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EFEITOS TOXICOLÓGICOS NEONATAL, TRANSGERACIONAL E CRÔNICO DE PIRETROIDES EM *Caenorhabditis elegans*

Santa Maria, RS
2022

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Dissertação apresentada ao Programa de Pós-Graduação em Farmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Farmacologia**.

Orientador: Prof. Dr. Leonardo José Gil Barcellos

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WAGNER ANTONIO TAMAGNO

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Aprovado em 16 de dezembro de 2022:

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Santa Maria, RS,
2022

Dedico esta dissertação aos meus três anjos do céu: Nona Inês, Nono Leonildo e Profe Rosilene

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“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota”.

(Madre Teresa de Calcuta)

RESUMO

EFEITOS TOXICOLÓGICOS NEONATAL, TRANSGERACIONAL E CRÔNICO DE PIRETROIDES EM *Caenorhabditis elegans*

AUTOR: Wagner Antonio Tamagno

ORIENTADOR: Leonardo José Gil Barcellos

O extenso uso de inseticidas domésticos e agropecuários para o controle de pragas domésticas, de lavoura e até mesmo de interesse zootécnico vem crescendo nos últimos anos. Uma das principais classes utilizadas na composição de inseticidas são os piretroides. Estes compostos possuem baixa ação neurotóxica em humanos, no entanto a exposição pode aumentar a predisposição a condições oxidativas e neurodegenerativas. O *Caenorhabditis elegans* é um verme de solo amplamente conhecido e utilizado em pesquisas bioquímicas, farmacológicas e toxicológicas pelo seu baixo custo, fácil manuseio, amplo índice de translacionalidade e rápido desenvolvimento. Desta forma, o objetivo deste trabalho foi avaliar o efeito tóxico de quatro diferentes inseticidas a base de piretroides em três diferentes protocolos de exposição em *C. elegans* (transgeracional, neonatal e crônico). Foram avaliados biomarcadores comportamentais, bioquímicos e o padrão da expressão fluorescente de proteínas do sistema antioxidante e da PolyQ40::YFP. Por fim, pôde-se concluir que os piretroides possuem distintos mecanismos de interferência no organismo animal, mas o que mais chama a atenção é o efeito toxicológico transgeracional que parece estar relacionado com o aumento da expressão de isoformas mutadas da proteína huntingtina e redução da atividade da AChE. Estes achados destacam uma maior predisposição ao aparecimento de condições neurodegenerativas e neurotóxicas em indivíduos filhos de pais expostos (F1). Além disso, diversos biomarcadores comportamentais foram alterados de maneira semelhante em exposições trangeracionais e crônicas, entretanto as razões fisiológicas alteradas pelos piretroides estão relacionadas com distintos mecanismos de ação. Destacamos o aumento do risco do desenvolvimento precoce da doença de Huntington de maneira transgeracional em indivíduos geneticamente predispostos.

Palavras-chave: Doença de Huntington. Neurodegeneração. Inseticidas. Estresse oxidativo. Comportamento. Sistema nervoso colinérgico.

ABSTRACT

NEONATAL, TRANSGENERATIONAL, AND LIFESPAM TOXICOLOGIC EFFECTS OF PIRETROIDS IN *Caenorhabditis elegans*

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The extensive use of domestic and agricultural insecticides to control domestic, crop and even zootechnical pests has been growing in recent years. One of the main classes used in the composition of insecticides are the pyrethroids. These compounds have low neurotoxic action in humans, however exposure may increase predisposition to oxidative and neurodegenerative conditions. *Caenorhabditis elegans* is a soil worm widely known and used in biochemical, pharmacological and toxicological research due to its low cost, easy handling, wide translational index and rapid development. Thus, the objective of this work was to evaluate the toxic effect of four different insecticides based on pyrethroids in three different exposure protocols in *C. elegans* (transgenerational, neonatal and lifespan). Behavioral, biochemical biomarkers and the pattern of fluorescent expression of proteins from the antioxidant system and PolyQ40::YFP were evaluated. Finally, it could be concluded that pyrethroids have different mechanisms of interference in the animal organism, but what most draws attention is the transgenerational toxicological effect that seems to be related to the increased expression of mutated isoforms of the huntingtin protein and reduced activity from AChE. These findings highlight a greater predisposition to the onset of neurodegenerative and neurotoxic conditions in children of exposed parents (F1). In addition, several behavioral biomarkers were similarly altered in cross-generational and chronic exposures, however the physiological reasons altered by pyrethroids are related to different mechanisms of action. We highlight the increased risk of early development of Huntington's disease in a transgenerational way in genetically predisposed individuals.

Keywords: Huntington’s disease. Neurodegeneration. Insecticides. Oxidative stress. Behavior. Cholinergic nervous system.

LISTA DE FIGURAS

CAPITULO 1

CAPITULO 2

CAPITULO 3

LISTA DE TABELAS

CAPITULO 3

LISTA DE ABREVIATURAS

YFP – *Yellow Fluorescent Protein*

GFP – *Green Fluorescent Protein*

PolyQ – *Polyglutamine*

PolyQ40 – *Polyglutamine higher than 40 nucleotides*

DH – Doença de Huntington

HD – *Huntington Disease*

ACh - *Acetylcholine*

AChE – *Acetylcholinesterase activity*

βA – Beta-amiloide

EO – Estresse oxidativo

ERO – Espécies reativas de oxigênio

ROS – *Reactive oxygen especies*

SOD – *Superoxide dismutase*

CAT - *Catalase*

GST – *Glutathione-S-transferase*

O_2^- - Ânion superóxido

H_2O_2 – Peróxido de hidrogênio

HSP – Heat shock protein

SNC – Sistema nervosa central

TG – *transgenerational*

NN – *Neonatal*

LS – *Lifespan*

T-BI – *Trasfluthrin-based insecticide*

P-BI – *Prallethrin-based insecticide*

λ-cBI – *λ-cihalothrin-based insecticide*

CyBI – *Cipermetrin-based insecticide*

NGM – *Nematode growth medium*

RCD – *Relative calculated dose*

RCHD – *Relative calculated half dose*

SUMÁRIO

1 INTRODUÇÃO

Os piretroides fazem parte de uma classe de compostos químicos com atividade inseticida, amplamente utilizado na agropecuária para controle de infestações de insetos na agricultura, assim como para o controle de ectoparasitas em animais de produção e domésticos (carrapatos, pulgas e etc.) (JOKANOVIĆ, 2018). Além disso, os piretroides são também eficazes nas dedetizações de residências e cidades com altos índices de infestação por mosquitos, especialmente os vetores de doenças como o *Aedes aegypti* (CHEN et al., 2020).

No Brasil, a dengue é uma das muitas infecções transmitidas pela picada de insetos, e segundo o ministério da saúde os casos, cresceram 36% nos dois primeiros meses de 2022 em comparação ao ano anterior (MINISTÉRIO DA SAÚDE, 2022). Este crescimento, fez aumentar o uso dos referidos inseticidas. Essa classe de inseticidas também possui uma ampla utilização no controle doméstico de insetos, para tal, encontram-se no mercado diversas formulações com distintas formas de utilizações domésticas (ROSE, 2001).

Os piretroides possuem sua ação sobre os canais de sódio neurais os quais se mantem abertos por mais tempo e acabam por hiperexcitar o sistema nervoso levando a morte do inseto (ZHU et al., 2020). A exposição aos piretroides é principalmente respiratória, distribuição plasmática e o efeito neuro-excitatório (MALLICK et al., 2020) observado em insetos só não é observada em seres humanos, devido à baixa concentração à qual se é exposto (EISENSTEIN, 2015). Exposições a baixas doses de piretroides em longo prazo pode ser um dos fatores para o desenvolvimento de patologias com razoes etiológicas ainda desconhecidas (FILIPPI et al., 2021).

As doenças neurodegenerativas possuem suas bases estabelecidas em desbalanços oxidativos caracterizados por quadros de estresse oxidativo (EO) (CARVALHO; MOREIRA, 2018). Além disso, debalanços na homeostasia nervosa podem estar relacionadas com exposições a toxicantes xenobióticos em baixas concentrações (TAMAGNO, 2022c).

Os biomarcadores fisiológicos podem estar relacionados com alterações comportamentais dos indivíduos. O comportamento é entendido como a expressão dos constituintes fisiológicos, bioquímicos e moleculares de um indivíduo em contato com o meio externo onde vive, bem como as suas respostas frente adaptações ao ambiente (SNOWDON C. T. 1999). As alterações comportamentais são o resultado de mudanças

fisiológicas que levam à um repertório comportamental incomum. A ligação entre o sistema nervoso colinérgico e comportamento já é bem esclarecida (TAMAGNO et al., 2022a; FENSKE et al. 2018) bem como as relações entre o sistema antioxidante e o comportamento já possuem sórdidas comprovações científicas (TAMAGNO et al., 2022c; KITA et al., 2009).

A relação entre o alterações do sistema nervoso por doenças degenerativas, não estão completamente elucidadas, e a ampla utilização de compostos, como inseticidas, nocivos às células nervosas, demonstra a relevância na sua investigação toxicológica (KONOVALOVA et al., 2019). Para tal, a utilização de modelos animais pode auxiliar nesta investigação. Entre os modelos amplamente utilizados em pesquisas toxicológicas temos os nematódeos *Caenorhabditis elegans (C. elegans*). O *C. elegans* são pequenos nematoides de solo (~5 mm), que possuem distintos comportamentos e vias metabólicas, fisiológicas e bioquímicas conservadas com o ser humano (JADIYA; S MIR; NAZIR, 2012; PARKER et al., 2015; SCHMEISSER; PARKER, 2018). A alta similaridade genética permite avaliar distintos biomarcadores de doenças ligadas ao sistema nervoso central (SNC) do verme e associar o seu efeito sobre o SNC humano. Em *C. elegans* mutantes é possível avaliar a expressão fluorescente de enzimas antioxidantes, bem como de agregados de PolyQ, o que facilita ainda mais a compreensão de condições de neurodegeneração e estresse celular e as suas respostas fisiológicas celulares (PEIXOTO et al., 2016).

Como mencionado anteriormente, existem poucas evidencias etiológicas do aparecimento e progressão de doenças neurodegenerativas relacionadas com o uso de inseticidas. Levando-se em consideração a grande utilização dos piretroides como inseticidas domésticos e agropecuários, avaliar a relação entre estes e as condições neurodegenerativas é de fundamental importância. Portanto, o presente estudo avaliou o efeito da exposição a quatro inseticidas da classe dos piretroides (dois domésticos e dois agropecuários) em diferentes estágios de vida do *C. elegans*, sendo trangeracional, neonatal e crônico. Adicionalmente, foram avaliados biomarcadores do sistema nervoso colinérgico, expressão de agregados de PolyQ40 e do sistema antioxidante, bem como o repertório comportamental de *C. elegans*.

2. REVISÃO BIBLIOGRÁFICA

2.1. PIRETROIDES

Com o advento da agricultura em larga escala, se fez necessário o desenvolvimento de diversas tecnologias químicas para o controle de pragas de lavouras como os inseticidas (RAIBANTE; ZAPPE, 2012). Por serem muito tóxicos, os primeiros inseticidas foram gradativamente substituídos por outras formulações de menor toxicidade. Os piretroides entraram no mercado com o intuito de substituir aqueles com elevada toxicidade, principalmente por não apresentarem toxicidade aguda em mamíferos, bem como não acumularem nos tecidos (ZHU et al., 2020). Derivados do ácido crisântemo, os piretroides ganharam usos além do agrícola no controle de pragas de lavoura, mas também pecuário e veterinário para o controle de ectoparasitas em animais de produção e domésticos. Outro uso é o domisanitário, no controle de pragas domésticas e em dedetizações de cidades enfestadas (JOKANOVIĆ, 2018).

Hoje, existem diversas formulações de piretroides para o uso doméstico, e tendo em vista o crescente aumento no número de doenças transmitidas por insetos hematófagos como a dengue, Chikunginha, zika e febre amarela (MINISTÉRIO DA SAÚDE, 2022), destaca-se o aumento do uso de inseticidas para controlar os vetores das doenças supracitadas. Desta forma, a exposição prolongada e aumentada aos piretroides tem despertado preocupações, uma vez que exposições a doses baixas por períodos prolongadas ainda não possuem seus efeitos toxicológicos bem esclarecidos (CHEN et al., 2020).

2.2. DOENÇA DE HUNTINGTON

O envelhecimento precoce é uma das vertentes da medicina que deve receber uma atenção especial. Com os avanços da tecnologia, os seres humanos estão vivendo cada vez mais, desta forma, doenças que acometem o sistema nervoso central e causam neurodegeneração devem receber extrema importância (HECHT; HODSHON, 2018). Dentre essas doenças, destacamos a Doença de Huntington (DH) que é uma afecção neurodegenerativas de caráter hereditário do sistema nervoso central, cujos sintomas são causados pela perda marcante de células cerebrais dos gânglios da base (MOHAPEL; REGO, 2011). Os principais efeitos são danos motores e cognitivos que atingem igualmente ambos os gêneros, no qual, os primeiros sintomas aparecem lenta e

gradualmente entre os 30 e 50 anos (BATES et al., 2015). As razões moleculares para o aparecimento da DH estão relacionadas com a expressão de uma forma mutante da huntingtina que acaba por sofrer alterações no seu dobramento causando a agregação (MARTELLI, 2014). A huntingtina normalmente está relacionada com o transporte intracelular (BARSOTTINI, 2007). A huntingtina mutante é mais resistente a degradação proteica e é causada por diversas repetições de nucleotídeos CAG (glutamina), sua formação causa depósito e degradação axonal formando o que é chamado de poliglutamina (PolyQ40) (SHANNON, 2004). O número de repetições CAG considerado normal situa-se entre 9 e 34, enquanto na DH o número de repetições é geralmente maior que 40 (BARSOTTINI, 2007). Apesar dos primeiros estudos terem focalizado na forma expandida (mutada) da huntingtina, cada vez mais dados propõem a perda de função da forma normal (*wild-type*) desta proteína como possível explicação para alguns aspectos da DH (MOHAPEL; REGO, 2011)

2.3. SISTEMA NERVOSO COLINÉRGICO

A sinalização colinérgica está envolvida na cognição, comportamento, raciocínio e aprendizado. O neurotransmissor do sistema nervoso parassimpático é a acetilcolina (ACh) e agonista de receptores muscarínicos e nicotínicos (SARTER; LUSTIG, 2020). Na fenda sináptica a ACh é clivada pela enzima acetilcolinesterase (AChE) em acetato e colina. A AChE é alvo de diversos compostos que possuem ação colinérgica neurotóxica pois acabam por inibir a atividade desta enzima a qual, então, não interrompe a sinalização causando morte por hiperexcitabilidade do sistema nervoso central (DISNEY; HIGLEY, 2020). Assim como na doença de Huntington, no Alzheimer a perda nervosa é acentuada, principalmente pela agregação de isoformas pouco solúveis da proteína beta-amilóide (βA) (KAIZER et al., 2008). Compostos como os piretroides não possuem ação conhecida direta sobre a AChE ou mesmo sobre a proteína βA, mas podem estar relacionadas tanto com condições de declínio cognitivo ou até mesmo com condições neurodegenerativas ainda pouco elucidadas.

2.4. SISTEMA ANTIOXIDANTE

O estresse oxidativo (EO) é caracterizado por um desbalanço entre moléculas oxidantes como as espécies reativas de oxigênio (EROs) e os compostos e/ou enzimas

antioxidantes. A produção de EROs ocorre naturalmente no organismo, mas devido, a superprodução das mesmas e/ou deficiência dos sistemas antioxidantes enzimáticos e não-enzimáticos elas acabam por causar danos aos componentes celulares, levando pôr fim a quadros oxidativos severos e morte celular. As principais enzimas antioxidantes que atuam na primeira linha de defesa contra as EROs são as enzimas superóxido dismutase (SOD) e catalase (CAT), que atuam reduzindo sequencialmente o ânion superóxido (O_2^-) à peróxido de hidrogênio (H_2O_2) e, por fim, à água e oxigênio (TAN et al., 2018). Outras vias de detoxificação de compostos xenobióticos tóxicos são encontradas e auxiliam na redução da produção de EROs, como a enzima Glutationa-S-transferase (GST), que insere grupamentos de glutationa em comopostos apolares aumentando assim a sua hidrossolubilidade facilitando a excreção (DAI; BUI; LODGE, 2021). Além das principais enzimas antioxidantes, os organismos apresentam ainda proteínas que auxiliam na redução do estresse, chamadas de chaperonas ou proteínas de estresse térmico (HSP - *heat shock protein*). As HSPs são transcritas em diversas células e possuem diversos intuitos, mas principalmente protegem os componentes celulares proteicos e lipídicos do dano oxidativo, térmico e xenobiótico (SURAI et al., 2019).

2.5. MODELOS DE AVAIAÇÃO TOXICOLÓGICA ANIMAL: *C. elegans*

Os *Caenorhabditis elegans* são nematoides de solo de vida livre amplamente utilizados em ensaios bioquímicos, toxicológicos e farmacológicos (BHAGAT; NISHIMURA; SHIMADA, 2021). Dentre as principais características positivas da utilização dos *C. elegans* na pesquisa destacam-se o baixo custo de utilização quando comparado com outros organismos modelo, alto índice translacional genético com o ser humano, facilidade de manuseio, rápido desenvolvimento e a transparência da cutícula (ASCHNER et al., 2017). Juntos, todas estas características garantem a este simples organismo vivo um potencial sem tamanho no entendimento de exposições a xenobióticos, dando uma clara noção das respostas fisiológicas intrínsecas ao organismo animal (TAMAGNO et al, 2022c).

Alguns biomarcadores fisiológicos são ainda mais interessantes de serem avaliados em *C. elegans*, devido terem sido alterados de maneira transgênica (GIRARD et al., 2007). Um bom exemplo é a expressão de proteínas humanas responsáveis por patologias distintas como a PolyQ40. Isso significa que, *C. elegans* selvagens (*wild type*) não expressam isoformas de PolyQ40, mas em *C. elegans* mutantes esta e muitas outras

proteínas foram inseridas agrupadas à um grupo fluorescente, o que facilita a localização e quantificação de taxas de agregação proteica nestes indivíduos (THABIT et al., 2018). Outros exemplos de inserções fluorescentes estão relacionados com proteínas já expressas em *C. elegans*, como é o caso das enzimas antioxidantes, SOD, CAT e GST, que possuem uma inserção de proteína fluorescente verde (GFP) e que quando expressas podem ser quantificadas (ISHIKADO et al., 2013).

Outra questão importante sobre as avaliações toxicológicas em *C. elegans* é em relação ao seu repertório comportamental que podem ser facilmente alterados por questões fisiológicas intrínsecas ao sistema nervoso central (TAMAGNO et al., 2022a) ou sistema antioxidante (TAMAGNO et al., 2022b) por exemplo. Desta forma, detectar alterações comportamentais é a base para o estabelecimento das alterações de raízes fisiológicas em *C. elegans*.

