogenates were centrifuged at 800 *x g* for 10 min at 4 °C. Some of these supernatants were used to evaluate AK activity; the pellets were discarded and the supernatants were once again centrifuged at 10.000 *x g* for 15 min at 4 °C; the supernatants resulting from this last centrifugation were collected for determination of PK activity. The supernatants were stored for no more than one week at -80 °C.

2.4. Hepatic oxidants

Hepatic ROS levels were determined by the DCFH oxidation method described by LeBel et al. (1992). Results were expressed as U DCF mg of protein^{-1}. Hepatic LOOH levels were measured as proposed by Monserrat et al. (2003). Levels were expressed as µmol CHP g of tissue^{-1}.

2.5. Hepatic antioxidants

Hepatic ACAP levels were measured according to the protocol established by Amado et al. (2009). Results were expressed as fluorescence units mg of protein^{-1}. Hepatic SOD activity was evaluated spectrophotometrically as described by Marklund and Marklund (1974). Enzymatic activity was expressed as U SOD mg of protein^{-1}. Hepatic GPx activity was measured according to the methodology described by Paglia and Valentine (1967). The activity was expressed as U GPx mg of protein^{-1}. Hepatic GST activity was measured based on the method described by Habig et al. (1974). Enzymatic activity was expressed as µmol GS-DNB min^{-1} mg of protein^{-1}.

2.6. Hepatic AK and PK

Hepatic AK activity was measured according to Dzeja et al. (1999). The activity was expressed as ηmol ATP formed min^{-1} mg of protein^{-1}. Hepatic PK activity was assayed according the protocol established by Leong et al. (1981). The activity was expressed as ηmol pyruvate formed min^{-1} mg of protein^{-1}.

2.7. Hepatic protein content

Hepatic protein content was evaluated by the method of Coomassie blue G dye (Read and Northcote, 1981), using serum bovine albumin as the standard.

2.8. Statistical analysis

The data met the assumption of parametric testing (normality and homoscedasticity) using Kolmogorov-Smirnov and Levene's tests, respectively. Subsequently, statistical analysis was performed using bilateral two-way analysis of variance (ANOVA) followed by the Tukey post-hoc test considering significance at $p < 0.05$.

3. Results

3.1. Hepatic oxidants levels

Hepatic ROS levels were higher ($p < 0.05$) in fish exposed to all treatments of eprinomectin (1.124, 1.809 and 3.976 µg L^{-1}) than in the control group (0.0 µg L^{-1}) at 24 and 48 h of exposure, remaining higher at 1.809 and 3.976 µg L^{-1} after 48 h recovery in eprinomectin-free water. ROS levels at the lowest concentration of eprinomectin (1.124 µg L^{-1}) were similar ($p > 0.05$) to those of control (0.0 µg L^{-1}) after 48 h recovery eprinomectin-free water. Fish exposed to the highest concentration of eprinomectin (3.976 µg L^{-1}) had higher levels of ROS ($p < 0.05$) than those exposed to other treatments at 24 and 48 h, even after 48 h of recovery in eprinomectin-free water (Fig. 1 - A).

Hepatic LOOH levels were higher ($p < 0.05$) in fish exposed to treatments with highest concentrations of eprinomectin (1.809 and 3.976 µg L^{-1}) compared to control group (0.0 µg L^{-1}) at 24 and 48 h of exposure, as well as after 48 h of recovery in eprinomectin-free water (Fig. 1 - B).

3.2. Hepatic antioxidants levels and activities

Hepatic ACAP levels were lower ($p < 0.05$) in fish exposed to the highest concentration of eprinomectin (3.976 µg L^{-1}) to those of control group (0.0 µg L^{-1}) at 24 and 48 h of exposure, as well as after 48 h of recovery to eprinomectin-free water (Fig. 2 - A).

Hepatic SOD activity was lower ($p < 0.05$) in fish exposed to the highest concentrations of eprinomectin (1.809 and 3.976 µg L^{-1}) than in the control group (0.0 µg L^{-1}) at 24 and 48 h of exposure, remaining lower after 48 h of recovery in eprinomectin-free water (Fig. 2 - B).

Hepatic GPx activity was lower ($p < 0.05$) in fish exposed to the highest concentration of eprinomectin (3.976 µg L^{-1}) compared to control group (0.0 µg L^{-1}) at 48 h of exposure,

and after 48 h of recovery in eprinomectin-free water (Fig. 2 - C). A similar result was found for hepatic GST activity (Fig. 2 - D).

3.3. Hepatic AK and PK activities

Hepatic AK activity was lower ($p < 0.05$) in fish exposed to the highest concentrations of eprinomectin (1.809 and 3.976 µg L^{-1}) than in the control group (0.0 µg L^{-1}) at 48 h of exposure, and remained lower after 48 h of recovery in eprinomectin-free water (Fig. 3 - A).

Hepatic PK activity was lower ($p < 0.05$) in fish exposed to the highest concentration of eprinomectin (3.976 µg L^{-1}) compared to control group (0.0 µg L^{-1}) at 48 h of exposure; there was recovery of PK enzyme activity ($p > 0.05$) for this treatment after 48 h of recovery in eprinomectin-free water (Fig. 3 - B).

4. Discussion

In this study, exposure of silver catfish (*R. quelen*) to water experimentally contaminated with eprinomectin, confirming the ability of this antiparasitic drug to cause oxidative stress and oxidative damage in hepatic tissue at up to 24 h, corroborating results obtained by Alak et al. (2017) in rainbow trout (*O. mykiss*). Increased ROS levels in liver, although not measured in the previous study cited with eprinomectin, or in other studies reporting oxidative stress in fish following exposure to other avermectins (Varó et al, 2010; Domingues et al., 2016; Oliveira et al., 2016), is an expected effect for a contaminant (Valavanidis et al., 2006; Slaninova et al., 2009), considering reports of the compromise of the antioxidant defense system and parameters of oxidative damage, that may also characterize oxidative stress related to increased levels of ROS (Slaninova et al., 2009; Li et al., 2013; Domingues et al., 2016; Oliveira et al., 2016; Alak et al., 2017). The increased generation of ROS following eprinomectin exposure may be explained by effects already described for the analogous molecule ivermectin that induces mitochondrial dysfunction by inhibiting the activity of the respiratory complex and suppressing mitochondrial respiration (Wang et al., 2018). The increases of ROS and oxidative damage in fish have already been associated with toxic effects of pesticides on respiratory mitochondrial activities (Slaninova et al., 2009).

Antioxidant defenses in general, measured as ACAP, were not efficient in avoiding oxidative damage (Amado et al., 2009). In general, eprinomectin is capable of affecting the antioxidant defense system by reducing the expression of antioxidant genes (Alak et al., 2017). The increase of ROS and the reduction in the activity of the antioxidant defense system may be associated with the increase in LOOH. Oxidative damage by lipoperoxidation caused by eprinomectin has been reported in rainbow trout liver (Alak et al., 2017). LOOH causes oxidative damages, with cell membranes being the most susceptible to these oxidation reactions. In LOOH, ROS react with structural lipids and cause the disruption or destruction of the integrity and function of cell membranes and essential organelles, including the maintenance of the osmotic and electrolytic gradients and, consequently the transmembrane flux of nutrients, metabolites and ions, as well as receptor-mediated signal transduction (Meagher and FitzGerald, 2000). Liver damages from various etiologies leads to irreversible conditions (Rašković et al., 2011), with possible consequent fish death.

Reduction of SOD and GPx activities caused by exposure to eprinomectin was also observed in *O. mykiss* liver by Alak et al. (2017). SOD is one of the principal enzymes involved in the elimination of ROS, formed during the bioactivation of xenobiotics in tissues (Sk and Bhattacharya, 2006) providing protection against oxidative damage (Pandey et al., 2003). According to Alak et al. (2017) the decrease in SOD activity can be attributed to inhibition of the formation of O_2^-, or to the increase in DNA damage attributed to ROS or caused by O_2^-, which, after transformation to H_2O_2 can cause oxidation of cysteine SOD. Reduction of GPx activity may also be linked to production or inhibition of O_2^-, due to its effects on enzyme activity, or even to the ability of xenobiotics to lower levels of reduced glutathione (GSH) and increase its conversion to oxidized glutathione (GSSG), affecting the regeneration of GSH. The increase in GSSG production would be responsible for the observed decrease in GPx activity.

The reduction in GST activity, observed in this study, may also be associated with the increase of GSSG induced by eprinomectin (Alak et al., 2017). The activity of GST is involved in the catalysis and conjugation of electrophilic substrates in the process of reduction of glutathione for the form of GSH (Van der Oost et al., 2003; Langiano and Martinez, 2008; Souza et al., 2018). Reduction of GST activity may affect the process of biotransformation, catalysis and conjugation of a variety of metabolites (including xenobiotics, including veterinary drugs, and lipoperoxidation products) in the process of transforming toxic

compounds into more easily excreted forms (Habig, 1974; Lushchak et al., 2009; Souza et al., 2018).

Varó et al. (2010) in the gilthead sea bream (*Sparus aurata*) demonstrated effects of oral administration of ivermectin on hepatic proteins involved in lipid metabolism (apoA-I) and responses to oxidative stress and energy generation (β-globin, ATP synthase-subunit β). In this study, we observed a significant reduction in the activity of AK and PK, suggesting a metabolic compromise between consumption and synthesis of ATP. In agreement with our observations, Baldissera et al. (2018), using the pesticide thiamethoxam, reported significant inhibition of hepatic AK and PK activities in silver catfish exposed to 1.125 and 3.75 µg L^{-1} over 24 and 96 h, concluding that the pesticide caused severe impairment of energetic metabolism linked to ATP production. Reduced activity of these enzymes may be associated with stress and oxidative damage in cells and cellular organelles (Baldissera et al., 2019). In accordance with our results, Serafini et al. (2019) observed that *R. quelen* exposed to water contaminated with eprinomectin show reduced sodium-potassium ATPase pump activity in the brain (Na^+/K^+-ATPase), an important enzyme for the neurotransmission that is strictly dependent on the generation and delivery of ATP. Impairments in activities of AK and PK have been associated with disorders in cellular functions, especially loss of osmoprotection (Gutierrez and Csonka, 1995) and cellular oxygen deficit (EL-Khaldi, 2010), respectively. It is important to emphasize that PK activity, an irreversible and important enzyme of the glycolytic pathway, was restored at baseline levels after 48 h of recovery in eprinomectin-free water, demonstrating that toxic effects of eprinomectin on PK activity are not permanent, and that this recovery can contribute to restoration of energetic metabolism linked to ATP caused by eprinomectin (Saks et al., 2006; Alekssev et al., 2012).

5. Conclusions

We conclude that silver catfish exposed to water experimentally-contaminated with the antiparasitic drug eprinomectin showed severe hepatic oxidative stress, confirmed by increased levels of reactive oxygen species and lipoperoxidation, associated with the absence of antioxidant response and inhibition of the enzymes superoxide dismutase, glutathione peroxidase and glutathione S-transferase, reflected in lower total antioxidant capacity in the liver. The silver catfish exposed to eprinomectin also had compromised phosphotransfer network, and consequently energy metabolism, as shown by the inhibition of the hepatic

enzymes adenylate kinase and pyruvate kinase. There was a residual negative effect (oxidative stress and impairment of adenosine triphosphate production) in the absence eprinomectin for up to 48 h. Therefore, eprinomectin represents a dangerous aquatic contaminant, as it compromises fish liver and its energetic functions by stress and oxidative damage, as well disrupting hepatic homeostasis. Future studies are needed, primarily to focus on long-term residual effects, and the effects on behavior, survival, growth and reproduction.

Ethics committee

This study was approved by the Ethical and Animal Welfare Committee of the Universidade do Estado de Santa Catarina (protocol number 4679260518).

Conflict of interest

Authors declare they have no conflict of interest.

Acknowledgments

The authors would like to thank the CAPES (Brazil) and CNPq (Brazil) for their technical and financial support.

References

Aksit, D., Korkut, O., Aksoz, E., Gokbulut, C., 2016. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. N. Z. Vet. J. 64, 207-211. https://doi.org/10.1080/00480169.2016.1146172

Alak, G., Yeltekin, A.Ç., Tas, I.H., Ucar, A., Parlak, V., Topal, A., Kocaman, E.M., Atamanalp, M., 2017. Investigation of 8-OHdG, CYP1A, HSP70 and transcriptional analyses of antioxidant defence system in liver tissues of rainbow trout exposed to eprinomectin. Fish Shellfish Immunol. 65, 136-144. https://doi.org/10.1016/j.fsi.2017.04.004

Alekssev, A.E., Reyes, S., Selivanov, V.A., Dzeja, P.P., Terzic, A., 2012. Compartmentation of membrane processes and nucleotide dynamics in diffusion-restricted cardiac cell

microenvironment. J. Mol. Cell. Cardiol. 52, 401-409. https://doi.org/10.1016/j.yjmcc.2011.06.007

Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas, R.F., Zafalon, B., Ferreira, J.L.R., Yunes, J.S., Montserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2115-2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Baiak, B.H.B., 2017. Estudo da resistência anti-helmíntica de bovinos aos nematódeos gastrintestinais: uma meta-análise. Master's Dissertation, Universidade Estadual de Ponta Grossa, Paraná, Brazil. https://tede2.uepg.br/jspui/handle/prefix/2382

Baldissera, M.D., Souza, C.F., Descovi, S.N., Zanella, R., Prestes, O.D., De Matos, A.F.I.M., Da Silva, A.S., Baldisserotto, B., Gris, A., Mendes, R.E., 2019. Disturbance of energetic homeostasis and oxidative damage provoked by trichlorfon as relevant toxicological mechanisms using silver catfish as experimental model. Chem. Biol. Interact. 299, 94-100. https://doi.org/10.1016/j.cbi.2018.11.015

Baldissera, M.D., Souza, C.F., Golombieski, J.I., Seben, D., Sippert, L.R., Salbego, J., Zanella, R., Baldisserotto, B., 2018. Thiamethoxam induced hepatic energy changes in silver catfish via impairment of the phosphoryl transfer network pathway: toxicological effects on energetics homeostasis. Environ. Toxicol. Pharmacol. 60, 1-4. https://doi.org/10.1016/j.etap.2018.04.002

Baldissera, M.D., Souza, C.F., Santos, R.C.V., Stefani, L.M., Moreira, K.L.S., Da Veiga, M.L., Da Rocha, M.I.U.M, Baldisserotto, B., 2017. *Pseudomonas aeruginosa* strain PA01 impairs enzymes of the phosphotransfer network in the gills of *Rhamdia quelen*. Vet. Microbiol. 201, 121-125. https://doi.org/10.1016/j.vetmic.2017.01.016

Baron, M.G., Mintram, K.S., Owen, S.F., Hetheridge, M.J., Moody, A.J., Purcell, W.M., Jackson, S.K., Jha, A.N., 2017. Pharmaceutical metabolism in fish: using a 3-D hepatic *in vitro* model to assess clearance. PLoS One 12, 1-13. https://doi.org/10.1371/journal.pone.0168837

Da Cunha, M.A., Zeppenfeld, C.C., Garcia, L.O., Loro, V.L., Da Fonseca, M.B., Emanuelli, T., Veeck, A.P.L., Copatti, C.E., Baldisserotto, B., 2010. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. Ciência Rural 40, 2107-2114. https://dx.doi.org/10.1590/S0103-84782010005000154

Davies, M.J., Fu, S., Wang, H., Dean, R.T., 1999. Stable markers of oxidant damage to proteins and their application in the study of human disease. Free Radic. Biol. Med. 27,

1151-1163. https://doi.org/10.1016/S0891-5849(99)00206-3

Diao, X., Jensen, J., Hansen, A.D., 2007. Toxicity of the anthelmintic abamectin to four species of soil invertebrates. Environ. Pollut. 148, 514-519. https://doi.org/10.1016/j.envpol.2006.12.002

Domingues, I., Oliveira, R., Soares, A.M.V.M., Amorim, M.J.B., 2016. Effects of ivermectin on *Danio rerio*: a multiple endpoint approach: behaviour, weight and subcellular markers. Ecotoxicology 25, 491-499. https://doi.org/10.1007/s10646-015-1607-5