3. OBJETIVOS

3.1. OBJETIVO GERAL

- Avaliar o efeito toxicológico de inseticidas domésticos e agropecuários a base de piretroides utilizados no comportamento e metabolismo de *Caenorhabditis elegans*.

3.2. OBJETIVOS ESPECÍFICOS

- Avaliar o efeito transgeracional, neonatal e crônico do inseticida doméstico a base de praletrina sobre o comportamento, fisiologia e expressão de proteínas relacionadas com a doença de Huntington em *Caenorhabditis elegans*.
- Avaliar o efeito transgeracional, neonatal e crônico do inseticida doméstico a base de transflutrina sobre o comportamento, fisiologia e expressão de proteínas relacionadas com a doença de Huntington em *Caenorhabditis elegans*.
- Avaliar o efeito transgeracional, neonatal e crônico de inseticidas agropecuários a base de λ-cialotrina e cipermetrina sobre o comportamento, fisiologia e expressão de proteínas relacionadas com a doença de Huntington em *Caenorhabditis elegans*.

4. DESENVOLVIMENTO

Os resultados obtidos estão dispostos em três capítulos, organizados no modelo de artigos científicos, redigidos em inglês e seguindo as normas recomendadas pelas revistas científicas específicas as quais estão ou serão submetidos para publicação.

No Capítulo 1, apresento o artigo 1, intitulado *"Household prallethrin-based insecticide toxicity on different C. elegans life stage: a possible sign of Huntington Disease"*, já publicado na revista científica *Environmental Pollution* em 25 de setembro de 2022, sobre o DOI: https://doi.org/10.1016/j.envpol.2022.120301. (Qualis A1 nas ciências biológicas II, fator de impacto 9.988).

E em sequência, o capítulo 2 que apresenta o artigo 2, intitulado *"Transfluthrin-based household effects of used and under-used doses on Caenorhabditis elegans metabolism"*, submetido em 07 de novembro de 2022 para a revista científica *Toxicology and Applyed Pharmacology* (Qualis A1 nas ciências biológicas II, fator de impacto 4.46). Situação em 28/11/2022: *under review* (ANEXO 01).

Por fim, o capítulo 3, apresenta o artigo 3 intitulado *"Pyrethroid-based insecticides on Caenorhabditis elegans exert transgenerational, persistent, and chronic physiological, behavioral, and neurodegenerative damage"*, submetido no dia 14 de novembro de 2022 para a revista científica *Environmental Toxicology and Pharmacology* (A1 nas ciências biológicas II, fator de impacto 5.785). Situação em 28/11/2022: *under review* (ANEXO 02).

4.1 CAPÍTULO 1 - HOUSEHOLD PRALLETHRIN-BASED INSECTICIDE TOXICITY ON DIFFERENT *C. elegans* LIFE STAGE: A POSSIBLE SIGN OF HUNTINGTON DISEASE

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Household prallethrin-based insecticide toxicity on different *C. elegans* life stage: A possible sign of Huntington Disease☆

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ABSTRACT

Household insecticide is largely used for insect and ectoparasite control, in city centers as well as in the countryside. The pyrethroids are the most used class of insecticide, these compounds in low doses have low toxicity for mammalians, in comparison to other compounds, with insecticide effects. The contact of these compounds in sublethal doses begins in early life and many cases, in intrauterine life. Considerable diseases still with undefined etiology, such as neurodegenerative conditions, and Huntington's Disease (HD) is one of them. HD is related to overexpression of Polyglutamine (PolyQ40), its aggregation, and non-solubilization, which leads to neural, behavioral, and cognitive damage. In our study, we evaluate the effect of two sublethal doses of a prallethrin-based insecticide (P-BI), in three different *Caenorhabditis elegans* life stages transgenerational, neonatal, and lifespan. We evaluated the Body bends and pharyngeal pumping rate, and social feeding as behavioral biomarkers. As well as acetylcholinesterase activity (AChE), PolyQ40 aggregation, antioxidant enzymes, and heat shock protein (HSP) expression. We observe that the toxic effect of P-BI is more pronounced on transgenerational and lifespan exposure. Both sublethal doses of P-BI decreased the AChE activity and retard the HSP expression as well as increased the PolyQ40 aggregates indicating a clear biomarker for possible effect in the progression of the HD, by the environmental contamination.

1. Introduction

The use of household insecticides has been increasing nowadays. This increase is due to the rise in the number of cases of zoonosis transmitted by hematophagous insects (van Balen et al., 2012). In Brazil, in 2022 the dengue cases increased accounting for approximately 113% in comparison to other years (Shimura and Nazarethdos, 2022). In this way, the use of insecticides becomes essential to living safely, especially in urban centers.

Among the main compounds used in the formulation of insecticides, there are pyrethroids. Pyrethroids have a wide advantage over other insecticides, such as low toxicity to mammals (Chrustek et al., 2018). Among the pyrethroids used in formulations to control insects and ectoparasites, prallethrin (Supplementary Fig. 1) ((S)-2-methyl-4-oxo-3-prop-2-ynylcyclopent-2-enyl(1R)-cis-trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropa-nencarboxylate) is a modified cyclopentadienone compound pyrethroid (Sarkar et al., 2018). Prallethrin has its mechanism of action related to the opening of sodium channels in nerve cells. The entry of Na^+ depolarizes the nerve bundles that release high concentrations of neurotransmitters into the synaptic cleft, overexciting the central nervous system (CNS).

One of the main neurotransmitters involved in cognition, learning, movement, and behavior is acetylcholine (ACh), which acts mainly on cholinergic nerve fibers and neuromuscular junctions (Kaizer et al., 2008). ACh is released through nerve depolarization and, by agonizing muscarinic and nicotinic receptors in the CNS, can transmit the nerve signal. The intense release of ACh leads to massive cholinergic activity

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Abbreviations

HD	Huntington disease
AChE	Acetylcholinesterase
CTL	Catalase
SOD	Superoxide dismutase
GST	Glutathione-S-transferase
GFP	Green fluorescent protein
P-BI	Prallethrin-based insecticide
TG	Transgenerational exposure
LS	Life span exposure
NN	Neonatal exposure
RCD	Relative calculated dose
RCHD	Relative calculated half dose
CNS	Central nervous system
ACh	Acetylcholine
PolyQ	Poly glutamine
NGM	Nematode growth medium
HSP	Heat shock protein
htt	huntingtin gene

that leads to the death of individuals. For ACh to be removed from the synaptic cleft, the enzyme acetylcholinesterase (AChE) catalyzes ACh into acetate and choline. Both are recaptured by the presynaptic terminal, thus ending the effect of ACh at the cholinergic terminal (Mathew et al., 2019).

The pharmacodynamic target of pyrethroids is not AChE, but studies report that their effect is not limited to the high discharge of neurotransmitters by nervous excitation, but also the decrease in the withdrawal of ACh from the synaptic cleft (Mužinić and Želježić, 2018). This effect may not be so pronounced, but it is believed that in neurodegenerative diseases it may be linked to their progression. Another important thing is that oxidative stress is related to accelerating the neurodegenerative process and many enzymes are affected by pesticide contact (Han et al., 2017).

Among the neurodegenerative diseases, Huntington's Disease (HD) stands out, characterized by the aggregation of poorly soluble isoforms of proteins derived from huntingtin, and polyglutamine (poly Q) expansions as a basis for cellular toxicity (Morley et al., 2002). This aggregation occurs in the body's smooth muscles, resulting in loss of motility, as well as muscle tone. The causes of HD are still poorly understood, and genetic, inflammatory, and environmental causes seem to be more related to these conditions of establishment and progression. What seems to be closely related to the progression of HD are high levels of free radicals, which end up oxidizing the body and progressing even faster in degenerative conditions (Sumathi et al., 2018).

Contamination by toxic compounds can be harmful to individuals, even when they are still in intrauterine development. Embryonic contaminations may be related to genetic mechanisms of transgenerational transmission, where their effects can be noticed late in adult life. The soil worm *Caenorhabditis elegans* is a model widely used for biochemical and pharmacological assays. Due to its high genetic similarity to human diseases, and the facility for maintenance, cultivation, and evaluation (Girard et al., 2007; Tamagno et al., 2021).

In this work, we evaluated the effects of the household pyrethroid prallethrin-based insecticide (P-BI), on the metabolism of *C. elegans* in three different life periods, transgenerational, neonatal, and, lifespan; observing the effects of behavioral, neurotoxicity and antioxidant.

2. Material and methods

2.1. Study strategy

To determine whether the household P-BI could affect biochemical and behavioral biomarkers in different periods of life of *C. elegans*, we carried out three protocols of exposure in the worms in three different life stages: transgenerational (TG), neonatal (NN), and lifespan (LS).

2.2. Exposure

For transgenerational (TG) exposure (Fig. 1), the worms were placed into contact with the pyrethroid before developing internal eggs and after developing the vulva (L4 life stage). They remained in contact with the toxicant during the oogenesis period (20 h). When the worms were at the with-eggs-adults stage they were synchronized, the P-BI was completed removed, and the eggs resulting were left to hatch in M9 buffer for 15 h until total hatching. When the F1 was at the L1 stage they were placed in NGM with *E. coli* (OP50) as a source of food until reached the L4 stage when were behavior and biochemical evaluated. The experiment is divided according to Fig. 1.

For neo-natal (NN) exposure (Fig. 1), the synchronized eggs (from non-exposed parents) were kept in contact with the pyrethroid in M9 buffer until they were completely hatched (20 h). After hatching, the neo-natal-L1 worms were washed for P-BI completely removal and placed in NGM with *E. coli* (OP50) as a source of food until reached the L4 stage when were behavior and biochemical evaluated.

For lifespan (LS) exposure (Fig. 1), the non-exposed worms were synchronized and kept just in the M9 buffer until reached the L1 stage. Then, they were placed in NGM with *E. coli* (OP50) and were kept in contact with the P-BI during their whole life with re-exposition every day (total time exposure of 48 h). When the worms reach the L4 stage, they were washed for total P-BI removal, and all the biochemical and behavioral biomarkers were evaluated.

2.3. Relative doses determination

Three groups were established for each exposure being, control (water), and relative calculated dose (RCD) (0.012 mg) and relative calculated half-dose (RCHD) (0.006 mg) of P-BI (commercial formulation). The used household insecticide was the SC Jhonson - Raid® electronic (Manaus, AM, Brazil) composed of 21.9 mL of prallethrin (1.6%) butylhydroxytoluene and vehicle. This insecticide is indicated for electric household use during the night or day 8 h per day. The indicated total duration of the product is 30 nights and the best effect is in a room with no more than 20 m^2 of area.

The relative calculated dose (RCD) and relative calculated half-dose (RCHD) were determined by calculating the total volume of the Raid® solution considering the number of nights (keeping turned on 8 h. $night^{-1}$) and the area of the room, relativizing the agar plate area (0.006 m^2) where the worms were exposed as following equation (1).

$$Relative\ concentration = \frac{((V \cdot C)/N)}{A} * pA \quad (1)$$

V = Total volume of the Raid® (mL).

C = Concentration of the active principle (prallethrin) on liquid (mg. mL^{-1}).

N = Number of nights indicated for use.

A = Area of the room (m^2).

Pa = Plate area.

2.4. Behavioral evaluations

2.4.1. Body bends and pharyngeal pumping

Twenty-four exposed worms (n = 24) in each specific group of treatment at the L4 life stage had the body bends rate and pharyngeal

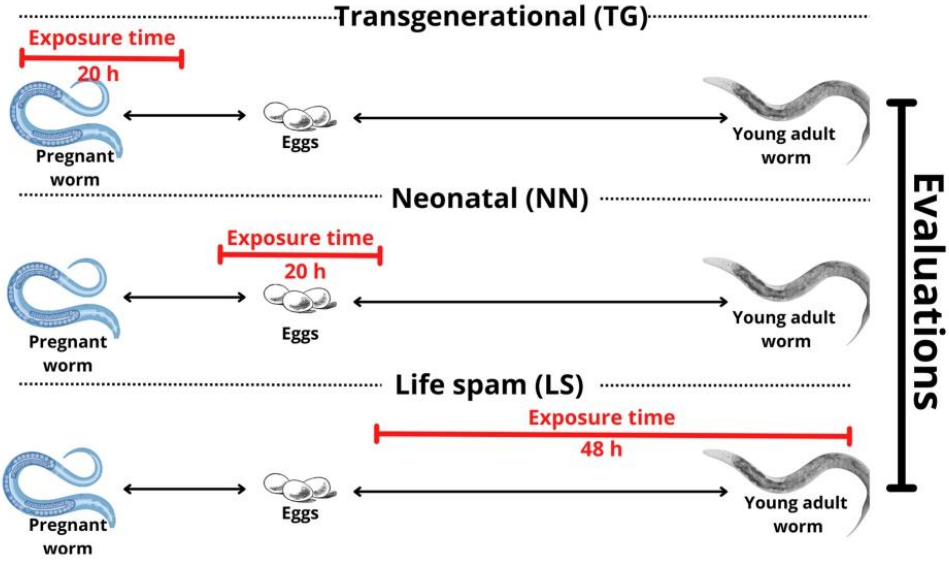

Fig. 1. Study strategy to the use of household insecticide in different period of life in C. elegans for toxicological evaluations. Image created by the author using Canva.pro/edu.

pumping evaluated as described by Tsalik and Hobert (2003) and Wang et al. (2008) respectively.

2.4.2. Feeding behavior

Borderline and agglomeration behaviors were quantified simultaneously as described previously by De Bono and Bargmann (1998); Gray et al. (2004); and Jang et al. (2017). Were used NGM plates previously prepared (48 h before) with OP50 *E. coli* just in the center of the plate. At the time of the analysis, one hundred (100) exposed worms were collected and placed in this central *E. coli* and they were left to acclimatize for 3 h at 20 °C. After 3 h, crowding behavior was measured by calculating the fraction of animals that were in contact with two or more other animals along at least 50% of the body length. At the same time, borderline behavior was measured by calculating the fraction of animals residing 2 mm from the edge of the bacterial turf. The result was expressed as the total animal eating together and total animals eating on the edge.

2.5. Biochemical evaluations

2.5.1. Acetylcholinesterase activity (AChE)

For AChE activity were used ten thousand (10,000) exposed worms as described in section 2.2 in a final volume of 1.5 mL of M9 buffer per pool, were prepared in 6 pools per concentration. The worms were sonicated and centrifuged according to Tamagno et al. (2021) and the supernatant was collected for protein determination using serum bovine as a pattern (Bradford, 1976). AChE activity was determined according to Cole et al. (2004). The spectrophotometric reading was carried out in quadruplicate per pool (n = 24). The final activity is expressed in μmol of ACh.h^{-1} mg of protein^{-1}.

2.5.2. Protein quantification by green fluorescent protein expression

The antioxidant enzymes superoxide dismutase (SOD), catalase (CTL), glutathione-S-transferase (GST), and heat shock protein 16.2 (HSP) were quantified in live transgenic worms (Tambara et al., 2020). For this, the transgenic strains of CF1553 [(muIs84 [(pAD76) sod-3p: GFP + rol-6(su1006)])] for *sod-3::GFP* expression, GA800 [(wuIs151 contains [ctl-1 + ctl-2 + ctl-3 + myo-2:GFP])] for *ctl-1,2,3::GFP* expressions, CL2166 [(dvIs19 [(pAF15)gst-4p:GFP:NLS])] for *gst-4::GFP* expression, and CL2070 [(dvIs70 [hsp-16.2p:GFP + rol-6(su1006)])] for *hsp-16.2::GFP* expression, were exposed as described in section 2.2. The fluorescent expression was determined using a microplate reader with 1000 worms-each well (well final volume 300 μL), ten wells were read per repeat, and all the exposures were carried out in triplicate (n = 30). The fluorescence was observed under an excitation of 485 nm and an emission of 530 nm (Tambara et al., 2020). The final result is expressed in fluorescence intensity.

2.6. PolyQ40 aggregates quantification

Fifty (50) exposed worms of the AM141 *(rmIs133[*unc-54p*::Q40::YFP])* strain were mounted on a glass slide with a drop of 10 mM sodium azide for paralysis. The slide was immediately subjected to fluorescence microscopy (λEx: 480/20 nm; λEm: 510/38 nm with 10X magnification) to quantify the number of *polyQ40::YFP* aggregates in the body wall muscle (Peixoto et al., 2016). The aggregates of 20 worms were quantified per slide, three sides were prepared by repeat and all the exposures were carried out in triplicate (n = 180). Results are presented a number of PolyQ40 aggregates.

2.7. Statistical analyzes

Data of three replicates were analyzed using one-way ANOVA followed by a post hoc of Tukey's test, or a Kruskal–Wallis's test followed by a post hoc Dunn's test, depending on the normality of the data (assessed by the Kolmogorov–Smirnov test).

3. Results

3.1. Behavioral evaluations

3.1.1. Body bends

The body bends rate in the transgenerational (TG) exposure (Fig. 2, A) was increased in the group treated with the relative calculated dose

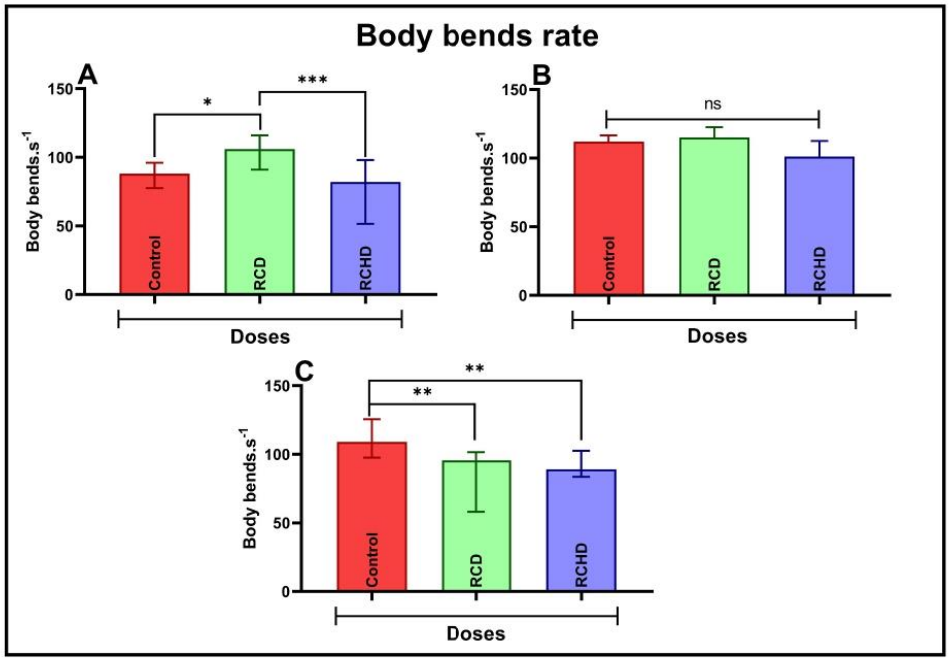

Fig. 2. Effect of indicated and half doses of P-BI in *Caenorhabditis elegans* body bends rate. Different times of exposure of transgenerational (A), neonatal (B), and lifespan (C). Data are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.0001$.