Dröge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82, 47-95. https://doi.org/10.1152/physrev.00018.2001

Dzeja, P.P., Terzic, A., 1998. Phosphotransfer reactions in the regulation of ATP- sensitive K^+ channels. FASEB J. 12, 523-529. https://doi.org/10.1096/fasebj.12.7.523

Dzeja, P.P., Terzic, A., 2003. Phosphotransfer networks and cellular energetics. J. Exp. Biol. 206, 2039-2047. https://doi.org/10.1242/jeb.00426

Dzeja, P.P., Vitkevicius, K.T., Redfield, M.M., Burnett, J.C., Terzic, A., 1999. Adenylate kinase-catalyzed phosphotransfer in the myocardium: increased contribution in heart failure. Circ. Res. 84, 1137-1143. https://doi.org/10.1161/01.RES.84.10.1137

EL-Khaldi, A.T.F., 2010. Effect of different stress factors on some physiological parameters of Nile tilapia (*Oreochromis niloticus*). Saudi J. Biol. Sci. 17, 241-246. https://doi.org/10.1016/j.sjbs.2010.04.009

Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A., Sherratt, T.N., 2005. Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. Annu. Rev. Entomol. 50, 153-179. https://doi.org/10.1146/annurev.ento.50.071803.130341

Grings, M., Parmeggiani, B., Moura, A.P., Alvorcem, L.M., Wyse, A.T.S., Wajner, M., Leipnitz, G., 2018. Evidence that thiosulfate inhibits creatine kinase activity in rat striatum via thiol group oxidation. Neurotox. Res. 34, 693-705. https://doi.org/10.1007/s12640-018-9934-y

Gutierrez, J.A., Csonka, L.N., 1995. Isolation and characterization of adenylate kinase (adk) mutations in *Salmonella typhimurium* which block the ability of glycine betaine to function as an osmoprotectant. J. Bacteriol. 177, 390-400. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC176603/

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139. https://www.jbc.org/content/249/22/7130.full.pdf

Halley, B.A., Winter, R., Yoon, S., Marley, S.E., Rehbein, S., 2005. The environmental safety of eprinomectin to earthworms. Vet. Parasitol. 128, 109-114. https://doi.org/10.1016/j.vetpar.2004.11.007

Holste, J.E., Smith, L.L., Hair, J.A., Lancaster, J.L., Lloyd, J.E., Langholff, W.K., Barrick, R.A., Eagleson, J.S., 1997. Eprinomectin: a novel avermectin for control of lice in all classes of cattle. Vet. Parasitol. 73, 153-16l. https://doi.org/10.1016/S0304-4017(97)00063-0

Horsberg, T.E., 2012. Avermectin use in aquaculture. Curr. Pharm. Biotechnol. 13, 1095-1102. https://doi.org/10.2174/138920112800399158

Høy, T., Horsberg, T.E., Nafstad, I., 1990. The disposition of ivermectin in Atlantic salmon (*Salmo salar*). Pharmacol. Toxicol. 67, 307-312. https://doi.org/10.1111/j.1600-0773.1990.tb00835.x

Jensen, J., Scott-Fordsmand, J.J., 2012. Ecotoxicity of the veterinary pharmaceutical ivermectin tested in a soil multi-species (SMS) system. Environ. Pollut. 171, 133-139. https://doi.org/10.1016/j.envpol.2012.07.014

Katharios, P., Iliopoulou-Georgudaki, J., Kapata-Zoumbos, K., Spiropoulos, S., 2001. Toxicity of intraperitoneally injected ivermectin in sea bream, *Sparus aurata*. Fish Physiol. Biochem. 25, 99-108. https://doi.org/10.1023/A:1020574810332

Langiano, V.C., Martinez, C.B.R., 2008. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. Comp. Biochem. Physiol. C. 147, 222-231. https://doi.org/10.1016/j.cbpc.2007.09.009

LeBel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227-231. https://doi.org/10.1021/tx00026a012

Leong, S.F., Lai, J.C.K., Lim, L., Clark, J.B., 1981. Energy-metabolising enzymes in brain regions of adult and aging rats. J. Neurochem. 37, 1548-1556. https://doi.org/10.1111/j.1471-4159.1981.tb06326.x

Li, M., You, T.-Z., Zhu, W.-J., Qu, J.-P., Liu, C., Zhao, B., Xu, S.-W., Li, S., 2013. Antioxidant response and histopathological changes in brain tissue of pigeon exposed to avermectin. Ecotoxicology 22, 1241-1254. https://doi.org/10.1007/s10646-013-1112-7

Litskas, V.D., Karamanlis, X.N., Batzias, G.C., Tsiouris, S.E., 2013. Are the parasiticidal avermectins resistant to dissipation in the environment? The case of eprinomectin. Environ. Int. 60, 48-55. https://doi.org/10.1016/j.envint.2013.07.017

Lumaret, J.-P., Errouissi, F., Floate, K., Römbke, J., Wardhaugh, K., 2012. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Curr. Pharm. Biotechnol. 13, 1004-1060. https://doi.org/10.2174/138920112800399257

Lushchak, O.V., Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I., 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76, 932-937. https://doi.org/10.1016/j.chemosphere.2009.04.045

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469-474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Meagher, E.A., FitzGerald, G.A., 2000. Indices of lipid peroxidation in vivo: strengths and limitations. Free Rad. Biol. Med. 28, 1745-1750. https://doi.org/10.1016/S0891-5849(00)00232-X

Merck, 1996. Ivomec Eprinex (Eprinomectin) Pour-On for Beef and Dairy Cattle: Environmental Assessment. Report NADA 141-079EA. Merck and Company, Rahway, NJ, USA.

Monserrat, J.M., Geracitano, L.A., Pinho, G.L.L., Vinagre, T.M., Faleiros, M., Alciati, J.C., Bianchini, A., 2003. Determination of lipid peroxides in invertebrates tissues using the Fe(III) xylenol orange complex formation. Arch. Environ. Contam. Toxicol. 45, 177-183. https://doi.org/10.1007/s00244-003-0073-x

Nieman, C.C., Floate, K.D., Düring, R.-A., Heinrich, A.P., Young, D.K., Schaefer, D.M., 2018. Eprinomectin from a sustained release formulation adversely affected dung breeding insects. PLoS One 13, 1-11. https://doi.org/10.1371/journal.pone.0201074

Oliveira, R., Grisolia, C.K., Monteiro, M.S., Soares, A.M.V.M., Domingues, I., 2016. Multilevel assessment of ivermectin effects using different zebrafish life stages. Comp. Biochem. Physiol. C. 187, 50-61. https://doi.org/10.1016/j.cbpc.2016.04.004

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158-169. https://www.translationalres.com/article/0022-2143(67)90076-5/abstract

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S., 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). Sci. Total Environ. 309, 105-115. https://doi.org/10.1016/S0048-9697(03)00006-8

Rašković, B.S., Stanković, M.B., Marković, Z.Z., Poleksić, V.D., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agricult. Sci. 56, 87-100. https://doi.org/10.2298/JAS1101087R

Read, S.M., Northcote, D.H., 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal. Biochem. 116, 53-64. https://doi.org/10.1016/0003-2697(81)90321-3

Rui, L., 2014. Energy metabolism in the liver. Compr. Physiol. 4, 177-197. https://doi.org/10.1002/cphy.c130024

Saks, V., Dzeja, P., Schlattner, U., Vendelin, M., Terzic, A., Wallimann, T., 2006. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. J. Physiol. 571, 253-273. https://doi.org/10.1113/jphysiol.2005.101444

Scheffczyk, A., Floate, K.D., Blanckenhorn, W.U., Düring, R.-A., Klockner, A., Lahr, J., Lumaret, J.-P., Salamon, J.-A., Tixier, T., Wohde, M., Römbke, J., 2016. Nontarget effects of ivermectin residues on earthworms and springtails dwelling beneath dung of treated cattle in four countries. Environ. Toxicol. Chem. 35, 1959-1969. https://doi.org/10.1002/etc.3306

Serafini, S., Souza, C.F., Baldissera, M.D., Baldisserotto, B., Segat, J.C., Baretta, D., Zanella, R., Da Silva, A.S., 2019. Fish exposed to water contaminated with eprinomectin show inhibition of the activities of AChE and Na^+/K^+-ATPase in the brain, and changes in natural behavior. Chemosphere 223, 124-130. https://doi.org/10.1016/j.chemosphere.2019.02.026

Sk, U.H., Bhattacharya, S., 2006. Prevention of cadmium induced lipid peroxidation, depletion of some antioxidative enzymes and glutathione by a series of novel organoselenocyanates. Environ. Toxicol. Pharmacol. 22, 298-308. https://doi.org/10.1016/j.etap.2006.04.004

Slaninova, A., Smutna, M., Modra, H., Svobodova, Z., 2009. A review: oxidative stress in fish induced by pesticides. Neuro. Endocrinol. Lett. 30, 2-12. https://www.researchgate.net/publication/40757648_A_review_Oxidative_stress_in_fish_induced_by_pesticides

Souza, C.F., Baldissera, M.D., Bianchini, A.E., Da Silva, E.G., Mourão, R.H.V., Da Silva, L.V.F., Schmidt, D., Heinzmann, B.M., Baldisserotto, B., 2018. Citral and linalool chemotypes of *Lippia alba* essential oil as anesthetics for fish: a detailed physiological analysis of side effects during anesthetic recovery in silver catfish (*Rhamdia quelen*). Fish

Physiol. Biochem. 44, 21-34. https://doi.org/10.1007/s10695-017-0410-z

Svendsen, T.S., Grønvold, J., Holter, P., Sommer, C., 2003. Field effects of ivermectin and fenbendazole on earthworm populations and the disappearance of dung pats from bolus-treated cattle. Appl. Soil Ecol. 24, 207-218. https://doi.org/10.1016/S0929-1393(03)00096-9

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotox. Environ. Saf. 64, 178-189. https://doi.org/10.1016/j.ecoenv.2005.03.013

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57-149. https://www.dbbe.fcen.uba.ar/contenido/objetos/vanderOostetal2003Fishbioaccumulationandbiomarkersreview_1465491986892607.pdf

Varó, I., Rigos, G., Navarro, J.C., Del Ramo, J., Calduch-Giner, J., Hernández, A., Pertusa, J., Torreblanca, A., 2010. Effect of ivermectin on the liver of gilthead sea bream *Sparus aurata*: a proteomic approach. Chemosphere 80, 570-577. https://doi.org/10.1016/j.chemosphere.2010.04.030

Vassilis, L.D., George, B.C., Charalampos, P.G., Athina, P.-V., Xanthippos, K.N., 2016. Mobility of pharmaceutical compounds in the terrestrial environment: adsorption kinetics of the macrocyclic lactone eprinomectin in soils. Chemosphere 144, 1201-1206. https://doi.org/10.1016/j.chemosphere.2015.09.100

Wang, H., Chu, W., Das, S.K., Ren, Q., Hasstedt, S.J., Elbein, S.C., 2002. Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in Northern European Caucasians. Diabetes 51, 2861-2865. https://doi.org/10.2337/diabetes.51.9.2861

Wang, J., Xu, Y., Wan, H., Hu, J., 2018. Antibiotic ivermectin selectively induces apoptosis in chronic myeloid leukemia through inducing mitochondrial dysfunction and oxidative stress. Biochem. Biophys. Res. Commun. 497, 241-247. https://doi.org/10.1016/j.bbrc.2018.02.063

Zortéa, T., Segat, J.C., Maccari, A.P., Sousa, J.P., Da Silva, A.S., Baretta, D., 2017. Toxicity of four veterinary pharmaceuticals on the survival and reproduction of *Folsomia candida* in tropical soils. Chemosphere 173, 460-465. https://doi.org/10.1016/j.chemosphere.2017.01.069

Table 1 - Actual concentrations of eprinomectin determined in the tanks water during the experimental period.

Eprinomectin nominal concentrations µg L^{-1}	Eprinomectin actual concentrations µg L^{-1}			
	0 h	24 h	48 h	48 h in eprinomectin free-water
0.0 (control)	-	-	-	-
0.0025	1.124	0.850	0.394	-
0.01	1.809	1.149	0.699	-
0.05	3.976	1.152	1.286	-
Ethanol	-	-	-	-

Figures legends

Fig. 1 - Hepatic reactive oxygen species (ROS) [A] and [B] lipoperoxidation (LOOH) levels in silver catfish (*Rhamdia quelen*), after 24 and 48 h of eprinomectin exposure in the water experimentally-contaminated, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) in six animals for each group. Capital letters indicate differences between times for the same treatment. Lowercase letters indicate differences between treatments for the same time. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by Tukey's test ($p < 0.05$; $n = 6$ per group). Treatments: 0.0 µg L^{-1} (control), 1.124 µg L^{-1}, 1.809 µg L^{-1}, 3.976 µg L^{-1} and dilution in ethanol used as control (Ethanol).

Fig. 2 - Hepatic antioxidant capacity against peroxyl radicals (ACAP) levels [A], and hepatic superoxide dismutase (SOD) [B], glutathione peroxidase (GPx) [C] and glutathione S-transferase (GST) [D] activities in silver catfish (*Rhamdia quelen*), after 24 and 48 h of eprinomectin exposure in the water experimentally-contaminated, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) in six animals for each group. Capital letters indicate differences between times for the same treatment. Lowercase letters indicate differences between treatments for the same time. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples followed by Tukey's test ($p < 0.05$; $n = 6$ per group). Treatments: 0.0 µg L^{-1} (control), 1.124 µg L^{-1}, 1.809 µg L^{-1}, 3.976 µg L^{-1} and dilution in ethanol used as control (Ethanol).

Fig. 3 - Hepatic adenylate kinase (AK) and pyruvate kinase (PK) activities in silver catfish (*Rhamdia quelen*), after 24 and 48 h of eprinomectin exposure in the water experimentally-contaminated, and after 48 h of recovery in eprinomectin-free water. The data are expressed as mean and standard deviation (┬) in six animals for each group. Capital letters indicate differences between times for the same treatment. Lowercase letters indicate differences between treatments for the same time. Different letters indicate statistically significant differences using bilateral two-way analysis of variance (ANOVA) for independent samples

followed by the Tukey's test ($p < 0.05$; $n = 6$ per group). Treatments: 0.0 µg L^{-1} (control), 1.124 µg L^{-1}, 1.809 µg L^{-1}, 3.976 µg L^{-1} and dilution in ethanol used as control (Ethanol).