(RCD) in comparison to control ($p < 0.05$) and relative calculated half dose (RCHD) ($p < 0.001$). In the neonatal (NN) exposure (Fig. 2, B) no changes were observed. In the lifespan (LP) exposure (Fig. 2, C) the body bends rate was decreased in the groups RCD ($p < 0.005$) and RCHD ($p < 0.001$) in comparison to the control group.

3.1.2. Pharyngeal pumping rate

Pharyngeal pumping rate, in the TG exposition (Fig. 3, A), was decreased in the group treated with the RCD in comparison to control ($p < 0.0001$) and RCHD ($p < 0.0001$), still the group treated with RCD was increased in comparison to control group ($p < 0.0001$). The NN exposure (Fig. 3, B) does not show any alteration. In the LS exposition (Fig. 3, C), the pharyngeal pumping was decreased in two groups treated with P-BI in the RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) doses in comparison to the control group.

3.1.3. Social behavioral related with feeding

The social behavior related to feeding in the TG exposure (Fig. 4, A1), was decreased in the groups treated with RCD ($p < 0.01$) and RCHD ($p < 0.0001$) in comparison to the control group, still the RCHD decreased in comparison to RCD ($p < 0.005$). The NN exposition (Fig. 4, A2) was not changed in any treatment. In the LS exposition (Fig. 4, A3) the worms ate less together in the RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) in comparison to the control group.

While the position of feeding in the TG exposition (Fig. 4, B1), the worms ate less in the edge in the group treated with RCD in comparison to control ($p < 0.001$) and RCHD ($p < 0.01$). In the NN exposition (Fig. 4, B2), the worms remain eaten less in the edge in the groups treated with RCD ($p < 0.05$) and RCHD ($p < 0.05$) in comparison to the control group. In the LS exposure (Fig. 4, B3), the worms remain eaten less in the border in the group treated with RCHD in comparison to control ($p < 0.05$) and RCD ($p < 0.001$).

3.2. Biochemical evaluations

3.2.1. Acetylcholinesterase activity

AChE activity, in the TG exposure (Fig. 5, A), was decreased in the group treated with RCHD in comparison to control ($p < 0.01$) and RCD ($p < 0.05$). In the NN exposition (Fig. 5, B) no alteration was observed. During the LS exposure (Fig. 5, C), AChE activity was decreased in the groups treated with RCHD ($p < 0.01$) in comparison to the control group.

3.2.2. Quantification of the PolyQ40 aggregates

The total fluorescent PolyQ40 aggregates in the whole body of *C. elegans* in the TG exposure (Fig. 6, A) was increased in the group RCD in comparison to control ($p < 0.001$) and RCHD ($p < 0.0001$). The NN exposure (Fig. 6, B) does not change in none exposure group. The number of aggregates in the LS exposition (Fig. 6, C) was increased in the group RCD in comparison to the control group ($p < 0.0001$) and RCHD ($p < 0.0001$).

3.2.3. Antioxidant system

SOD fluorescent expression in the TG exposition (Fig. 7, A1) was increased in the group treated with RCD in comparison to the control ($p < 0.0001$) and RCHD ($p < 0.0001$). In the NN exposure (Fig. 7, A2), the RCD was decreased in comparison to control ($p < 0.01$) and RCHD ($p < 0.0001$), as well as the RCHD, was increased ($p < 0.0001$) in comparison to the control group. In the LS exposition (Fig. 7, A3), the SOD activity was increased in the group treated with RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) in comparison to the control, as well as increased in the group RCHD ($p < 0.01$) in comparison to RCD.

GST fluorescent expression in TG exposition (Fig. 7, B1) was decreased in the group treated with RCD in comparison to control ($p < 0.0001$) and RCHD ($p < 0.0001$). In the NN exposure (Fig. 7, B2) no

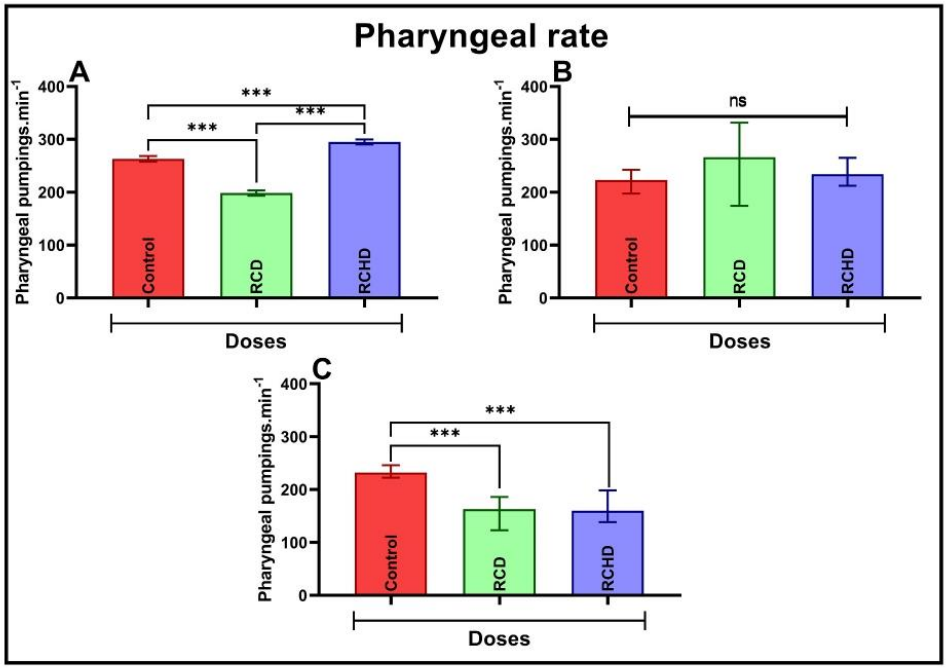

Fig. 3. – Effect of indicated and half doses of P-BI in *Caenorhabditis elegans* pharyngeal pumping rate. Different times of exposure of transgenerational (A), neonatal (B), and lifespam (C). Data in panel A is expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. Data in panel B and C are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. ***$p < 0.0001$.

change was observed. In the LS exposure (Fig. 7, B3), both groups RCD and RCHD decreased in comparison to the control group ($p < 0.0001$, $p < 0.0001$ respectively).

CTL fluorescent expression in the TG exposure (Fig. 7, C1) was decreased in the group treated with RCHD in comparison to the control ($p < 0.01$) and RCD ($p < 0.0001$). In the NN exposition (Fig. 7, C2) no alteration was observed among the exposed groups. In the LS exposure (Fig. 7, C3) CTL was increased in the RCHD group in comparison to the control ($p < 0.0005$) and RCHD ($p < 0.0001$) and decreased in the group treated with RCHD in comparison to the control group ($p < 0.01$).

HSP 16.2 fluorescent expression in TG exposition (Fig. 7, D1) was decreased in the RCD ($p < 0.0001$) and increased in the RCHD ($p < 0.05$) in comparison to control group. The NN exposition (Fig. 7, D2) was increased in the RCD group in comparison to control ($p < 0.0001$) and RCHD ($p < 0.0001$) as well as increased in the group treated with RCD ($p < 0.05$) in comparison to RCHD. The LS exposition (Fig. 7, D3) had the HSP identification decreased in the group RCHD in comparison to control ($p < 0.0001$) and RCD ($p < 0.0001$) as well as the RCD increased in comparison to control ($p < 0.05$).

4. Discussion

Here we show the effect of household P-BI in different life stages of *C. elegans.*, changing the behavioral parameters evaluated, when *C. elegans* and its progenitors are exposed, as well as when adult worms are chronically exposed (life span) to P-BI. These same exposition protocols increase the cholinergic signaling to critical levels and increase the PolyQ40 aggregates, which might be related to Huntington's Disease (HD) establishment and progression.

The effects in transgenerational (TG) and life span (LS) expositions, might suggest a possible sign of a deleterious effect of exposed progenitors during the reproductive period. On neonatal (NN) exposition effect was less pronounced than the other expositions, suggesting an increased resistance of *C. elegans* to toxicants in early life.

Regarding behavior, the body bends increased in the RCD, pharyngeal pumping decreased in RCD, and increased in RCHD in the TN group. In another way, observed decreased body bends and pharyngeal bumping in the LS group led to conclude, that the effect of P-BI is related to the organism absorption of the compound. In addition, some mechanisms of parental transference or embryonic malformation could be related to these alterations observed on TG and LS expositions.

In the TN as well as LS groups, the P-BI remains in the plate with the bacterial food, and thus, the worms eat and absorb an increased amount of the compound. Differently, in the NN protocol, P-BI remains in the water, when the worm is protected by the egg, and does not eat the compound, until birth. The egg of the worm is resistant and can protect the worm to absorb the substances in the environment they were.

Studies with prallethrin are scarce, especially regarding the evaluation of prallethrin effects in *C. elegans*. In previous studies with pyrethroids, bifenthrin was a significant acceleration of the hatching process, and morphological impairments in zebrafish embryos and larvae (Jin et al., 2009; Yang et al., 2018). In invertebrates, pyrethroid exposure in adult female honeybees causes a diminution in egg production as well developmental disorders in the next generation. Evidenced by higher egg weight, the lower success rate of egg development, and delayed hatch time of the eggs (Dai et al., 2010). In *C. elegans,* cypermethrin induced feeding inhibition (Shashikumar and Rajini, 2010). Here we show that the P-BI embryonic exposure in *C. elegans* is related to neurological alterations, as well as behavioral changes. AChE decreases and in mutant worms for HD an increase in the expression of PolyQ40.

We observe more alterations in the behavior of F1 of adult worms when exposed to P-BI than when the worms were exposed in early life.

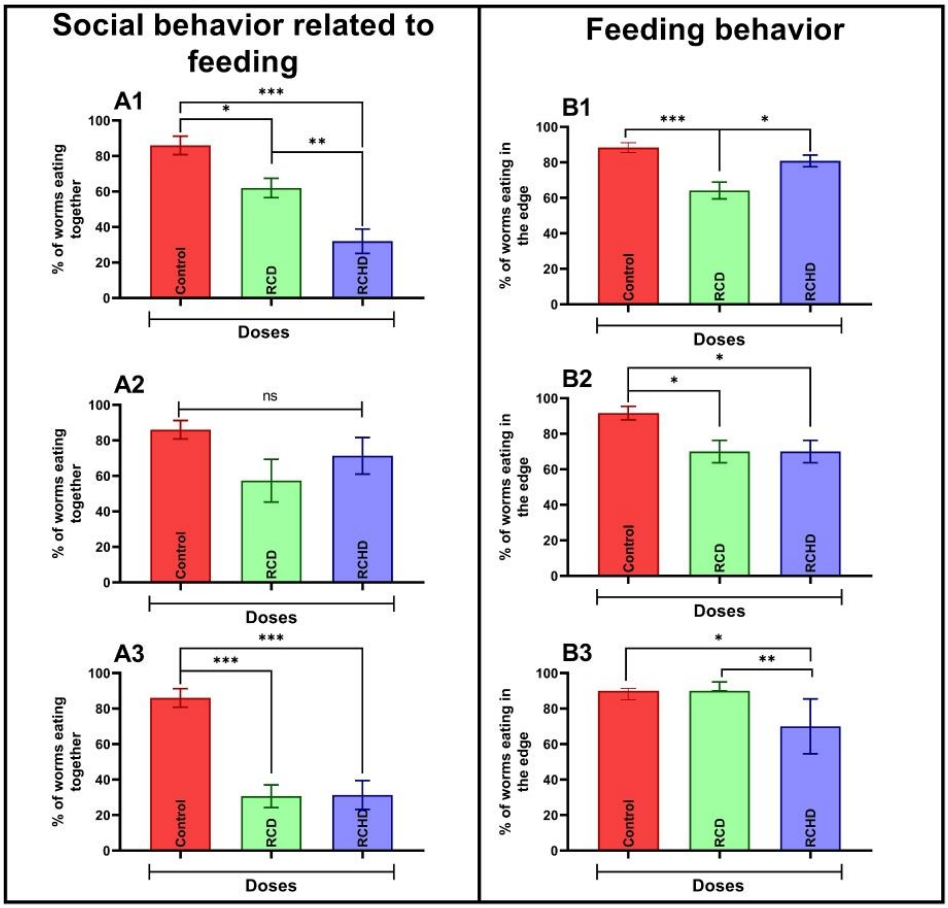

Fig. 4. - Effect of indicated and half doses of P-BI in *Caenorhabditis elegans* social behavior related to feeding (A) and feeding behavior (B) of different times of exposure of transgenerational (1), neonatal (2), and lifespam (3). Data in panel A1, A2, A3, B1, and B2 are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. Data in panel B3 is expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. *p < 0.05, **p < 0.01, and ***p < 0.001.

Social behavior related to the feeding and edge feeding was decreased in the TN and LS groups highlighting the same observed for other behaviors. Social feeding is related to calmodulin levels, which have an important role in social interaction while feeding in *C. elegans* due to regulating the expression of the calcineurin gene, and this is related to the increased risk of schizophrenia (Dwyer et al., 2015). In addition, dopamine is an important neurotransmitter related to social feeding, and that dopamine and calmodulin regulate the gap junction activity that is prominent in the neurons mediating social feeding (Zou et al., 2014). Taken together, the pronounced effect on social behavior and edge feeding are related to central nervous dopaminergic signaling quality (De Bono and Bargmann, 1998).

Another important signaling pathway that implies social feeding behavior is cholinergic signaling. In addition, both body bends and pharyngeal pumping behaviors are modulated by the cholinergic nervous system. In our study, the levels of AChE activity were decreased in the TG and LS exposure protocols. The enzyme AChE clivates the neurotransmitter acetylcholine (ACh) in the synaptic cleft into choline and acetate which are recaptured by the neuron (Parvaz et al., 2020). In the neurotoxicity framework, the AChE activity is inhibited and the ACh remains longer in the synaptic cleft activating the post-neuronal termination. In this situation occurs a cholinergic breakdown and leads in to organism's death.

Most the insecticides cause a neural breakdown in animals; so, why are not humans affected? The explanation is that the amount necessary to kill an insect is smaller than the necessary to affect a human. The question that remains, is whether even in low concentrations, the insecticide cannot affect the organism's physiology? The decrease in the AChE levels was not enough to kill *C. elegans*, but the changes in this system can be related to dementia, Alzheimer's disease, and other neurodegenerative diseases as well as Huntington's. The observed for AChE on TG might be related to the down-regulation of the gene *ache* (encodes AChE) on F1 by pyrethroids that could exert developmental toxicity by disrupting the cholinergic system (Wu et al., 2017). The reduction observed in the group LS might be related to the neurotoxicity of pyrethroids on the cholinergic nervous system due to continuous expositions during their whole life (Vu et al., 2020). Pyrethroids are not related to decreasing the AChE activity as the main action mechanism, but several studies describe that pyrethroid can leads an AChE decrease (Hasan et al., 2017; Saoudi et al., 2017; Simaremare et al., 2020) and

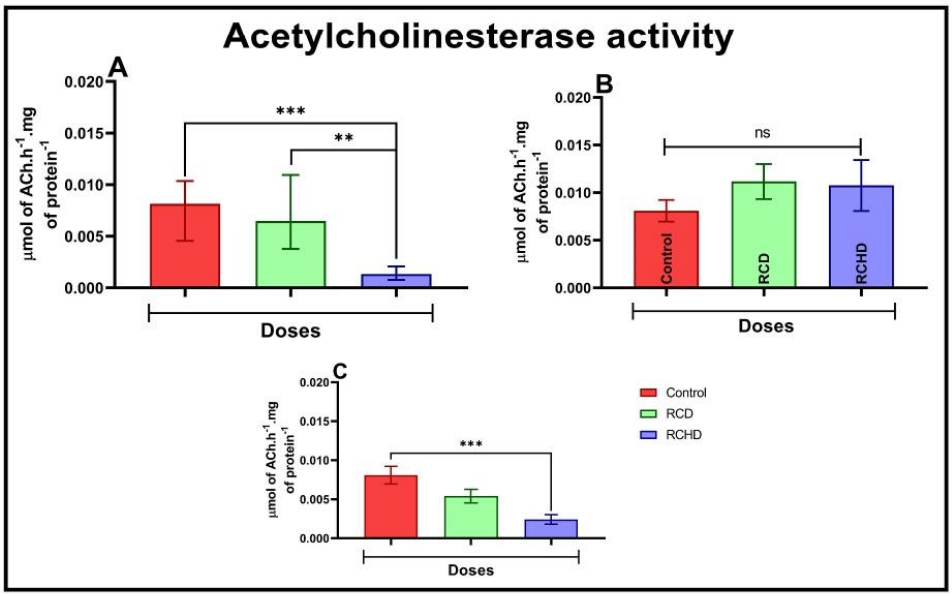

Fig. 5. - Effect of indicated and half doses of P-BI in *Caenorhabditis elegans* AChE activity of different times of exposure of transgenerational (A), neonatal (B), and lifespam (C). Data in panel B and C are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. Data in panel A is expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

this can potentiate the neurotoxic effect. In studies carried out with humans chronically exposed to prallethrin, a reduction in the AChE activity was observed (Narendra et al., 2008).

AChE reduction is related to neurodegeneration as well as Huntington's Disease (HD) (Manyam et al., 1990). HD is a neurodegenerative disease caused by a dominantly inherited CAG (glutamine encode) repeated expansion (PolyQ mode than 40) in exon 1 for the huntingtin gene (*htt*). In addition, the environmental toxicants are related to CAG overexpression or non-solubilization and removal from neurons (Fig. 8). HD is characterized by progressive involuntary choreiform movements, behavioral and psychiatric disturbances, and dementia1 (Tabrizi et al., 2020). Thus, in our study, we observed PolyQ40 aggregates on LS and TG exposures. Here, the number of muscular aggregates was increased in mutant worms.

In Fig. 7 we observe the pathway for huntingtin expression. All three represented pathways start with the expression of the *htt* gene, if the pathway does not have high expression of CAG (glutamine) the protein formed will not have the ability to aggregate. The aggregation is represented by two other pathways, genetics, when due to heredity the individual overexpresses the CAG termination or when overexpression occurs through environmental contact with harmful compounds, and both end up generating aggregatable groups of Poly-glutamine (PolyQ).