Fig. 1

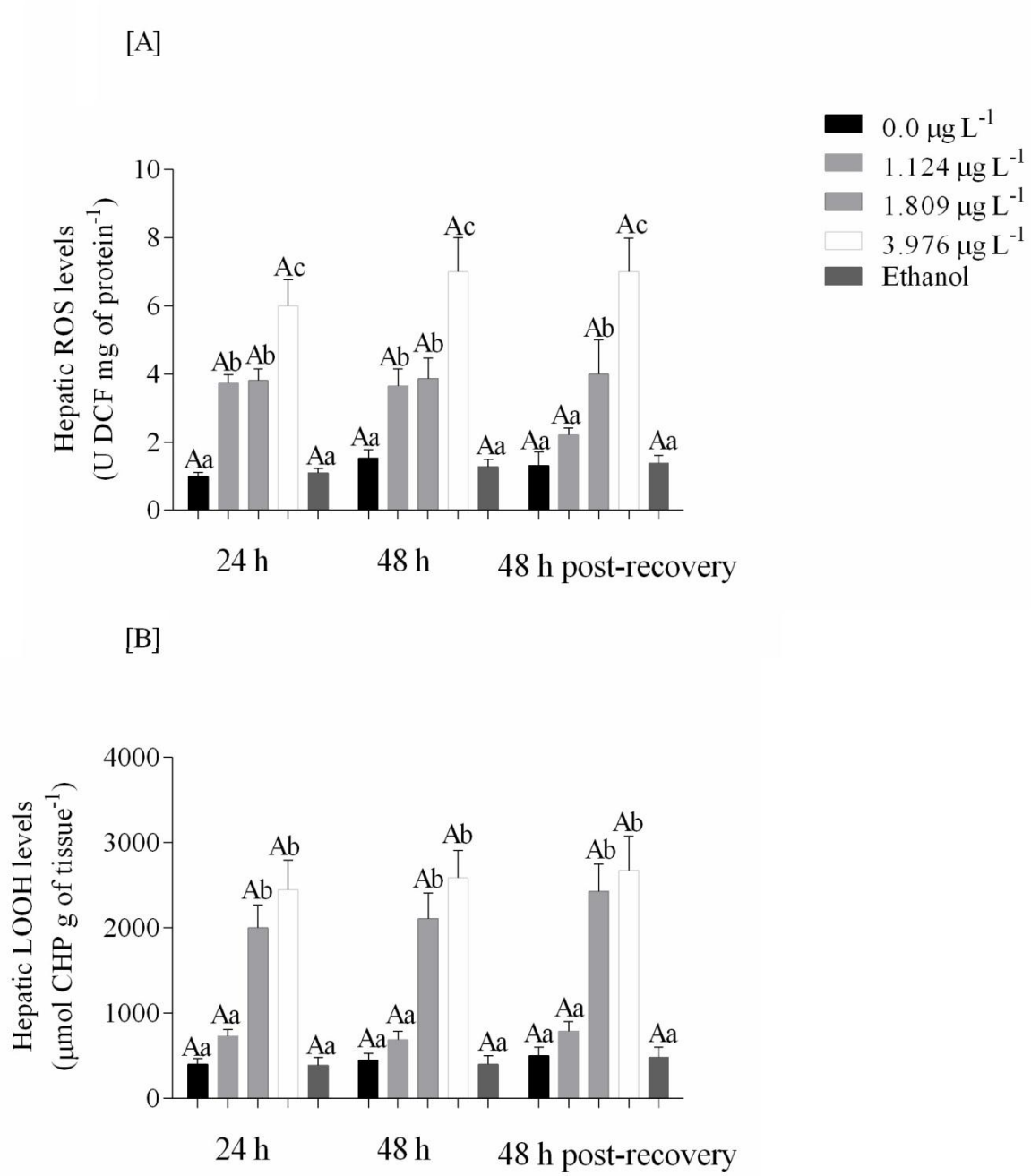

[A]
0.0 µg L-1
1.124 µg L-1
1.809 µg L-1
3.976 µg L-1
Ethanol
Hepatic ROS levels
(U DCF mg of protein-1)
10
8
6
4
2
0
Aa
Ab
Ac
24 h
48 h
48 h post-recovery
[B]
Hepatic LOOH levels
(µmol CHP g of tissue-1)
4000
3000
2000
1000
0
Aa
Ab
24 h
48 h
48 h post-recovery

Fig. 2

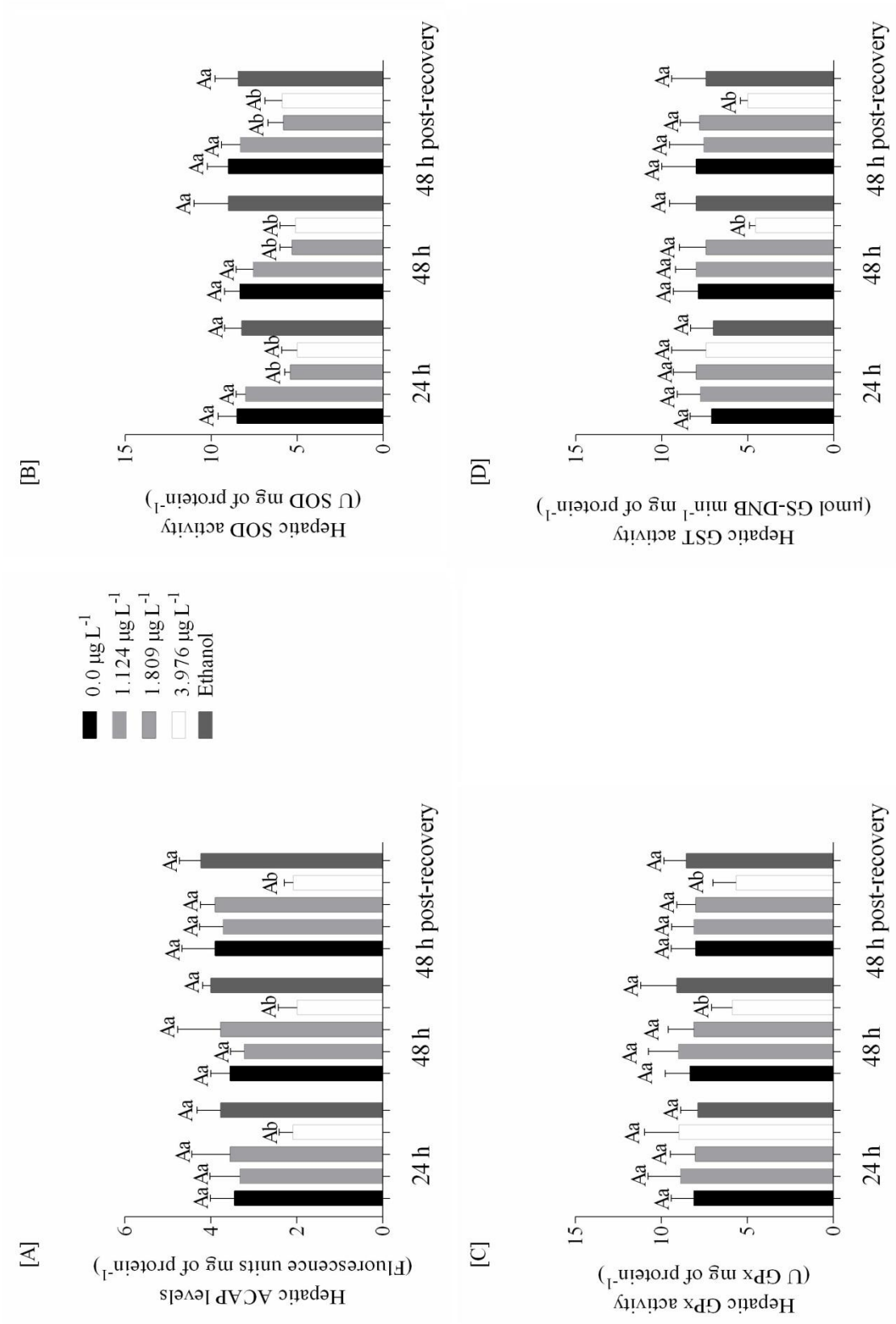
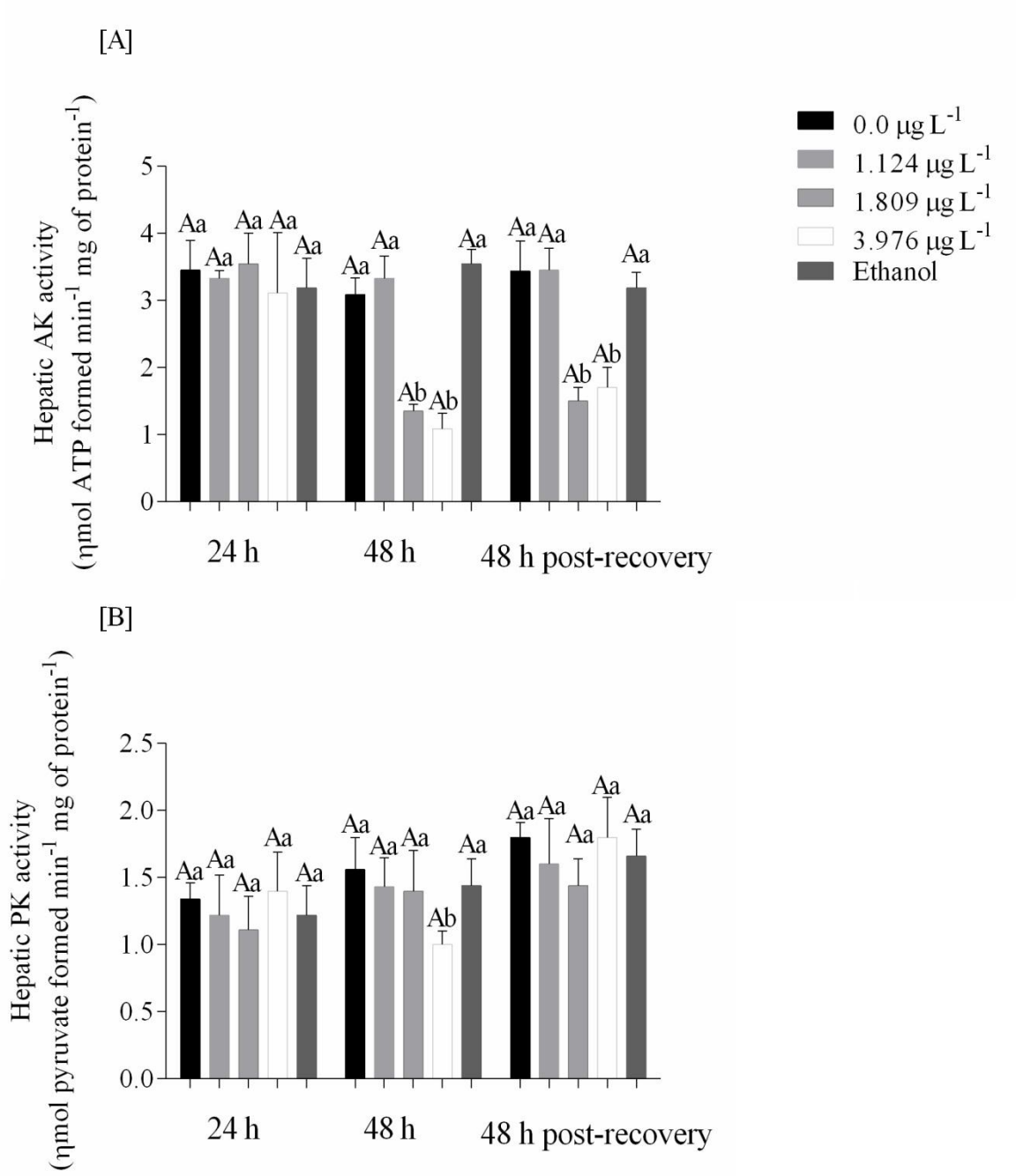

[A]
Hepatic ACAP levels
(Fluorescence units mg of protein-1)
[B]
Hepatic SOD activity
(U SOD mg of protein-1)
[C]
Hepatic GPx activity
(U GPx mg of protein-1)
[D]
Hepatic GST activity
(µmol GS-DNB min-1 mg of protein-1)
24 h
48 h
48 h post-recovery
0.0 µg L-1
1.124 µg L-1
1.809 µg L-1
3.976 µg L-1
Ethanol

Fig. 3


[A]
Hepatic AK activity
(ηmol ATP formed min-1 mg of protein-1)
0.0 μg L-1
1.124 μg L-1
1.809 μg L-1
3.976 μg L-1
Ethanol
24 h
48 h
48 h post-recovery
[B]
Hepatic PK activity
(ηmol pyruvate formed min-1 mg of protein-1)
24 h
48 h
48 h post-recovery

3. CONSIDERAÇÕES FINAIS

A partir dos resultados obtidos com a realização deste estudo, houve contribuição quanto ao entendimento dos efeitos ecotoxicológicos causados pelo antiparasitário eprinomectina sobre colêmbolos e peixes, organismos da fauna edáfica e aquáticos, respectivamente. Quanto às diferenças de intensidade sobre parâmetros toxicológicos agudos e crônicos, bem como a relação dos efeitos em diferentes solos e na água.

Nossos resultados evidenciam a periculosidade da eprinomectina para o meio ambiente, pois demonstram efeitos do antiparasitário sobre sobrevivência, reprodução e comportamento de fuga de colêmbolos, organismos com importância na prestação de serviços ecossistêmicos, assim como uma maior intensidade dos efeitos tóxicos em solo de textura mais arenosa, típico em regiões subtropicais e especialmente tropicais.

Também demonstram a capacidade da eprinomectina em causar comprometimento cerebral e afetar a neurotransmissão de peixes, com consequências sobre alterações do comportamento natural destes animais. Além de comprometimento do fígado e suas funções energéticas, por extresse oxidativo. Bem como efeitos tóxicos residuais sobre estes parâmetros, mesmo na ausência da contaminação aquática pelo antiparasitário, prejudicando a homeostase do organismo. Um resultado de grande importância a ser também considerado é a inadequação do medicamento veterinário comercial à base de eprinomectina utilizado no estudo, quanto à concentração da molécula farmacológica estar acima da relatada em bula. Isto foi encontrado durante a avaliação da concentração de eprinomectina na água no experimento aquático.

Nossos resultados também abrem portas para outros estudos com o antiparasitário eprinomectina, cujos conhecimentos ecotoxicológicos ainda são limitados e necessitam de atenção. Futuramente poderão ser realizados estudos quanto aos efeitos dos metabólitos da eprinomectina após administração em animais de produção, biomagnificação no ambiente, diferenças ecorregionais, impactos sobre a biodiversidade do solo e serviços ecossistêmicos, relação do comprometimento fisiológico em peixes nos sistemas produtivos e nos sistemas naturais, impactos sobre a cadeia alimentar, entre outras muitas possibilidades.

REFERÊNCIAS

ABBAS, R. Z., et al. Acaricide resistance in cattle ticks and approaches to its management: the state of play. **Veterinary Parasitology**, v. 203, n. 1-2, p. 6-20, 2014.

AKSIT, D., et al. Plasma disposition and faecal excretion of eprinomectin following topical and subcutaneous administration in non-lactating dairy cattle. **The New Zealand Veterinary Journal**, v. 64, n. 4, p. 207-211, 2016.

ALAK, G., et al. Investigation of 8-OHdG, CYP1A, HSP70 and transcriptional analyses of antioxidant defence system in liver tissues of rainbow trout exposed to eprinomectin. **Fish and Shellfish Immunology**, v. 65, p. 136-144, 2017.

ANDRADE, S. F. **Manual de terapêutica veterinária**. São Paulo: Roca, 454 p., 2008.

ANDREA, M. M. Abordagens em ecotoxicologia terrestre no Brasil. In: XII Congresso Brasileiro de Ecotoxicologia, 882 p., 2012. Porto de Galinhas, PE. **Anais**... Porto de Galinhas: Sociedade Brasileira de Ecotoxicologia, p. 1, 2012.

AZEVEDO, F. A.; CHASIN, A. A. M. **As bases toxicológicas da ecotoxicologia**. São Paulo: RiMa, 322 p., 2003.

BIANCHINI, A.; MARTINS, S. E.; JORGE, M. B. **O modelo ligante biótico e suas aplicações em ecotoxicologia**. 2009. Disponível em: <http://www.inct-ta.furg.br/english/difusao/BLMM.pdf>. Acesso em: 20 nov. 2018.

BNDES SETORIAL. **Panorama da indústria farmacêutica veterinária**. 2007. Disponível em: <https://www.bndes.gov.br/SiteBNDES/export/sites/default/bndes_pt/Galerias/Arquivos/conhecimento/bnset/set2506.pdf>. Acesso em: 20 nov. 2018.

BORGES, F. A., et al. Anthelmintic resistance impact on tropical beef cattle productivity: effect on weight gain of weaned calves. **Tropical Animal Health and Production**, v. 45, n. 3, p. 723-727, 2013.

BOTTINELLI, N., et al. Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? **Soil and Tillage Research**, v. 146, p. 118-124, 2015.

BOXALL, A. B. A., et al. Review of veterinary medicines in the environment. **Technical Report European Environment Agency**. Almondsbury, Bristol, United Kingdom, 251 p., 2002.

BUENO, A. F.; FREITAS, S. Effect of the insecticides abamectin and lufenuron on eggs and larvae of *Chrysoperla externa* under laboratory conditions. **Journal of Biological Control**, v. 49, n. 3, p. 277-283, 2004.

BURG, R. W., et al. Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. **Antimicrobial Agents and Chemotherapy**, v. 15, n. 3, p. 361-367, 1979.

CHRISTIAN, T., et al. Determination of antibiotic residues in manure, soil, and surface waters. **Acta Hydrochimica et Hydrobiologica**, v. 31, n. 1, p. 36-44, 2003.