The decrease in cholinergic signaling, as well as the increase on PolyQ40 aggregates, are linked to neurodegenerative disease establishments and progression. Polyglutamine expansion (poly Q) diseases comprise several neurodegenerative or neuromuscular disorders such as Huntington's disease (HD) and spinocerebellar ataxia (Webster et al., 2019). These aggregations are linked to the expansion of CAG (cytosine-adenine-guanine) triplets in the coding region of seemingly unrelated genes encoding proteins with expanded glutamine stretches that are prone to aggregate (Markaki and Tavernarakis, 2020). An expansion of glutamine repeats beyond a critical length of Q40 results in aggregate formation and cellular dysfunction. Stress to toxicants such as pyrethroids can lead to a high expression of PolyQ with large chains (Q35 to Q40) and which have difficult solubility (Kishimoto et al., 2017), even in the reproductive period, these aggregates can be transmitted to the embryo as a form of protection and initiate aggregation on adulthood (Takamatsu et al., 2019). In our study, we indicate increased aggregation of PolyQ40 in worms exposed in the LS period, and this highlights the overexpression of Polyglutamyl aggregatable forms (PolyQ40) under stressful contact with P-BI. Taken together, the reduced AChE activity and increased PolyQ40 aggregation is related to neurotoxicity and neurodegeneration by P-BI exposition. In addition, there is a relationship between the PolyQ expression, and AChE disbalance with Alzheimer's disease as well as with schizophrenia, both neurological conditions (Takamatsu et al., 2019).

Another important biomarker of the homeostatic imbalance is oxidative status. The antioxidant enzymes actuate in a line of defense to transform toxic and reactive compounds into less dangerous ones. Prallethrin is considered an oxidant compound because can destabilize the oxidative balance (Dalmolin et al., 2020). Pyrethroids and their esters, from cyanohydrins, decompose to cyanides and aldehydes. Cyanide ions are mainly converted to thiocyanate and CO_2. The aldehydes and other lipophilic conjugates may produce oxidative stress in pyrethroid toxicity (Narendra et al., 2008).

In this way after the production of the H_2O_2 by SOD activity, the catalase (CTL) transforms the reactive H_2O_2 in water (H_2O) and oxygen (O_2) that can be used for the organism (Dalmolin et al., 2020; James and Xu, 2012). CTL fluorescent expression was decreased in the RCHD group on TG exposition and in the RCD group on NN exposition, these reductions might be related to the decrease in gene expression for CTL, even in the group treated with RCHD on NN exposure the CTL expression was increased, highlighting the increased production of H_2O_2 by SOD. However, to transform all the H_2O_2 produced by increased SOD expression another metabolic route could be activated, the GPx (glutathione peroxidase) for example which eliminate different hydroperoxides such as H_2O_2 (Panday et al., 2020). The GST activity decreased when in contact with the pyrethroid is related to two different mechanisms. First, due to the binding affinity of pyrethroid insecticides to the catalytic site (Ribeiro et al., 2022). Here upon, the pyrethroids can

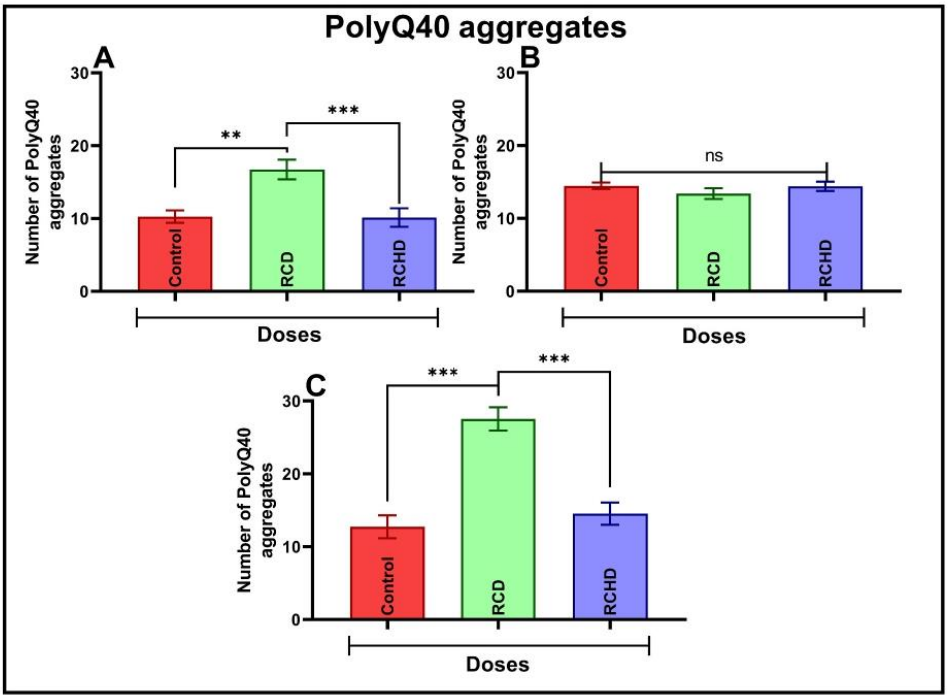

Fig. 6. - Effect of indicated and half doses of P-BI on PolyQ40 aggregates on AM141 transgenic strain of *Caenorhabditis elegans*. The worms were exposed in transgenerational (A), neonatal (B), and lifespam (C). Data are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. $^{**}p < 0.01$, and $^{***}p < 0.001$.

couple to the GST-A chain generating lower binding energy, the more favorable the interaction of the ligands with the amino acid residues of the active site of the target enzyme. In our study, we determine the fluoresce expression of the enzyme GST, here the second endpoint for decrease, in this way the observed reduction might be related to the downregulation of the expression of GST gene occurred by P-BI intoxication (Awoyemi et al., 2019). The decreased behavior was observed on TG and LS highlighting the dangerous effect of parental and continuous contamination.

Even like GST, was measured the SOD fluorescent expression in the mutant worm, and in this enzyme, the expression was increased in all exposures (TG, NN, and LS). Superoxide dismutase acts by reducing the superoxide radical ($O_2\bullet^-$), an important oxidant in the organism, to hydrogen peroxide (H_2O_2). Its activity, as its transcription, are modulated by the increase in the amount of $O_2\bullet^-$ and when increased highlight that had occurred an increase in reactive oxygen species (ROS).

Another observation about stress status in *C. elegans* is the HSP (*hsp-16.2*) expression. HSP induction is a response to proteotoxicity on the organisms, which can repair partly denatured proteins (Hallare et al., 2004). In addition, HSP is responsible for the re-establishment of proteostasis following stress exposure, and the prolonged overexpression of HSP is reported to be detrimental to cell growth and division (Chen et al., 2019). We observed that P-BI reduced the HSP expression against heat stress in TG and LS while on NN the expression was increased, these results are similar to those already found in the other parameters. In *C. elegans* was observed that other pyrethroids (permethrin) increase the time for HSP expression and consecutively retard the stress response. The increased time for HSP is due to the instability of the reporter gene product (Shashikumar and Rajini, 2010). In other studies, with sub-lethal doses of pesticides and fungicides, the HSP reporter gene is decreased and this is also related to HSP decrease (Gupta et al., 2005). Another important factor that directly effects on cell toxicity is the lipophilic characteristic of this compound (Supplementary Fig. 1) that allows it to easily pass through the plasma membrane and alter vital cellular function before interacting with cellular proteins, denaturing them and triggering the induction of stress proteins (Narendra et al., 2008).

In addition to the processes of genetic alteration, now is well recognized that misfolding and aggregation of specific proteins are associated with most of these diseases, but their role in aggravating the symptoms is not so well understood. The literature describes the intrinsic relationship of changes in complexes involved in energy balance, mainly related to mitochondrial complexes I and II. It is already known that pesticides can affect these complexes by interfering with the speed of aggregation of wrong protein forms causing neurotoxicity and neurodegeneration(Deshmukh et al., 2012; Dominah et al., 2017). In our study, we observed that the P-BI is then related to neurotoxicity and the reason for this might be due to mitochondrial alteration and oxidative dysfunction that interact with the protein folding process such as findings in mammalians, highlighting that even mammalians and *C. elegans* have similar physiology on HD development.

So, it is possible to observe that the P-BI are related to neurotoxicity, especially to the HD once that was observed AChE reduction, PolyQ40 aggregates increased, and retard HSP expression. Taken together, the change in biomarkers in the P-BI exposition, leads a warning for the development and use of less toxic compounds.

5. Conclusion

Here we show the dangerous effect of using household P-BI mainly

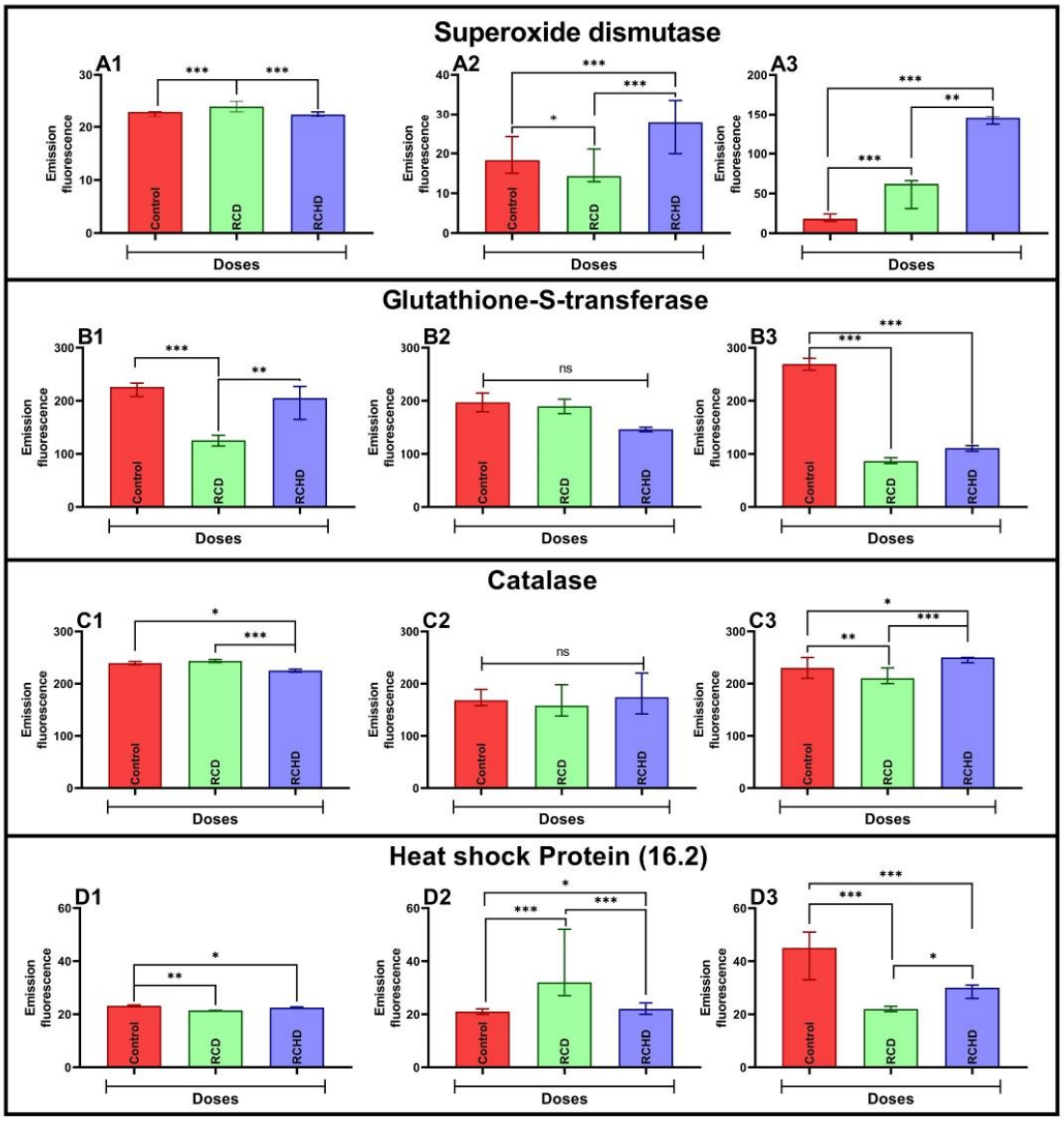

Fig. 7. - Effect of indicated and half doses of P-BI on SOD fluorescent expression (A) (strain CF1553), GST fluorescent expression (B) (strain CL2166), CTL fluorescent expression (C) (strain GA800), and HSP 16.2 fluorescent expression (D) (strain CL2070) of *Caenorhabditis elegans*. The worms were exposed in transgenerational (1), neonatal (2), and lifespam (3). Data in the panels A1, C1, and D1 are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. Data in panels A2, A3, B1, B2, B3, C2, C3, D2, and D3 are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. *p < 0.05, **p < 0.01, and ***p < 0.001.

during the uterine-embryological period of *C. elegans*. We also show that even contamination during embryological development is responsible for increasing the PolyQ40 aggregates in adult life. Even AChE is not a target mechanism P-BI was largely affected, including transgenerational effects. The retard on HSP expression might reduce the efficiency of mechanisms related to stress avoidance. We can, thus, relate these effects to HD appearance and progression. During early life, during eggs and the first larval stage, *C. elegans* show increased resistance to the toxicants. Our results lead to suggest that the increase of using household insecticide might be indicative of pathologies with unknown etiology.

Founding

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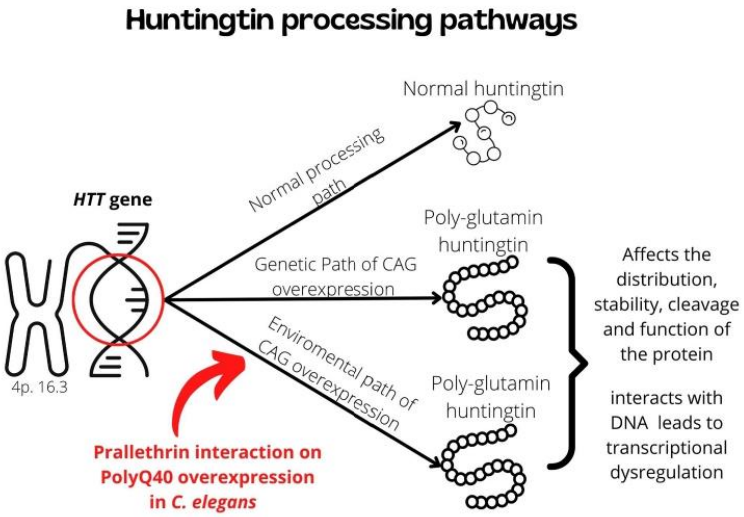

Fig. 8. Polyglutamine (PolyQ) processing pathways for nervous and behavioral effects. Source created by the author himself on canva.edu/.

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Ethical approval

This work was not carried out with vertebrate animals. *Caenorhabditis elegans* is a soil nematode and does not need any ethical approval to be studied.

Patient consent to participate

Not applicable.

Permission to reproduce material prim other sources

Not applicable.

Authors contributions

W A Tamagno: Conceptualization; Data curation; Formal analysis; Writing –original draft; Investigation; Project. **C Alves**: Methodology, Writing – review & editing; Data curation; Formal analysis. **A Pompermaier**: Methodology, Writing – review & editing; Data curation; Formal analysis. **A P Vanin**: Methodology. **Leonardo J G Barcellos**: Supervision; Writing – original draft; Writing – review & editing; Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120301.

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4.2. CAPÍTULO 2 - TRANSFLUTHRIN-BASED HOUSEHOLD EFFECTS OF USED AND UNDER-USED DOSES ON *Caenorhabditis elegans* METABOLISM

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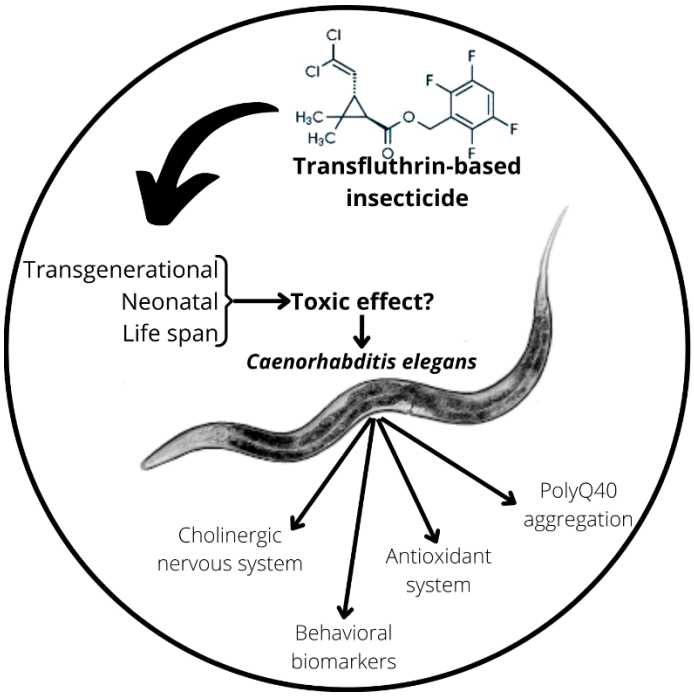
Graphical abstract



Abstract

Insect control is a public health problem, and due to this to control insects and mosquitoes developed different compounds with insecticides properties. Pyrethroids are one of the most used classes of insecticides in urban and rural environments. The mechanism of action of these compounds is related to nervous hyperexcitability and might interact with several physiological processes leading to oxidative disbalance, behavioral alterations, and neurodegenerative conditions. In this work, we carried out three different exposure protocols (transgenerational, neonatal, and lifespan) in the nematode *Caenorhabditis elegans* to transfluthrin-based insecticide (T-BI) a largely used household pyrethroid. We evaluated behavioral changes as well as nervous biomarkers on cholinergic signaling, PolyQ40 aggregations, and the status of the antioxidant system. Here we could observe that the T-BI reduced the health spam of worms treated during their whole life and changed biochemical and behavioral patterns in worms that were exposed in the progenitor's uterus and in a neonatal way. Here we highlight that the T-BI has effects that can be considered transgenerational and persistent and can be harmful to non-target animals such as humans that largely use these compounds.

Keywords: Huntington's disease, transfluthrin-based insecticide, *C. elegans*, cholinergic system, redox status.

1. Introduction

Insect control is a public health problem. The number of parasites and different zoonoses transmitted by mosquitoes to humans is increasing (Bauer et al., 2021; Cong and Elsheikha, 2021; Lednicky et al., 2021). Because of diseases transmitted by blood-sucking insects, especially in tropical and underdeveloped regions, the number is alarming. To contain these infestations, it is necessary to pay attention to the vectors, controlling their proliferation. In Brazil, the cases of dengue raised 113.7% in 2022 in comparison to 2021 and this rate of infection rises even more when added to other infections transmitted by insects (ANVISA, 2022).