CNQ CUT – CONFEDERAÇÃO NACIONAL DO RAMO QUÍMICO. **Panorama da indústria farmacêutica veterinária**. 2015. Disponível em: <http://cnq.org.br/system/uploads/publication/9aee2f902857d5d6467b924555af8983/file/panorama-industria-farmaceutica-b.pdf>. Acesso em: 20 nov. 2018.

COOP, R. L.; KYRIAZAKIS, I. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. **Trends in Parasitology**, v. 17, n. 7, p. 325-330, 2001.

COUMENDOUROS, K., et al. Eficácia anti-helmíntica da eprinomectina no controle de nematóides gastrintestinais de bovinos. **Revista Brasileira de Parasitologia Veterinária**, v. 12, n. 3, p. 121-124, 2003.

CRAMER, L. G., et al. Persistent efficacy of topical eprinomectin against nematode parasites in cattle. **Parasitology Research**, v. 86, n. 11, p. 944-946, 2000.

CVETOVICH, R. J., et al. Synthesis of 4"-epi-amino-4"-deoxyavermectins B1. **Journal of Organic Chemistry**, v. 59, p. 7704-7708, 1994.

DIAO, X.; JENSEN, J.; DUUS HANSEN, A. Toxicity of the anthelmintic abamectin to four species of soil invertebrates. **Environmental Pollution**, v. 148, n. 2, p. 514-519, 2007.

DOMINGUES, I., et al. Effects of ivermectin on *Danio rerio*: a multiple endpoint approach: behaviour, weight and subcellular markers. **Ecotoxicology**, v. 25, n. 3, p. 491-499, 2016.

DORNY, P., et al. Control of gastrointestinal nematodes in first season grazing calves by two strategic treatments with eprinomectin. **Veterinary Parasitology**, v. 89, n. 4, p. 277-286, 2000.

DOURMISHEV, A. L.; DOURMISHEV, L. A.; SCHWARTZ, R. A. Ivermectin: pharmacology and application in dermatology. **International Journal of Dermatology**, v. 44, n. 12, p. 981-988, 2005.

EMEA - EUROPEAN MEDICINES AGENCY. **Eprinomectin, summary report**. EMEA/MRL/114/96-FINAL. Committee for Veterinary Medicinal Products, 5 p., 1996.

EPE, C., et al. Strategic control of gastrointestinal nematode and lungworm infections with eprinomectin at turnout and eight weeks later. **Veterinary Record**, v. 144, n. 14, p. 380-382, 1999.

FLOATE, K. D., et al. Fecal residues of veterinary parasiticides: nontarget effects in the pasture environment. **Annual Review of Entomology**, v. 50, p. 153-179, 2005.

GAWOR, J.; BORECKA, A.; MALCZEWSKI, A. Use of eprinomectin (Eprinex Pour-On) to control natural infection by gastro-intestinal nematodes in goats. **Medycyna Weterynaryjna**, v. 56, n. 6, p. 398-400, 2000.

GEARY, T. G. Ivermectin 20 years on: maturation of a wonder drug. **Trends Parasitology**, v. 21, n. 11, p. 530-532, 2005.

GEARY, T. G.; MORENO, Y. Macrocyclic lactone anthelmintics: spectrum of activity and mechanism of action. **Current Pharmaceutical Biotechnology**, v. 13, n. 6, p. 866-872, 2012.

GERENUTTI, M.; SPINOSA, H. S. Avermectinas: revisão do uso e da ação sobre o SNC. **Biotemas**, v. 10, n. 2, p. 1-27, 1997.

GREENSLADE, P.; VAUGHAN, G. T. A comparison of Collembola species for toxicity testing of Australian soils. **Pedobiologia**, v. 47, n. 2, p. 171-179, 2003.

GRISI, L., et al. Reassessment of the potential economic impact of cattle parasites in Brazil. **Brazilian Journal Veterinary Parasitology**, v. 23, n. 2, p. 150-156, 2014.

HALLEY, B. A., et al. The environmental safety of eprinomectin to earthworms. **Veterinary Parasitology**, v. 128, n. 1-2, p. 109-114, 2005.

HALLEY, B. A.; VANDENHEUVEL, W. J.; WISLOCKI, P. G. Environmental effects of the usage of avermectins in livestock. **Veterinary Parasitology**, v. 48, n. 1-4, p. 109-125, 1993.

HALLING-SØRENSEN, B., et al. Occurrence, fate and effects of pharmaceutical substances in the environment - a review. **Chemosphere**, v. 32, n. 2, p. 357-393, 1998.

HAVLICEK, E. Soil biodiversity and bioindication: from complex thinking to simple acting. **European Journal of Soil Biology**, v. 49, p. 80-84, 2012.

HOKE, R., et al. Review of laboratory-based terrestrial bioaccumulation assessment approaches for organic chemicals: current status and future possibilities. **Integrated Environmental Assessment and Management**, v. 12, n. 1, p. 109-122, 2016.

HOPKINS, A. L.; WITTY, M. J.; NWAKA, S. Mission possible. **Nature**, v. 449, n. 7159, p. 166-169, 2007.

ISLAM, M. D. Behaviour of veterinary drugs in soil, plant and water system: transport investigation of antiparasitic drugs in soil-plant-water. Dissertação (Mestrado). University of Natural Resources and Life Sciences,Vienna, 2013.

IWASA, M., et al. Nontarget effects of ivermectin on coprophagous insects in Japan. **Environmental Entomology**, v. 34, n. 6, p. 1485-1492, 2005.

JENSEN, J.; SCOTT-FORDSMAND, J. J. Ecotoxicity of the veterinary pharmaceutical ivermectin tested in a soil multi-species (SMS) system. **Environmental Pollution**, v. 171, 133-139, 2012.

JENSEN, J.; DIAO, X.; HANSEN, A. D. Single- and two-species tests to study effects of the anthelmintics ivermectin and morantel, and the coccidiostatic monensin on soil invertebrates. **Environmental Toxicology and Chemistry**, v. 8, n. 2, p. 316-323, 2009.

KÖHLER, P. The biochemical basis of anthelmintic action and resistance. **International Journal for Parasitology**, v. 31, n. 4, p. 336-345, 2001.

LIMA, C. A. Avaliação de risco ambiental como ferramenta para o descomissionamento de uma indústria de metalúrgica de zinco. Tese (Doutorado), Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2009.

LITSKAS, V., et al. Are the parasiticidal avermectins resistant to dissipation in the environment? The case of eprinomectin. **Environment International**, v. 60, p. 48-55, 2013.

LITSKAS, V., et al. Sorption of the antiparasitic drug eprinomectin in three soils. **Chemosphere**, v. 82, n. 2, p.193-198, 2011.

LUMARET, J. P., et al. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. **Current Pharmaceutical Biotechnology**, v. 13, n. 6, p. 1004-1060, 2012.

MANDAL, K.; SINGH, B. Persistence of fipronil and its metabolites in sandy loam and clay loam soils under laboratory conditions. **Chemosphere**, v. 91, p. 1596-1603, 2013.

MATAMOROS, V.; BAYONA, J. Elimination of pharmaceutical and personal care products in subsurface flow constructed wetlands. **Environmental Science and Technology**, v. 4, n. 8, p. 811-816, 2006.

MCKELLAR, Q. A. Ecotoxicology and residues of anthelmintic compounds. **Veterinary Parasitology**, v. 72, n. 3-4, p. 413-426, 1997.

MCKELLAR, Q. A.; JACKSON, F. Veterinary anthelmintics: old and new. **Trends in Parasitology**, v. 20, n. 10, p. 456-461, 2004.

MERCK. **Ivomec eprinex (eprinomectin) pour-on for beef and dairy cattle**. Environmental Assessment. Report NADA 141-079EA. Rahway, Merck and Company, New Jersey, United States of America, 1996.

MOLENTO, M. B. Resistência parasitária em helmintos de equídeos e propostas de manejo. **Ciência Rural**, v. 35, n. 6, p. 1469-1477, 2005.

MONTEIRO, S. G. **Parasitologia na medicina veterinária**. São Paulo: Roca, 356 p., 2011.

NETTO, D. P., et al. Levantamento dos principais fármacos utilizados no rebanho leiteiro do Estado do Paraná. **Acta Scientiarum: Animal Sciences**, v. 27, n. 1, p. 145-151, 2005.