For the control of mosquitoes, there is the intense use of the substance with insecticidal activity. Pyrethroids are classes of insecticides widely used to control insects externally in spraying cities and streams as well as in domestic use (Ravula and Yenugu, 2021). The use of these compounds is increasing more and more. Among the main pyrethroids is transfluthrin. Transfluthrin-based insecticides (T-BI) have several

commercial formulations, from electrical and programmed release to spray, which facilitate their use in closed rooms, increasing their effectiveness.

Several studies have reported the deleterious effect of pyrethroids on different animal organisms such as embryo-larval zebrafish (Awoyemi et al., 2019), strial cells (Dominah et al., 2017), rats (Hossain et al., 2004), and humans (Lucero and Muñoz-Quezada 2021; Narendra et al., 2008). The effects are explicit neurotoxicity and physiological changes in non-target organisms as well as humans. Teratogenicity has already been observed due to contamination of pyrethroids used in agriculture (Lucero and Muñoz-Quezada, 2021) and livestock (Burns and LaKind, 2021). In agriculturally used pyrethroids, many end up reaching lotic and lentic environments and affect the physiology of aquatic organisms and soil (Awoyemi et al., 2019). Thus, it is interesting to investigate the potential impact of insecticides present in general on the environment.

The half-life of transfluthrin in the environment varies from where it is found, for example, suspended in the air is around 2.4 days and in water is around 14 days (Luo et al., 2010). In insects, transfluthrin, like other pyrethroid-based insecticides, interacts with voltage-gated sodium channels keeping them open longer. This opening causes a continuous depolarization of the neuron which causes an intense discharge of neurotransmitters causing the death of the individual due to the hyperexcitability of the nervous system (Ensley, 2018).

To test the physiological effects of T-BI, the soil nematodes *Caenorhabditis elegans* (*C. elegans*) are essential due to their low maintenance and production costs, they are also excellent models for pharmacology (Tambara et al., 2020), toxicology (Bortoli et al., 2018) and biochemistry (Tamagno et al., 2021) assays. However, they can perfectly elucidate the effect of these compounds present in the environment in the interaction with non-target individuals.

Here we evaluated the effects of a commercial formulation of T-BI, an electrical household insecticide, on the physiology and behavior of *C. elegans* exposed at three different life stages to usual doses and sub-doses. The three life stages evaluated were: transgenerational (TG), neonatal (NN), and lifespan (LS). We evaluated the effect of the compound on body curvature rate, pharyngeal beat, aggregation, border behavior, acetylcholinesterase activity, number of PolyQ40 aggregations in muscle, and antioxidant system status.

2. Material and methods

2.1. Study strategy

To determine whether the household transfluthrin-based insecticide (T-BI) could affect biochemical and behavioral biomarkers on *C. elegans*, exposed in different periods of life, we exposed the worms in three different protocols: transgenerational (TG), neonatal (NN), and life span (LS) as described in section 2.3.

2.2. *Caenorhabditis elegans* husbandry

The strains N2 (Wild-type), CL2070 [(*dvIs70[hsp-16.2p::GFP+rol-6(su1006)]*)], CF1553 [(*muIs84[(pAD76)sod-3p::GFP+rol-6(su1006)]*)], GA800 [*(wuIs151[ctl-1+ctl-2+ctl-3+myo-2::GFP)])*], CL2166 [(*dvIs19[(pAF15)gst-4p::GFP::NLS])*], and AM141 *[(rmIs133[unc-54p::Q40::YFP])]* of *C. elegans* were acquired from the Caenorhabditis Genetic Center (Minnesota University, EUA). *C. elegans* were kept on nematode growth medium (NGM) and fed with *E. coli* (OP50) at 20 °C.

2.3. Exposition

For transgenerational (TG), neo-natal (NN) and lifespan (LS) exposure (Figure 1), we utilizing the same methodology as in a previous work (Tamagno et al., 2022).

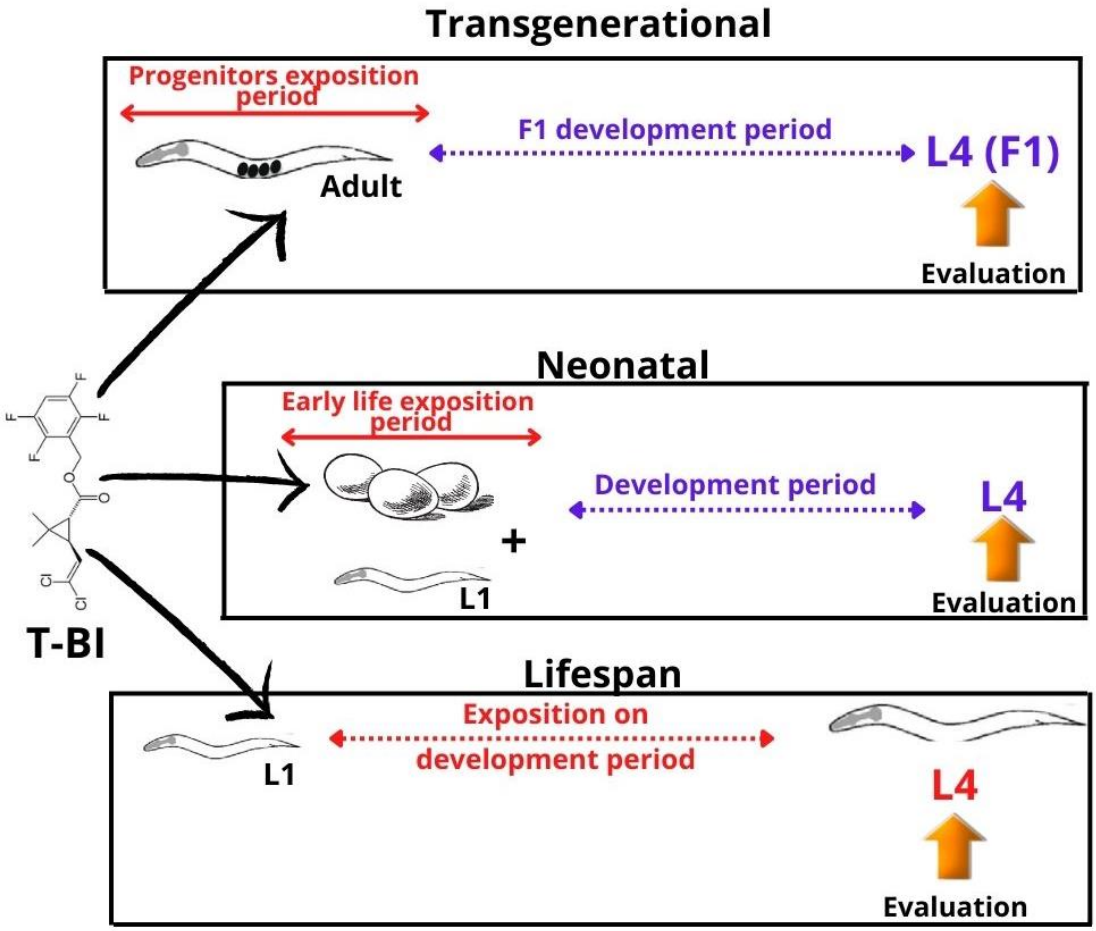


Figure 1 – Study strategy. Three ways of exposure to Caenorhabditis elegans: Transgenerational with exposition in progenitors (in red), neonatal with exposition in early life (in red), and lifespan with exposition during the whole development period (in red).

For each exposition (TG, NN and LS), was established three concentrations of T-BI being, control (water), relative calculated dose (RCD) (0.012 mg), and relative calculated half-dose (RCHD) (0.006 mg) of T-BI (commercial formulation) according to Tamagno et al., 2022 with adaptations regarding to the compound. The household insecticide was the SBP® Liquid Electric Repellent (Cachoeirinha, RS, Brazil) composed of 8 % of transfluthrin, antioxidants, and vehicle. This insecticide is indicated for electric household use during the night or day 8 h per day. The indicated total duration of the product is 45 nights and the best effect is in a room with no more than 20 m² of area.

The relative calculated dose (RCD) and relative calculated half-dose (RCHD) were determined by calculating the total volume of the SBP® solution considering the number of nights (keeping turned on 8 hours.night^{-1}) and the area of the room, relativizing the agar plate area (0.006 m²) where the worms were exposed as following the equation 1.

Equation 1. $Relative\ concentration = \frac{((V \cdot C)/N)}{A} * pA$

V = Total volume of the SBP® (mL)

C = Concentration of the active principle (transfluthrin) on liquid ($mg.mL^{-1}$)

N = Number of nights indicated for use

A = Area of the room (m^2)

Pa = Plate area

For a better understanding of the established concentrations, the RCD is the usual concentration that humans are exposed to in household use, relatively calculated for the plate area. In this way, the RCHD is here considered a sub-dose (50 %) of the usual exposure that humans and animals are exposed to in the household environment

2.4. Behavioral biomarkers

2.4.1. Body bends and pharyngeal pumping rate

For body bends, twenty-four (n = 24) exposed worms per treatment at the L4 stage were evaluated as described by Tsalik and Hobert (2003). For pharyngeal pumping, twenty-four (n = 24) exposed worms per treatment at the L4 life stage were evaluated as described by Wang et al. (2008).

2.4.2. Feeding behavior

Borderline and agglomeration behaviors were quantified simultaneously as described previously by De Bono and Bargmann, (1998); Gray et al., (2004); Jang et al., (2017). As previously described in Tamagno et al., 2022.

2.5. Biochemical biomarkers

2.5.1. Acetylcholinesterase activity

For AChE activity were used 10000 exposed worms per pool as described in section 2.3. and after we procced as described by Tamagno et al. (2021 & 2022). The final activity is expressed in µmol of $ACh.h^{-1}$ mg of $protein^{-1}$.

2.5.2. PolyQ40 aggregates quantification

The number of *polyQ40::YFP* muscle aggregates was counted as described by Peixoto et al., (2016) and adapted by Tamagno et al, 2022. Results are presented as several *polyQ40::YFP* aggregates.

2.5.3. Heat shock protein and antioxidant enzymes fluorescent expression quantification

Heat shock protein (*hsp-16.2*), superoxide dismutase (*sod-3*), catalase (*ctl-1, 2, and 3*), and Glutathione-S-transferase (*gst-4*) were quantified as described by Tamagno et al., (2022); Tambara et al., (2020) with some adaptations.

2.6. Statistical analyze

Data of three replicates were analyzed using one-way ANOVA followed by a post hoc of Dunnet's test, or a Kruskal–Wallis's test followed by a post hoc Dunn's test, depending on the normality of the data (assessed by the Kolmogorov–Smirnov test). The software used for all analyzes was the Graph Pad Prism 8.0.1.

3. Results

3.1. Behavioral biomarkers

3.1.1. Body bends rate

In the transgenerational (TG) exposition (Fig. 2, A) the body bends rate was increased in the group treated with RCD in comparison to RCHD ($p < 0.01$), but no changes were observed related to control. In the neonatal (NN) exposition (Fig. 2, B), the group treated with RCD had the body bends rate decreased in comparison to control ($p < 0.001$) and RCHD ($p < 0.01$). In the group exposed during life span (LS) (Fig. 2, C), the body bends rate was increased in the group treated with RCD in comparison to the control ($p < 0.05$).

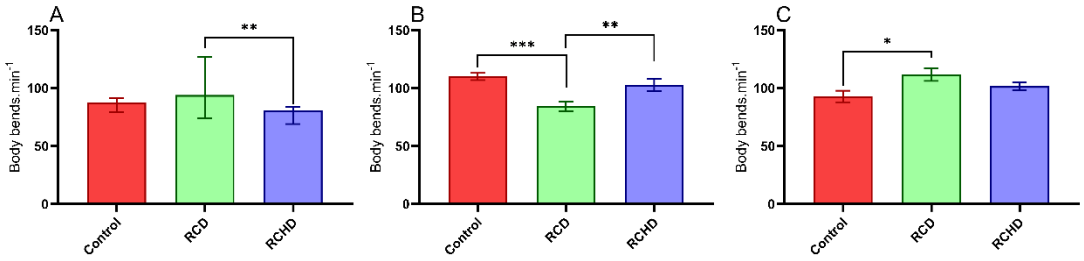


Fig. 2 – Body bends rate of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panel A is expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panels B and C are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3.1.2. Pharyngeal pumping rate

Pharyngeal pumping rate in the TG way of exposition (Fig. 3, A) was increased in the RCD group in comparison to control ($p < 0.05$) and to RCHD ($p < 0.01$). In the NN (Fig. 3, B) the RCD group was decreased in comparison to the control ($p < 0.05$). In the LS (Fig. 3, C) the group treated with RCHD had the pharyngeal pumping rate increased in comparison to control ($p < 0.0001$) and RCD ($p < 0.0001$).

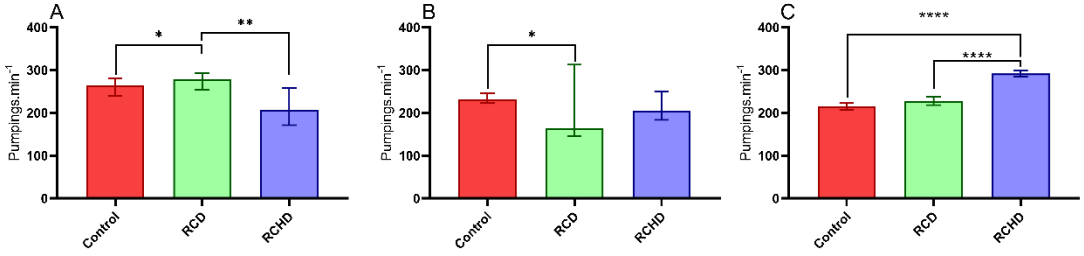


Fig. 3 – Pharyngeal pumping rate of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels A and B are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panel C is expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *p < 0.05; **p < 0.01; ****p < 0.0001.

3.1.3. Behavior related to feeding

The TG (Fig. 4, 1A) exposition reduced the number of worms that were eating together in both RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) groups in comparison to the control. In this same exposition way (Fig. 4, 2A), the worms ate less in the edge of the bacteria in the RCHD ($p < 0.05$) in comparison to the control. In the NN exposition (Fig. 4, 1B, and 2B), the worms did not show a difference in the pattern of social feeding as well as edge feeding. In the LS exposition (Fig. 4, 1C), the worms eaten less in group in both RCD ($p < 0.0001$) and RCHD ($p < 0.001$) groups in comparison to control. Regarding edge feeding (Fig. 4, 2C), the group treated with RCHD decreased the preference for the edge in comparison to the control ($p < 0.05$) and RCD ($p < 0.01$) groups.

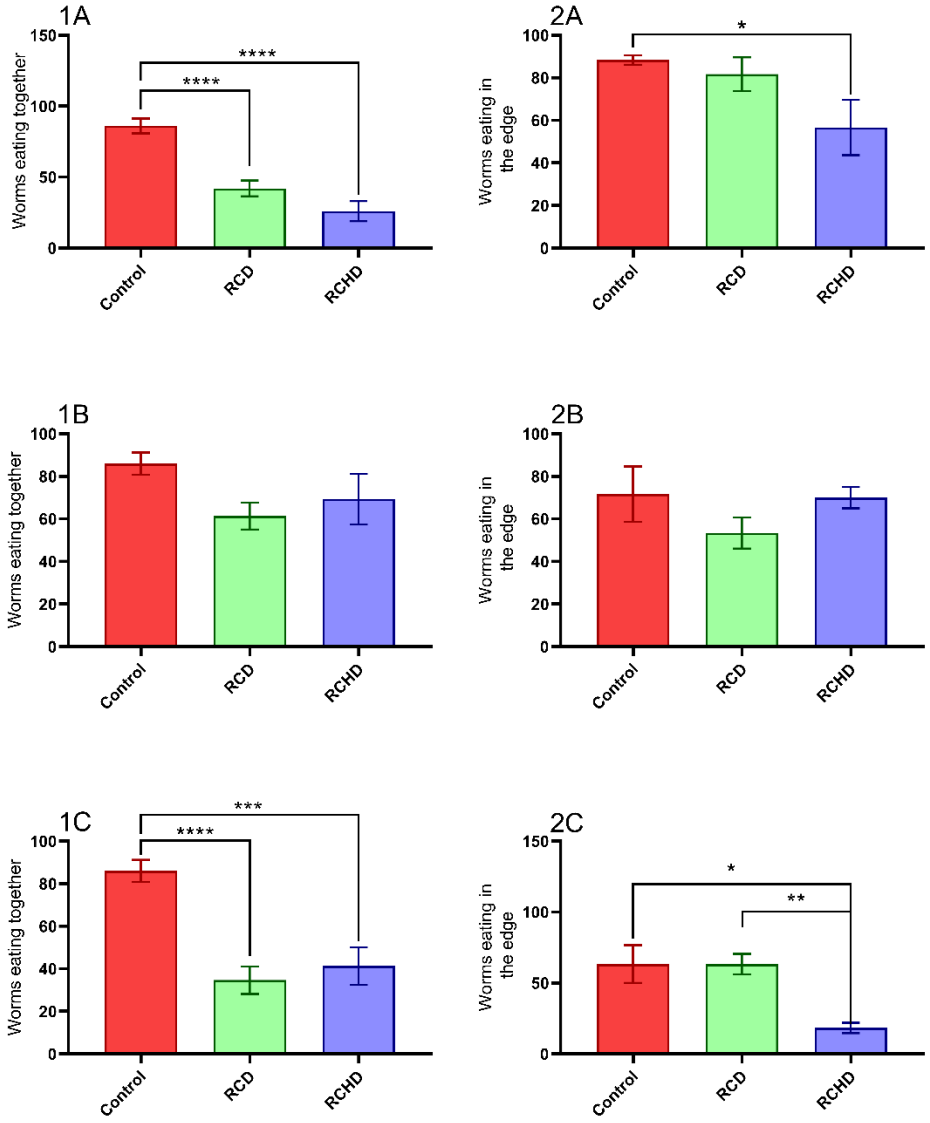


Fig. 4 – Social behavior related to feeding (1) and feeding behavior (2) of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels 2A, 2B, and 2C are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panels 1A, 2A, and 2C are expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

3.2. Biochemical biomarkers

3.2.1. Acetylcholinesterase activity

AChE activity in the TG exposition (Fig. 5, A) was decreased in the group treated with RCD ($p < 0.05$) and increased in the group with RCHD ($p < 0.01$) both in comparison to control. In addition, the group treated with RCHD was increased in comparison to RCD ($p < 0.0001$). In the NN exposition (Fig. 5, B) the group treated with RCD increased the AChE activity in comparison to control ($p < 0.0001$) and RCHD ($p < 0.0001$). In the

group LS group (Fig. 5, C), the AChE activity was decreased in the group RCD ($p < 0.0001$) in comparison to control and in comparison to RCHD ($p < 0.01$).