NIEMAN, C.C., et al. Eprinomectin from a sustained release formulation adversely affected dung breeding insects. **PlosOne**, v. 13, n. 8, p. 1-11, 2018.

OLIVEIRA, P. A., et al. Doenças parasitárias em bovinos e ovinos no sul do Brasil: frequência e estimativa de perdas econômicas. **Pesquisa Veterinária Brasileira**, v. 37, n. 8, p. 797-801, 2017.

OLIVEIRA, R., et al. Multilevel assessment of ivermectin effects using different zebrafish life stages. **Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology**, v. 187, p. 50-61, 2016.

PALMER, S. J.; BEAVERS, J. B. **Reported (Appendix D-9) in Ivomec® Eprinex® (eprinomectin) pour on for beef and dairy cattle**. Environmental Assessment, United States of America, 1995. Disponível em: < http//:www.fda.gov/CVM/efoi/EA_Files/141-079EA.PDF >. Acesso em: 20 nov. 2018.

PERRY, B. D.; RANDOLPH, T. F. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. **Veterinary Parasitology**, v. 84, n. 3-4, p. 145-168, 1999.

POLAT, H., et al. Investigation of acute toxicity of beta-cypermethrin on guppies *Poecilia reticulata*. **Chemosphere**, v. 49, n. 1, p. 39-44, 2002.

RUAS, J. L.; BERNE, M. E. A. Parasitoses por nematódeos gastrintestinais em bovinos e ovinos, p. 584-604. In: RIET-CORREA, F., et al. (Eds), **Doenças de ruminantes e equídeos**, v. 1, Campo Grande: Equali, 722 p., 2007.

SANGSTER, N. C. Managing parasiticide resistance. **Veterinary Parasitology**, v. 98, n. 1-3, p. 89-109, 2001.

SARMAH, A. K., et al. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. **Chemosphere**, v. 65, n. 5, p. 725-759, 2006.

SCHENCK, F. J.; LAGMAN, L. H. Multiresidue determination of abamectin, doramectin, ivermectin, and moxidectin in milk using liquid chromatography and fluorescence detection. **Journal of AOAC International**, v. 82, n. 6, p. 1340-1344, 1999.

SHALABY, H. A., Anthelmintics resistance; how to overcome it? **Iranian Journal of Parasitology**, v. 8, n. 1, p. 18-32, 2013.

SHOOP, W. L., et al. Eprinomectin: a novel avermectin for use as atopical endectocide for cattle. **International Journal for Parasitology**, v. 26, n. 11, p. 1237-1242, 1996.

SHOOP, W. L; MROZIK, H.; FISHER, M. H. Structure and activity of avermectins and milbemycins in animal health. **Veterinary Parasitology**, v. 59, n. 2, p. 139-156, 1995.

SHOOP, W.; SOLL. M. Ivermectin abamectin and eprinomectin. In: VERCRUYSSE, J.; REW, R. (Eds.), **Macrocyclic lactones in antiparasitic therapy**. Oxon, United Kingdom: CABI Publishing, 29 p., 2002.

SINDAN – SINDICATO NACIONAL DA INDÚSTRIA DE PRODUTOS PARA SAÚDE ANIMAL. **Mercado Brasil 2017**. 2017. Disponível em: <http://www.sindan.org.br/mercado-brasil-2017/>. Acesso em: 20 nov. 2018.

STOTZER, E. S., et al. Impacto econômico das doenças parasitárias na pecuária. **Revista Brasileira de Higiene e Sanidade Animal**, v. 8, n. 3, p. 98-121, 2014.

SUAREZ, M., et al. High impact and effectiveness of Gavac™ vaccine in the national program for control of bovine ticks *Rhipicephalus microplus* in Venezuela. **Livestock Science**, v. 187, p. 48-52, 2016.

SVENDSEN, T. S., et al. Field effects of ivermectin and fenbendazole on earthworm populations and the disappearance of dung pats from bolus-treated cattle. **Applied Soil Ecology**, v. 24, n. 3, p. 207-218, 2003.

TEREKHOVA, V. A. Soil bioassay: problems and approaches. **Eurasian Soil Science**, v. 44, p. 173-179, 2011.

TREMBLAY, L. A.; WRATTEN, S. D. 2002. **Effects of ivermectin in dairy discharges on terrestrial and aquatic invertebrates**. Department of Conservation, Wellington, New Zealand, 13 p., 2002.

TRETTER, V.; STEPHEN, J. M. $GABA_A$ receptor dynamics and constructing GABAergic synapses. **Frontiers in Molecular Neuroscience**, v. 1, n. 7, 2008.

VASSILIS, L. D., et al. Mobility of pharmaceutical compounds in the terrestrial environment: adsorption kinetics of the macrocyclic lactone eprinomectin in soils. **Chemosphere**, v. 144, p. 1201-1206, 2016.

VERDÚ, J. R., et al. Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. **Scientific Reports**, v. 5, p. 1-10, 2015.

WOLSTENHOLME, A. J.; ROGERS, A. T. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. **Parasitology**, v. 131, p. 85-95, 2005.

WOLSTENHOLME, A. J., et al. Drug resistance in veterinary helminthes. **Trends in Parasitology**, v. 20, n. 10, p. 469-476, 2004.

WOODS, D. J.; WILLIAMS, T. M. 2007. The challenges of developing novel antiparasitic drugs. **Invertebrate Neuroscience**, v. 7, n. 4, p. 245-250, 2007.

ZHANG, H., et al. Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. **Chemosphere**, v. 152, p. 229-237, 2016.

ZORTÉA, T., et al. 2018. Ecotoxicological effect of fipronil and its metabolites on *Folsomia candida* in tropical soils. **Environmental Toxicology and Pharmacology**, v. 62, p. 203-209, 2018.

ZORTÉA, T., et al. Influence of cypermethrin on avoidance behavior, survival and reproduction of *Folsomia candida* in soil. **Chemosphere**, v. 122, p. 94-98, 2015.

ZORTÉA, T., et al. Toxicity of four veterinary pharmaceuticals on the survival and reproduction of *Folsomia candida* in tropical soils. **Chemosphere**, v. 173, p. 460-465, 2017.

ANEXOS

ANEXO I

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Efeitos bioquímicos, oxidativos, energéticos e comportamentais de jundiás expostos a concentrações subletais de eprinomectina", protocolada sob o CEUA nº 4679260518 (ID 000647), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 06/06/2018.

We certify that the proposal "Biochemical, oxidative, energetic and behavioral effects of jundias exposed to sublethal concentrations of eprinomectin", utilizing 96 Fishes (males and females), protocol number CEUA 4679260518 (ID 000647), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 06/06/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2018 a 10/2018 Área: Zootecnia

Origem:	Animais de proprietários						
Espécie:	Peixes	sexo:	Machos e Fêmeas	idade:	40 a 50 dias	N:	96
Linhagem:	Jundia			Peso:	10 a 15 g		

Local do experimento: O experimento ocorrerá na UDESC Oeste na sala de Experimentação, prédio Zootecnia.

Lages, 21 de janeiro de 2019

Marcia Regina Pfuetzenreiter
Coordenadora da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Prof. Dr. Ubirajara Maciel da Costa
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

ANEXO II

Chemosphere 223 (2019) 124–130

Contents lists available at ScienceDirect

Chemosphere

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

Fish exposed to water contaminated with eprinomectin show inhibition of the activities of AChE and Na^+/K^+-ATPase in the brain, and changes in natural behavior

Suélen Serafini [a], Carine de Freitas Souza [b], Matheus Dellaméa Baldissera [b], Bernardo Baldisserotto [b], Julia Corá Segat [a], Dilmar Baretta [a, c, *], Renato Zanella [d], Aleksandro Schafer da Silva [a, c, **]

[a] Graduate Program of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil
[b] Graduate Program of Pharmacology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[c] Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil
[d] Department of Chemistry, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

HIGHLIGHTS

- Eprinomectin may be a contaminant aquatic even in low concentrations.
- Eprinomectin cause inhibition of cerebral AChE activity in fish.
- Eprinomectin cause inhibition of cerebral Na^+/K^+-ATPase activity in fish.
- Inhibition of AChE and Na^+/K^+-ATPase activities affects the natural behavior of fish.
- Behavioral changes in fish are hyperlocomotion and longer times on the surface.