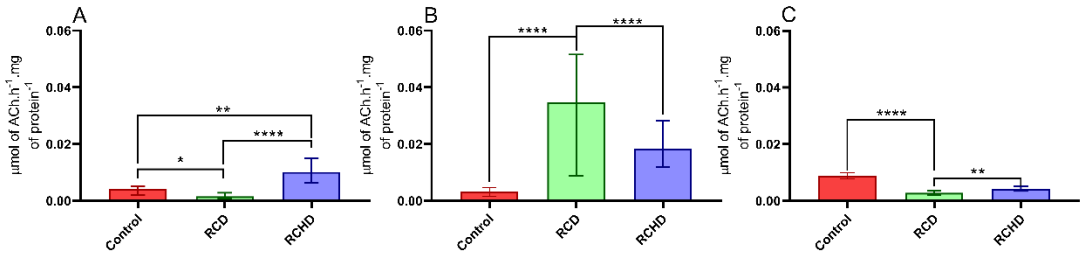


Fig. 5 – Acetylcholinesterase activity of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels A and B are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panel C is expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$.

3.2.2. Number of PolyQ40 aggregates

The number of PolyQ40 aggregates in the TG (Fig. 6, A) increased in the groups exposed to RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) in comparison to control. In the NN exposition (Fig. 6, B) the group treated with RCD increased the PolyQ40 aggregates in comparison to RCHD ($p < 0.05$). In the LS exposition (Fig. 6, C) the number of PolyQ40 aggregates was decreased in the group treated with RCHD in comparison to control ($p < 0.0001$) and in comparison to RCD ($p < 0.001$).

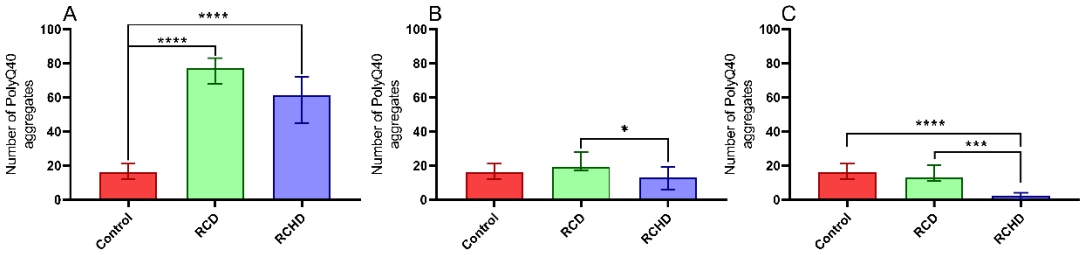


Fig. 6 – Number of PolyQ40 aggregates on muscles marked with GFP in mutant AM141 strain of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels A and C are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panel B is expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$.

3.2.2. Number of PolyQ40 aggregates

Heat shock protein (HSP) in the TG exposure (Fig. 7, A) was decreased in the group treated with RCD ($p < 0.05$) and RCHD ($p < 0.01$) in comparison to the control group. In the NN (Fig. 7, B) HSP increased the expression in the RCD ($p < 0.0001$) and RCHD ($p < 0.001$) in comparison to control group. In the LS exposition (Fig. 7, C), the

HSP expression was increased in the groups treated with RCD ($p < 0.0001$) and RCHD ($p < 0.01$) in comparison to the control. In addition, the group treated with RCD increased the HSP expression in comparison to RCHD ($p < 0.0001$).

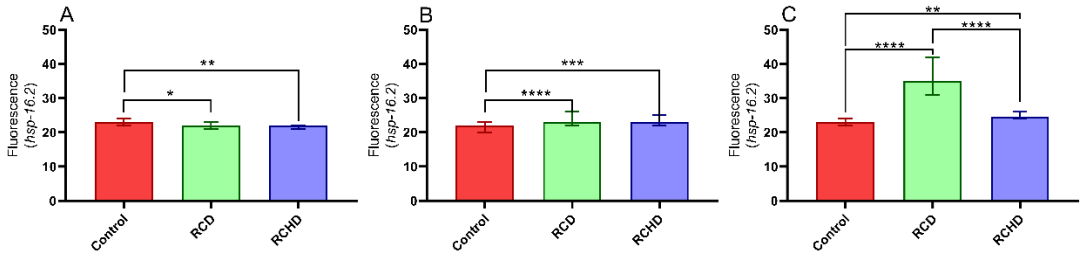


Fig. 7 – Heat shock protein (*hsp-16.2*) expression by GFP quantification in mutant CL2070 strain of *Caenorhabditis elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels B and C are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. Data in panel A is expressed as the mean ± SEM analyzed by ANOVA One-way followed by Tukey's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

3.2.3. Antioxidant system

In the antioxidant system, the SOD GFP expression (Fig. 8, 1) in the TG exposition (Fig. 8, 1A) was increased in the groups treated with RCD ($p < 0.0001$) and RCHD ($p < 0.0001$). In the NN exposition (Fig. 8, 1B) SOD expression was increased in the groups treated with RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) in comparison to control. In the LS exposition (Fig. 8, 1C) the group treated with RCHD increased in comparison to control ($p < 0.0001$) and RCD ($p < 0.0001$).

In the CTL GFP expression, in the TG exposition (Fig. 8, 2A) the group treated with RCHD decreased the CTL expression in comparison to control ($p < 0.01$) and in comparison to RCD ($p < 0.01$). In the NN exposition (Fig. 8, 2B) both groups RCD ($p < 0.0001$) and RCHD ($p< 0.0001$) decreased in comparison to control group. In the LS exposition (Fig. 8, 2C) the group RCD decrease the CTL activity in comparison to control ($p < 0.0001$) and RCHD ($p < 0.0001$).

The GST GFP expression was decreased in the TG (Fig. 8, 3A) in both RCD ($p < 0.0001$) and RCHD ($p < 0.0001$) in comparison to control group. In the NN exposition (Fig. 8, 3B), the group treated with RCHD decreased in comparison to control ($p < 0.01$) and RCD ($p < 0.05$). In the LS exposition (Fig. 8, 3C), the GST expression was increased in the group treated with RCD in comparison to control ($p < 0.05$) and RCHD ($p < 0.0001$). The RCHD decreased in comparison to the control group ($p < 0.0001$).

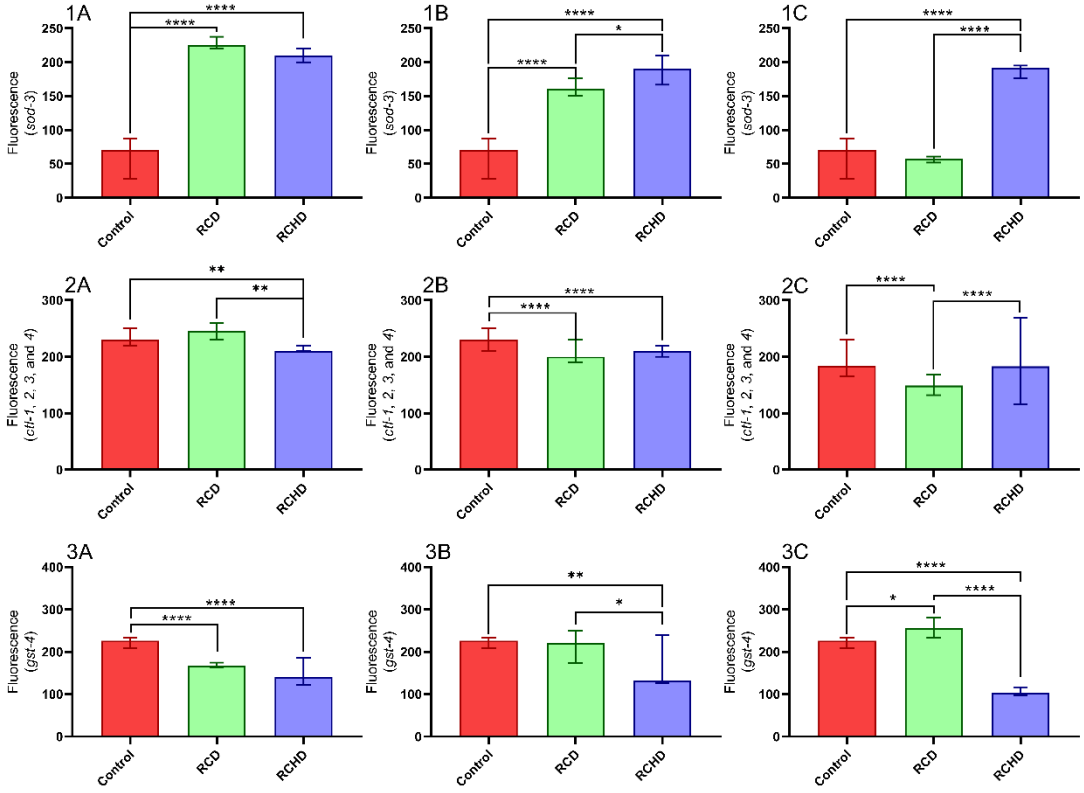


Fig. 8 – Superoxide dismutase (1), catalase (2), and glutathione-S-transferase (GST) expression by GFP quantification in mutant CF1553 (*sod-3*), GA800 (*ctl-1, 2, and 3*), and CL2166 (*gst-4*) strains of *C. elegans* exposed to relative calculated dose (RCD) and relative calculated half-dose (RCHD) of T-BI. Three different ways of expositions were carried out in different periods of life, transgenerational (A), neonatal (B), and life span (C). Data in panels are expressed as the median ± interquartile range and analyzed by the Kruskal-Wallis test followed by Dunn's test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

4. Discussion

The T-BI is one of the most used active principles for insecticide household. Although, its safety is not completely understood especially regarding neural harmful effects. Here we show that the behavior of *C. elegans* was changed especially when the worms are exposed to the uterus of their progenitors (before and during the oogenesis process). It suggests that the main effect is not in the current generation, and more pronounced in the offspring, characterizing a transgenerational effect of the T-BI.

The pharyngeal pumping was increased in the F1 generation when progenitors are exposed to T-BI. In addition, when the worms are exposed during life span (LS) even body bends and pharyngeal pumping are increased. In this way, the observed TG effect is similar to that observed in LS. Pharyngeal pumping and body bends are behavioral parameters related to health span of the worm (Rollins et al., 2017; Tamagno et al., 2021),

changes in its patterns are related to physiological changes as well as neural activity dysfunction (Bortoli et al., 2018).

Furthermore, the social behavior related to feeding was decreased in these two expositions ways (TG and LS), which means that the worms exposed to T-BI remain far from its conspecific while feeding, an unexpected behavior. Moreover, these worms that remain far during the feeding process, also ended up eating far away from the edges than untreated worms.

The NN behavior occurred oppositely to that observed in TG and LS, in NN we observe a reduction in motility observing the body bends, as well as on the pharyngeal pumping. Regarding the social behavior related to feeding, no changes were observed. The reduction in pharyngeal pumping and motility is linked with senile aging, which highlights the possible effects in early aging in organisms exposed in NN way (Bortoli et al., 2018; Hosono et al., 1980; Tamagno et al., 2022). In addition, we believe that few physiological changes in this group in comparison to TG and LS can be due to the worms exposed in NN, remain in contact with T-BI for a period inside the egg with its protection. And after the hatch they remain without food, that could reduce the T-BI ingestion and then reducing the intoxication.

The explanation for the reduced motility on NN might be related to AChE increased levels. The enzyme AChE is related to cholinergic signaling and is one of the most important key enzymes involved in neurodegenerative diseases, especially that related to the cholinergic nervous system. This enzyme works in the synaptic cleft hydrolyzing the neurotransmitter acetylcholine (ACh) ending with cholinergic signaling (Kaizer et al., 2008; (Lewis et al., 2013)). AChE is the mechanism of action of many insecticides such as organophosphates that decrease their activity until critical levels end up causing hyperexcitability of the nervous system causing the organism's death (Adeyinka et al., 2018). Pyrethroids have their mechanism of action related to the opening of sodium channels that cause an action potential and an increased release of neurotransmitters that hyper-excite the nervous system causing death (Zhu et al., 2020).

As we can observe, the mechanism of action of transfluthrin does not involve AChE, but the increase in neurotransmitter releases by membrane destabilization can cause it. These findings in the AChE pattern were already found in honeybees, after pyrethroid exposure the AChE activity increased (Badiou and Belzunces, 2008). As in other studies, the observed increase in AChE in the T-BI-treated group can be explained by two different mechanisms. First, T-BI, like other pyrethroids, increases hippocampal

ACh release due to nerve fiber hyperexcitability (Hossain et al., 2004), thereby inducing regulatory overcompensation by increasing AChE. Second, based on studies with the *Schistosoma mansonni* trematode, it is observed that the release of AChE from the membrane by Phosphoinositide-Specific Phospholipase C (PI-PLC) in the parasite causes a *de novo* synthesis mediated by diacylglycerol, associated with a compensation, probably to replace the AChE removed from the cell membrane surface (Espinoza et al., 1991). This study supports the hypothesis that T-BI activates an endogenous phospholipase. In *C. elegans*, the action of PI-PLC can induce an AChE deficit in the cell membrane, triggering an adaptive upregulation to replace the released AChE, as in honeybee (Badiou and Belzunces, 2008). The question that remains is how does this overcompensation start in the neonatal period and can it persistently maintained increased?

Furthermore, increased AChE might be related to the reduced behavior observed on NN. An interesting observation is in the TG and LS ways of exposure, once these groups had increased body bends and pharyngeal pumping and a reduction in AChE activity. In our study, the reduction in this preference highlights the cholinergic signaling disbalance.

Another observation is regarding the *PolyQ40::YFP* aggregates on muscles. There is an increased number of aggregations on the TG , which means that when parents are exposed to the T-BI during the pregnancy, F1 generation increases the *PolyQ40::YFP* aggregates when they reach the adult age. *PolyQ40::YFP* aggregates are related to HD (Priya and Gromiha, 2019). HD is a neurodegenerative and hereditary disease that is hypothesized by the huntingtin gene alteration and formation of agreeable forms of poly-glutamine (PolyQ). In an organism predisposed to manifest HD, the gene that transcribes huntingtin has many CAG repeats (which encodes glutamate), usually more than 35 repeats. These repeats are transcribed together with the huntingtin gene and because they are very large, they are poorly soluble and end up aggregating in muscles and the central nervous system (Markaki and Tavernarakis, 2020; Tabrizi et al., 2020; Tan et al., 2015). In our study, we observed that in worms with the insertion of the altered gene for huntingtin (*PolyQ40*) when exposed to T-BI, their offspring increase the expression of these aggregates. HD is expected to be initiated during aging, but there are cases in which the effects start early, it is believed that the environment has a large effect on the triggering of these effects in early life. In worms with de insertion of *PolyQ40::YFP* gene that was treated during their whole life (LS) or just in the neonatal life (NN) is no observed

increase in this biomarker, highlighting the harmful effect of this compound on pregnancy.

Another important biomarker is the antioxidant system, related to neurodegenerative conditions (Collin, 2019). Reactive oxygen species (ROS) can affect cells due to high instability and reactivity; they are generated during whole life by the organisms. For this, the cell has an intrinsic antioxidant enzymatic system that removes the increased amount of ROS from the cell before generating damage (Aziz et al., 2019). The first enzyme reacting with ROS is SOD (superoxide dismutase) which acts in the front line of defense reacting with the anion superoxide (O_2^-), one of the harmful free radicals, reducing it to hydrogen peroxide (H_2O_2) with less toxicity but still dangerous in excess (Ighodaro and Akinloye, 2018). In our study, we observed an increased *sod-3::GFP* expression in all treatment groups, Following enzyme catalase (CTL) clivate the H_2O_2 resulting from SOD to water and oxygen two non-toxic products. In our study, we observed a reduction in *ctl-1,2,3::GFP* expression which suggests that had occurred an activation of another H_2O_2 scavenger pathway such as GPx or even another non-enzimatic antioxidant pathway (Chaudiere et al., 1987; Chen et al., 2021).

Glutathione-S-transferase (GST) is another important enzyme is antioxidant defense, GST reacts with toxic compounds by inserting glutathione terminals, reducing the toxicity of its compounds (Vaish et al., 2020). Here, we observed a reduced *gst-4::GFP* expression on F1 from exposed parents. It is highlight the low capacity of the second line antioxidant defense against harmful compounds. A further important mechanism of stress protection is the heat shock protein (HSP) system, these proteins are expressed under heat or environmental stress conditions (Chen et al., 2018) and help the cell to fight against ROS avoiding lipid and genetic damage. Here we evaluate the *hsp-16.2::GFP* expression that is related to protein precipitation in the nervous system and neurodegeneration avoidance (DanQing et al., 2021). We observed in our study a reduced expression of HSP in TG, indicating a reducing resistance to stress. Regarding the NN and especially LS the *hsp-16.2::GFP* was increased highlighting current and persistent stress exerted by T-BI on cells.

Finally, we observe the importance of establishment on politics for correct use of insecticides. Even in agricultural fields or livestock animals in the countryside. But even more important in house consumption. All ages people use some compounds to avoid insects, but are under possible contamination. That could be cause transgenerational and persistent effects on non-target individuals and offspring.

5. Conclusion

Taken together, all results highlight the harmful effect of the T-BI concentrations to humans and animals are exposed. Here we highlight that the exposition during the pregnancy might be related to the gene changes and the results are severally pronounced in adulthood of the F1 generation. The negative effects on AChE, and the antioxidant system results in behavioral alteration in the worm. Another observation is from the environmental point of view, here we show that in a soil organism the effect is quite harmful highlighting that the incorrect disposal of T-BI can remain in the environment for a long period and be dangerous for many organisms.

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Conflict of interest

The authors declare that they have no competing interests.

Authors contributions

W A Tamagno: Conceptualization; Data curation; Formal analysis; Writing –original draft; Investigation; Project. **C Alves:** Methodology, Writing –review & editing; Data curation; Formal analysis. **A Pompermaier:** Methodology, Writing –review & editing; Data curation; Formal analysis. **H C Santos:** Methodology. **L J G Barcellos:** Supervision; Writing–original draft; Writing–review& editing; Funding acquisition.

Founding

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Ethical approval

This work was not carried out with vertebrate animals. *Caenorhabditis elegans* is a soil nematode and does not need any ethical approval to be studied.

Patient consent to participate

Not applicable.

Permission to reproduce material prim other sources

Not applicable.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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4.3. CAPÍTULO 3 - PYRETHROID-BASED INSECTICIDES ON *Caenorhabditis elegans* EXERT TRANSGENERATIONAL, PERSISTENT, AND CHRONIC PHYSIOLOGICAL, BEHAVIORAL, AND NEURODEGENERATIVE DAMAGE

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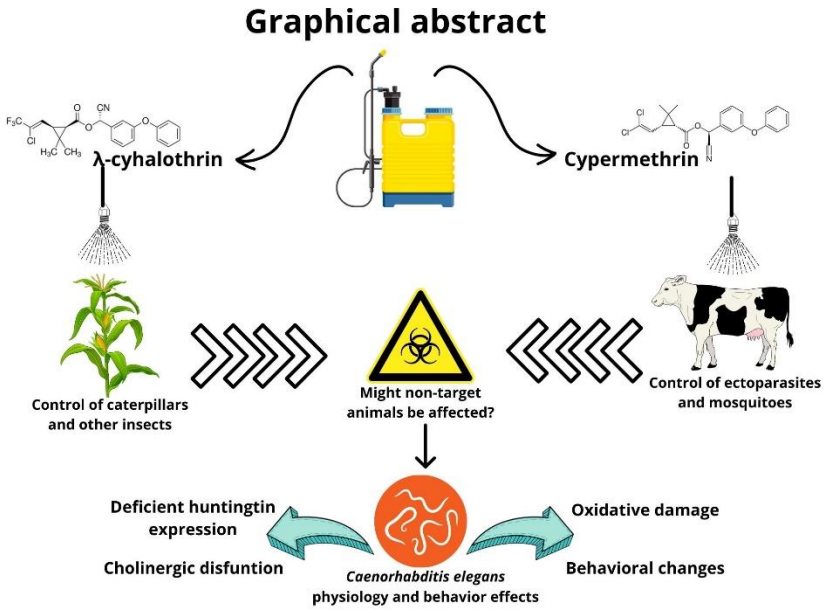
Graphical abstract



Abstract

The use of agrichemical pyrethroi-based insecticides to combat infestations in crop plants as well as ectoparasites in animals is increasing. In this context, two pyrethroid-based insecticides are widely used, λ-cyhalothrin and Cypermethrin. The mechanism of action of these insecticides is characterized by the opening of ion channels and death by neural hyperexcitability. In non-target organisms such as *Caenorhabditis elegans* the mechanism of action may involve more complex mechanisms and differ from the usual mechanisms. In this work we evaluated the toxicological effect of the two insecticides λ-cyhalothrin and Cypermethrin in three different exposure protocols in *C. elegans* aiming to evaluate the effect of these compounds in a transgenerational (TG), neonatal (NN) and lifespan (LS) way. Behavioral biomarkers, fluorescent expression of antioxidant enzymes, fluorescent expression of PolyQ40 aggregates, and the activity of the enzyme acetylcholinesterase were evaluated. Finally, we observed that the compounds have a similar deleterious behavioral effect in the TG and LS protocols, but that the mechanism that caused the change is different. We conclude that the compounds have effect related with transgenerational and chronic toxicity. Compounds influenced the increase in the probability of the expression of PolyQ40 muscle aggregates in mutant worms, being related to the increased probability of the incidence of Huntington's Disease in genetically predisposed patients.

Keywords: Cypermethrin, λ-cihalothrin; huntingtin; oxidative stress biomarkers; *C. elegans*.

Founding

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1. Introduction

The increase in crop cultures and animal production is due to an increased need for food worldwide. Brazil is among the main countries in the world in the ranking of food

production, whether of plant or animal origin. In the same way that there is an increase in food production, there is a growing development of products that guarantee effective productivity, products that can reduce biotic interferences that lead to productivity losses (FAO, 2018). One of the main interferers in crop plants and animal production are insects. In crop plants, they imply infestations that can lead to productivity collapse due to the use of plants as a food source. In animals, ectoparasites and opportunistic insects cause, in addition to productivity losses, animal death and disease transmission.

To control these insects, chemicals called insecticides such as pyrethroids are used. Pyrethroids are a very old and widely used class of insecticides because of their low toxicity in vertebrate animals. With pyrethroids it is possible to develop agricultural crops and livestock in an easier way (Carter, 1989). However, the high incidence of pyrethroid-resistant insects and the use of overdoses as well as the very recurrent application become a public health problem (World Health Organization, 2005).

Both in crops and in animal livestock, the application of these compounds ends up reaching non-target organisms such as fish in rivers and lakes, molluscs in water and soil and free-living nematodes that make up the soil biota (Anadón et al., 2009; Diabate et al., 2002). Among the non-target organisms, we have the soil nematode *Caenorhabditis elegans*, which in addition to being a non-target organism for the use of pyrethroid insecticides, is a clear bioindicator of the toxicological and physiological effect of different compounds such as pyrethroids (Oya et al., 2022; Tamagno et al., 2022).

In this study, we evaluated the toxic effect of two pyrethroid-based insecticides, used in agriculture and livestock, λ-cyhalothrin (λ-cBI) and cypermethrin (CyBI). The reason for choosing these chemicals were due to the increased use of the λ-cBI in the agriculture in a wide variety of cultures. And as well as of the CyBI is the most common ectoparasite-control pyrethroid, in livestock animals (Anbalagan et al., 2013; Ruan et al., 2009). To understand the toxicological effect of the compounds and whether have harmful effect in a specific period of the life, we carried out three independent protocols of experimentation. Were evaluated the transgenerational effect (TG) when hermaphrodite parents were exposed and later, we evaluated the F1 of worms; the second way is the neonatal effect (NN) where worms were exposed during the egg-life stage and in the early life before starting to eat; the third way was assessed by exposing the worms during whole-life (LS). In all parameters were evaluated behavioral biomarkers, antioxidant enzymes fluorescent expression, PolyQ40 fluorescent expression aggregates as well as the acetylcholinesterase activity.

We highlight the importance of this work due to the increased use of agrichemicals and the obedience of the 17 Sustainable Development Goals (SDG) of the United Nations specially the SDG-3 that highlight zero hunger for this develop a sustainable agriculture to end hunger, achieve food security and improved nutrition, as well as the SDG-6 that highlight the importance of consciously use water so that everyone who needs it can have access to clean and treated water.

2. Material and methods

2.1. Study strategy

To determine whether the agrichemicals λ-cihalothrin- (λ-cBI) and cypermethrin-based pyrerthroid insecticide (CyBI) affects biochemical and behavioral biomarkers on *C. elegans*, exposed in different period of life (ways of exposure), we exposed the worms in three different protocols: transgenerational (TG), neo natal (NN), and life spam (LS) as fully described in the section 2.3.

2.2. Caenorhabditis elegans husbandry

The strains N2 (Wild-type), CL2070 [(dvIs70 [hsp-16.2p::GFP + rol-6(su1006)])], CF1553 [(muIs84 [(pAD76) sod-3p::GFP + rol-6(su1006)])], GA800 [(wuIs151 contains [ctl-1 + ctl-2 + ctl-3 + myo-2::GFP])], CL2166 [(dvIs19 [(pAF15)gst-4p::GFP::NLS])], and AM141 (rmIs133[unc-54p::Q40::YFP]) of *C. elegans* were acquired from the Caenorhabditis Genetic Center (Minnesota University, EUA). *C. elegans* were kept on nematode growth medium (NGM) and fed with *E. coli* (OP50) and kept at 20 °C.

2.3. Exposure protocols

Exposure protocols for TG, NN and LS was carried out as described by Tamagno et al., (2022a). For TG protocol, the worms were placed into contact with the λ-cBI or CyBI before developing internal eggs and after develop vulva (L4). They stayed in contact with the toxicant during all the oogenesis period (~20 h).

For NN protocol, the synchronized eggs (from non-exposed parents) were kept in contact with the λ-cBI or CyBI in M9 buffer until they were completely hatched (~20 h). The neo-natal-L1 worms were placed in NGM with *E. coli* (OP50) as source of food until reach the L4 stage when were behavior and biochemical evaluated.

For LS protocol, the non-exposed worms were synchronized and kept just in M9 buffer until reach the L1 stage. Then, they were placed in NGM with *E. coli* (OP50) where were kept in contact with the λ-cBI or CyBI during L1 to L4 period of the post-embryonic

development with re-exposure (re-administration of the chemicals in the same plate) every day (total time exposure 48 h). When the worms reach the L4 stage all the biochemical and behavioral biomarkers were evaluated.

For each protocol and for each insecticide were established three concentrations of λ-cBI and CyBI being, control (M9), indicated dose on recipe (100%) and half of the indicated dose (50%) of each commercial formulation as follow in the table 1.

Table 1 – Technical information about the pyrethroyd-based insecticides used in this experiment.

Compound	Abbreviation	Commercial name and manufacturer	Specifications	Compositions	Indications for use
λ-cihalothrin-based insecticide	λ-cBI	Karate Zeon® 250 Cs Manufactured by Syngenta proteção de cultivo Ltda. São Paulo, SP, Brazil.	Pyrethroid used for the control of insects such as caterpillars, bed bugs, flies and cicadas in cotton, rice, peanuts, potato, coffee, onion, citrus, cabbage, chrysanthemum, beans, tobacco, gerbera, watermelon, melon, corn, strawberry, soybean, wheat and grape.	λ-Cyhalothrin 250 $g.L^{-1}$ 5.0 % (m/v) Other ingredients 975 $g.L^{-1}$ 97.5 % (m/v)	Field application prepare a emulsion in the concentration of 0.004 $mL.L^{-1}$ for pulverization in the rate of 2.000 $L.Ha^{-1}$ The dosage application will vary depending of the insect and the culture of application.
Cypermethrin-based insecticide	CyBI	Colosso® pulverization Manufactured by Ouro Fino Saúde Animal Ltda. Cravinhos, SP, Brazil.	Pyrethroid anti ectoparasite for use in chicken aviaries, cattle and swine (carrapaticidal, mosquicidal, bernicidal, sarnicidal, piolhicidal, repellent)	Cypermethrin (150 $g.L^{-1}$) Chlorpyrifos (250 $g.L^{-1}$) Citronella (10 $g.L^{-1}$) Vehicle (1 L)	Cattle: 1.25 $mL.L^{-1}$ Swine: 2.25 $mL.L^{-1}$ Chicken aviaries: 2.5 $mL.L^{-1}$

In this way, the used concentrations for the exposure in worms were calculated according to the volume (20 mL) and/or area (0.006 m^2) of the plate where the worms were exposed. The respective concentrations were 0.004 $mL.Ha^{-1}$ (100%) and 0.004 $mL.L^{-1}$ (50%) of λ-CBI; and 2.5 $mL.L^{-1}$ (100%) and 1.25 $mL.L^{-1}$ (50%) for CyBI.

2.4. Behavioral biomarkers

2.4.1. Body bends and pharyngeal pumping rate

Twenty-four exposed worms in each specific group of treatment at the L4 stage had the body bends rate and pharyngeal pumping evaluated as described by Tsalik and Hobert (2003) and Wang et al. (2008) respectively.

2.4.2. Agglomeration and borderline behavior

Borderline and agglomeration behaviors were quantified simultaneously as described previously by De Bono and Bargmann (1998); Gray et al. (2004); Jang et al. (2017), and adapted from Tamagno et al., 2022.The result was expressed as the total animal eating together and total animals eating in the edge.

2.5. Biochemical biomarkers

2.5.1. Acetylcholinesterase activity

For AChE activity were used 10000 exposed worms per pool as described in section 2.3. and after we procced as described by Tamagno et al. (2021 & 2022a). *2.5.2. PolyQ40 aggregates quantification*

The number of *polyQ40::YFP* muscle aggregates was counted as described by Peixoto et al., (2016) and adapted by Tamagno et al, 2022a.

2.5.3. Heat shock protein and antioxidant enzymes fluorescent expression quantification

Heat shock protein (*hsp-16.2*), superoxide dismutase (*sod-3*), catalase (*ctl-1, 2, and 3*), and Glutathione-S-transferase (*gst-4*) were quantified as described by Tamagno et al., (2022a); Tambara et al., (2020) with some adaptations.

2.6. Statistical analysis

Data of three replicates were analyzed using one-way ANOVA followed by a post hoc of Dunnet's test, or a Kruskal–Wallis's test followed by a post hoc Dunn's test, depending on the normality of the data (assessed by the Kolmogorov–Smirnov test). The software used for all analyzes was the Graph Pad Prism 8.0.1.

3. Results

3.1.Behavioral evaluations

3.1.1. Body bends

The body bends rate in the transgenerational (TG) exposure way (Fig. 1, A1) was decreased in the group exposed with 100% of λ-cBI in comparison to control ($p < 0.0001$) and with 50% λ-cBI ($p < 0.0001$). The same behavior of decrease was observed in the 100% CyBI (Fig. 1, A2) in comparison to control ($p < 0.001$) and 50% Cyp ($p < 0.01$). In the neonatal (NN) exposure way, when in contact with λ-cBI (Fig. 1, B1), the worms were not able to hatch and growth in both concentrations. In the NN contact with CyBI (Fig. 1, B2) the concentration 50% decreased the rate of body bends in comparison to 100 % ($p < 0.05$). In the life span (LS) exposure way, in the contact with λ-cBI (Fig. 1, C1) the worms decreased the body bends rate in the 100% ($p < 0.05$) and 50% ($p < 0.01$) concentrations in comparison to control. In the LS exposure way in contact with CyBI (Fig. 1, C2) the worms treated with both concentrations of 100% and 50% decreased the rate in comparison to control (respectively $p < 0.001$and $p < 0.0001$).

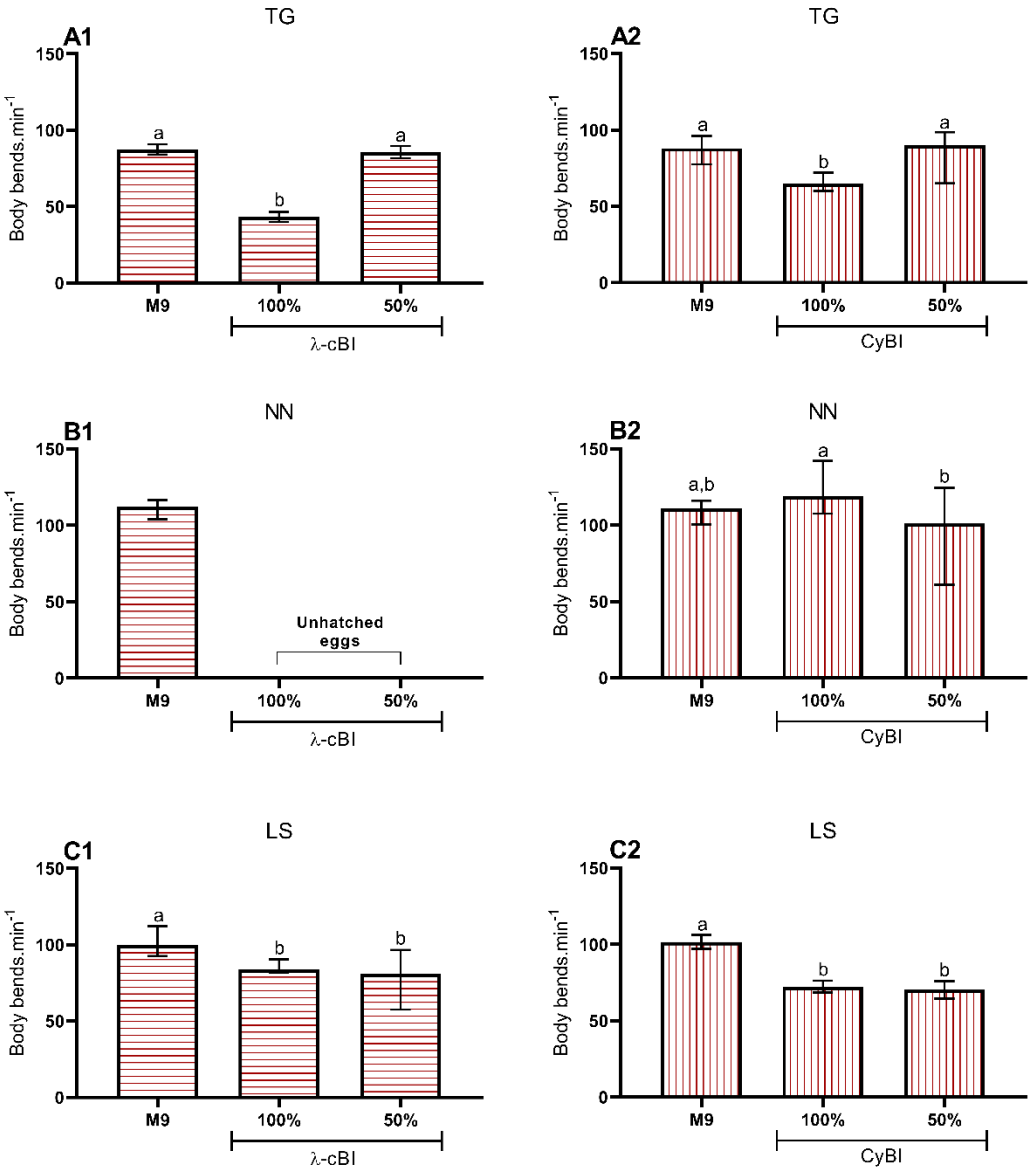

Fig. 1 – Body bends rate of transgenerational (TG)(A), neonatal (NN)(B), and life span (LS)(C) exposed *C. elegans* (N2 strain) to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI)(2). Data in the panels A2, B1, B2, and C1 are expressed as median ± interquartile range. Data in the panels A1 and C2 are expressed as mean ± SD. Different letters means statistical difference.

3.1.2. Pharyngeal pumping

Pharyngeal pumping in the TG exposure way in contact with λ-cBI (Fig. 2, A1) was increased in the concentration 50% in comparison to control ($p < 0.0001$) and 100% ($p < 0.0001$). In the worms exposed in the TG way to CyBI (Fig. 2, A2) the concentration 50% was increased in comparison to control ($p < 0.0001$) and 100% ($p < 0.0001$). In the NN exposure way, the contact to CyBI (Fig. 2, B2) decreased the pharyngeal pumping rate in the 50% in comparison to control ($p < 0.0001$) and 100% ($p < 0.0001$). In the LS exposure way, the contact

to λ-cBI (Fig. 2, C1) at the concentration of 100% had the rate decreased in comparison to control group ($p < 0.01$). In the LS exposure way to CyBI (Fig. 2, C2) the pharyngeal pumping decreased in both concentrations of 100% ($p < 0.0001$) and 50% ($p < 0.01$) in comparison to control group.

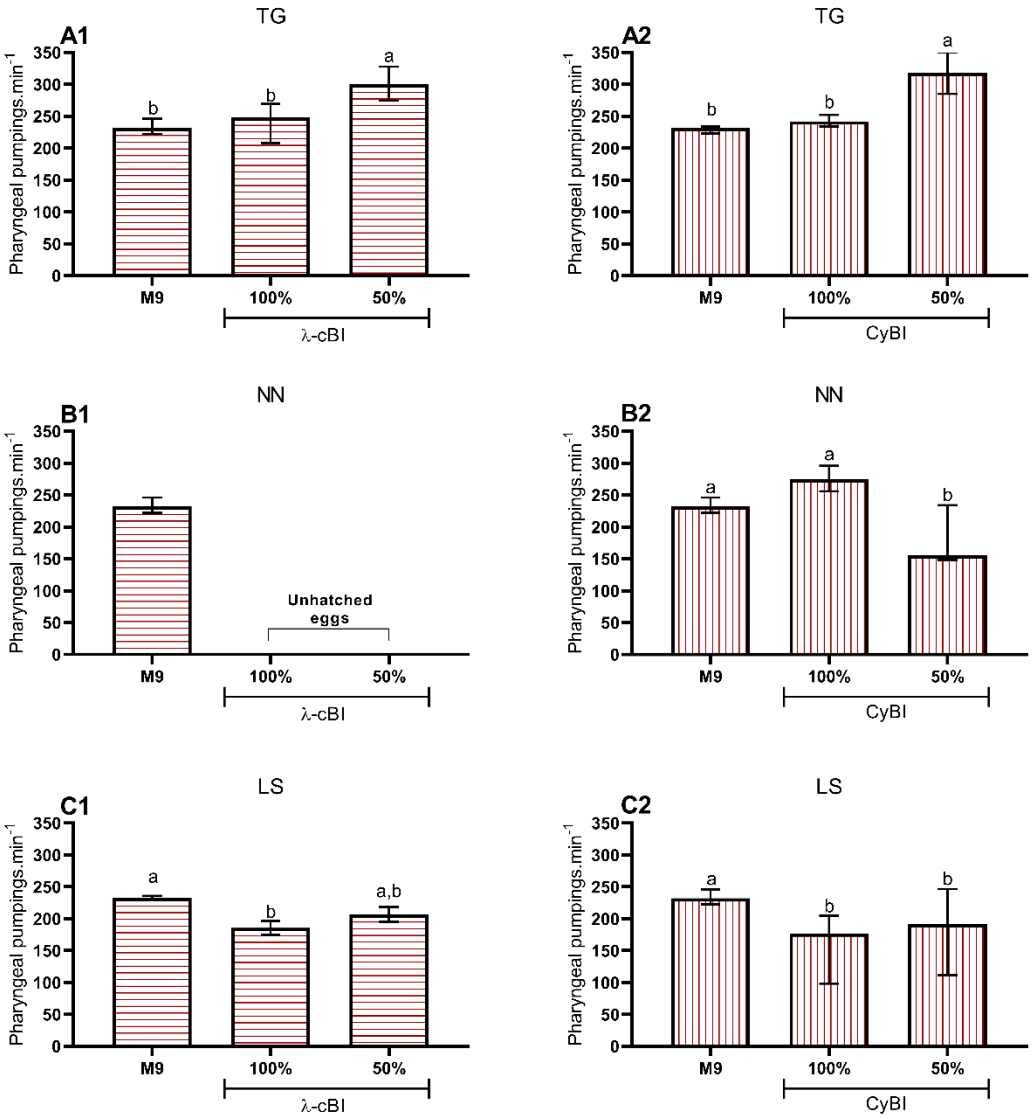

Fig. 2 – Pharyngeal pumping rate of transgenerational (TG)(A), neonatal (NN)(B), and life span (LS)(C) exposed *C. elegans* (N2 strain) to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI)(2). Data in the panels A1, A2, B1, B2, and C2 are expressed as median ± interquartile range. Data in the panel C1 is expressed as mean ± SD. Different letters means statistical difference.

3.1.3. Agglomeration behavior

Agglomeration behavior in *C. elegans* in contact to λ-cBI on TG way (Fig. 3, A1) decreased the amounts that were eaten together in two concentrations of 100% ($p < 0.05$) and 50% ($p < 0.05$) in comparison to control. In the CyBI TG exposed worms (Fig. 3, A2) just the

concentration of 50% decreased the number of worms eaten together in comparison to control ($p < 0.05$). In the NN exposed worms to CyBI (Fig. 3, B2) in the concentration 100% the worms remain far from other worms when compared to control ($p < 0.001$) as well as to 50% ($p < 0.05$). In the LS exposed worms to λ-cBI (Fig. 3, C1) they remains eaten far from other worms in both concentrations of 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control group. The last observation is about the LS exposed worms to CyBI (Fig. 3, C2) that reduced the social behavior while feeding in the concentrations of 100% ($p < 0.0001$) and 50 % ($p < 0.0001$) in comparison to control.

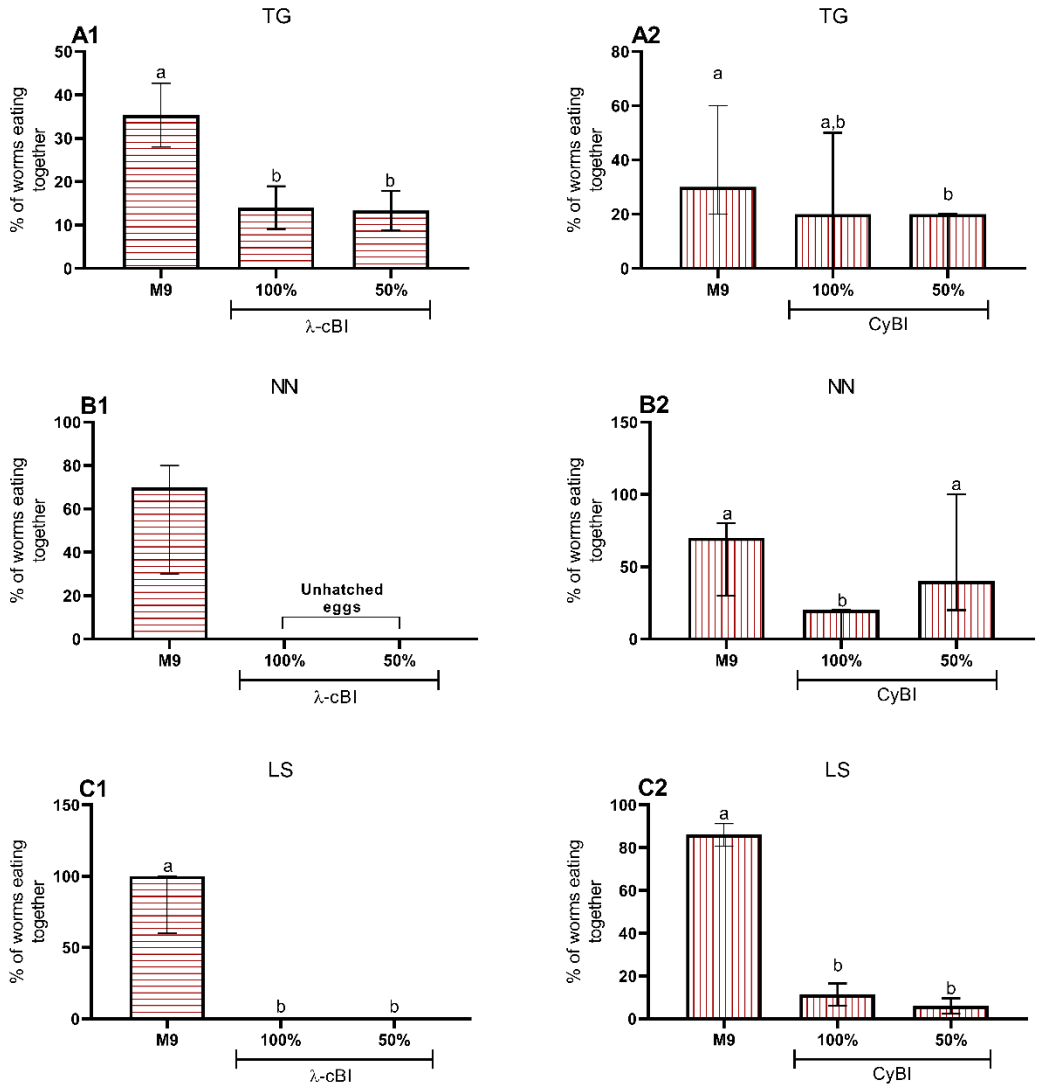

Fig. 3 – Agglomeration behavior pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI)(2). Data in the panels A2, B1, B2, and C1 are expressed as median ± interquartile range. Data in the panels A1 and C2 are expressed as mean ± SD. Different letters means statistical difference.

3.1.4. Borderline behavior

The borderline behavior in the TG exposure was decreased in the λ-cBI (Fig. 4, A1) at the concentration of 100% in comparison to control ($p < 0.001$) and 50% ($p < 0.001$). The group treated with CyBI in the TG exposure way (Fig. 4, A2) have not shown changes on borderline behavior. As NN, in the CyBI group (Fig. 4, B2) either have not shown significant difference. The LS exposure group at the λ-cBI (Fig. 4, C1) the concentration 100% reduced the borderline behavior in comparison to control ($p < 0.05$). In the LS treated with CyBI (Fig. 4, C2) the borderline behavior was either decreased in the 100% concentration in comparison to control ($p < 0.05$).

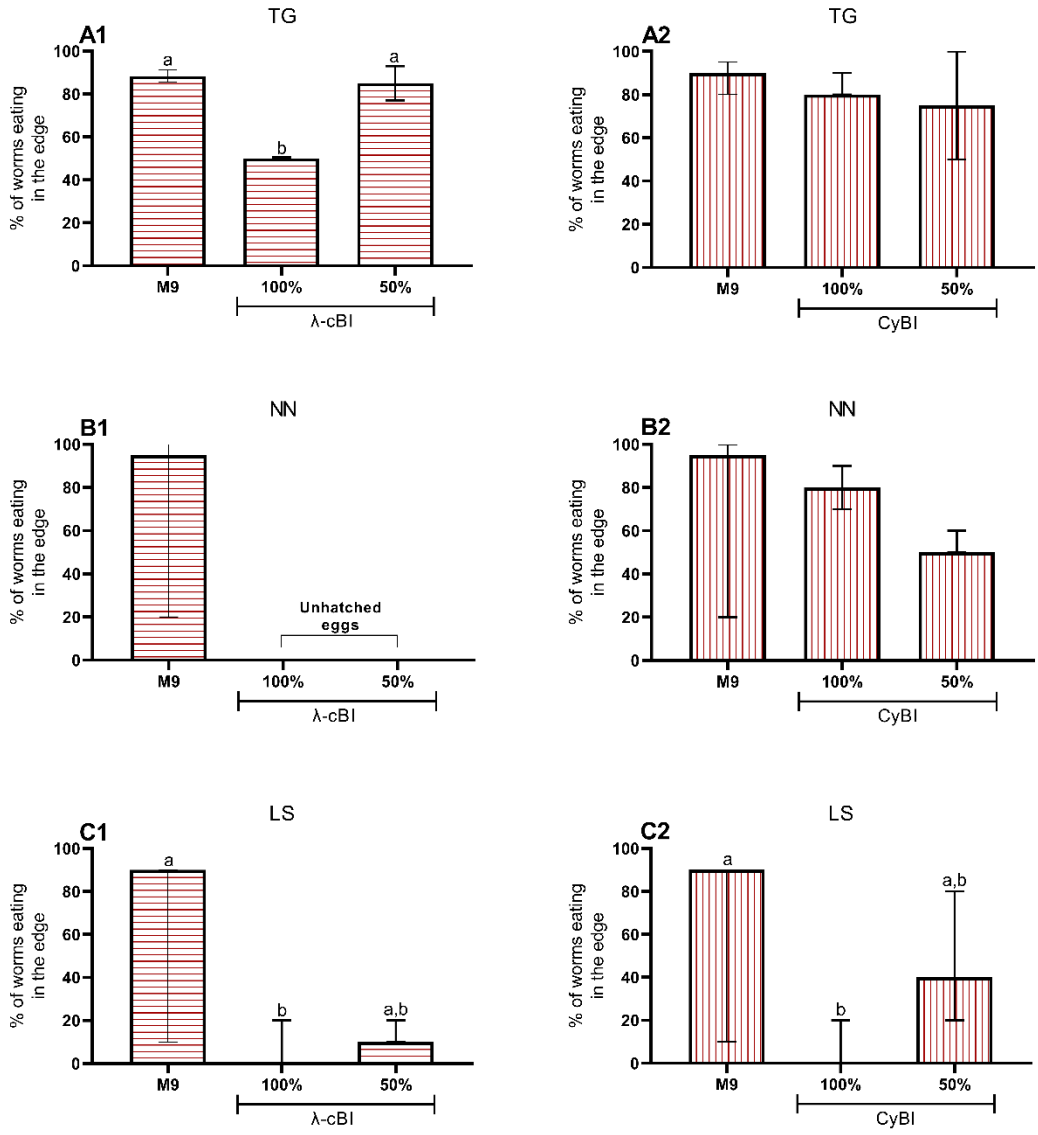

Fig. 4 - Borderline behavior of transgenerational (TG)(A), neonatal (NN)(B), and life span (LS)(C) exposed *C. elegans* (N2 strain) to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI) (2). Data in the panels A2, B1, B2, C1, and C2 are expressed as median ± interquartile range. Data in the panel A1 is expressed as mean ± SD. Different letters means statistical difference.

3.2. Biochemical biomarkers

3.2.1. Acetylcholinesterase activity

AChE activity in the TG exposure way in contact to λ-cBI (Fig. 5, A1) at the concentration of 100% was decreased in comparison to control ($p < 0.01$) and 50% ($p < 0.0001$). In the CyBI (Fig. 5, A2) the activity was than decreased ($p<0.01$). In the NN exposure, in the CyBI (Fig. 5, B2) on the concentration 50% the activity was decreased in comparison to control ($P < 0.01$). In the LS exposure way at the λ-cBI contact (Fig. 5, C1) the AChE activity was decreased in the group treated with 100% in comparison to control ($p < 0.05$). In the CyBI (Fig. 5, C2), the AChE activity was not changed in comparison to control group.

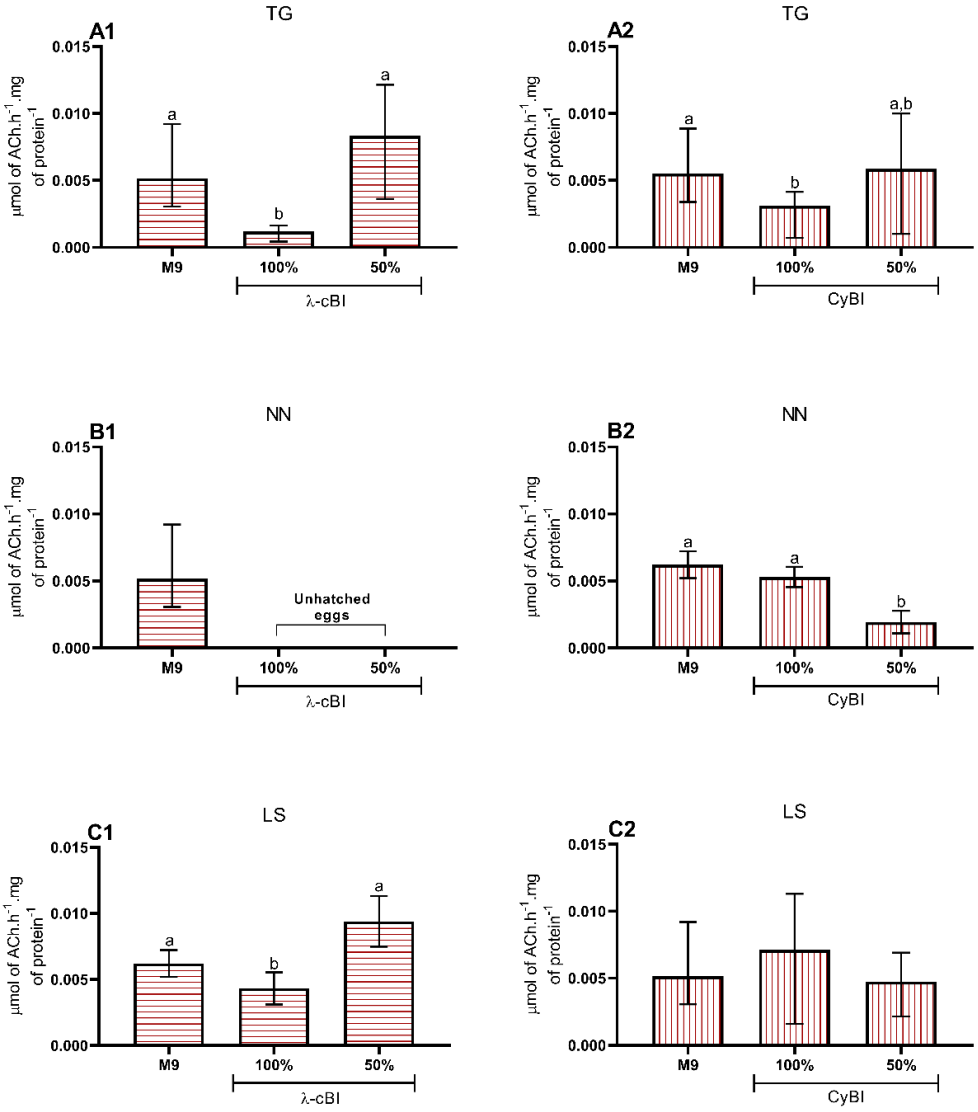

Fig. 5 – Acetylcholinesterase activity of transgenerational (TG)(A), neonatal (NN)(B), and life span (LS)(C) exposed *C. elegans* (N2 strain) to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI)(2). Data in the panels A1, A2, B1, and C2 are expressed as median ± interquartile range. Data in the panels B2 and C1 are expressed as mean ± SD. Different letters means statistical difference.

3.2.2. PolyQ40::YFP aggregation

The PolyQ40::YFP aggregation in the TG (Fig. 6, A1) way of exposure was observed that λ-cBI increased the PolyQ40 aggregation expression on 100% ($p < 0.0001$) and 50% ($p < 0.01$) in comparison to control. In this same exposure way, the CyBI (Fig. 6, A2) was not able to disestablish the PolyQ40 expression in both concentrations. The NN exposure way in the CyBI contact (Fig. 6, B2), the group treated with 100% had the PolyQ40 aggregates increased in comparison to control ($p < 0.0001$) and with 50% ($p < 0.01$). In the LS exposure way in the λ-cBI contact (Fig. 6, C1) the concentration of 100% killed all the individuals and it was just observed at this concentration as well as in this strain, it might be due to the increased instability of these strain once have the insertion of the human transgene for PloyQ40 what makes it more susceptible for death. In the concentration 50% was observed a decrease in the PolyQ40 expression in comparison to control group ($p < 0.001$). In the CyBI exposure (Fig. 6, C2) the concentration of 100% increased the amount of the PolyQ40 aggregation in the muscle in comparison to control ($p < 0.01$).

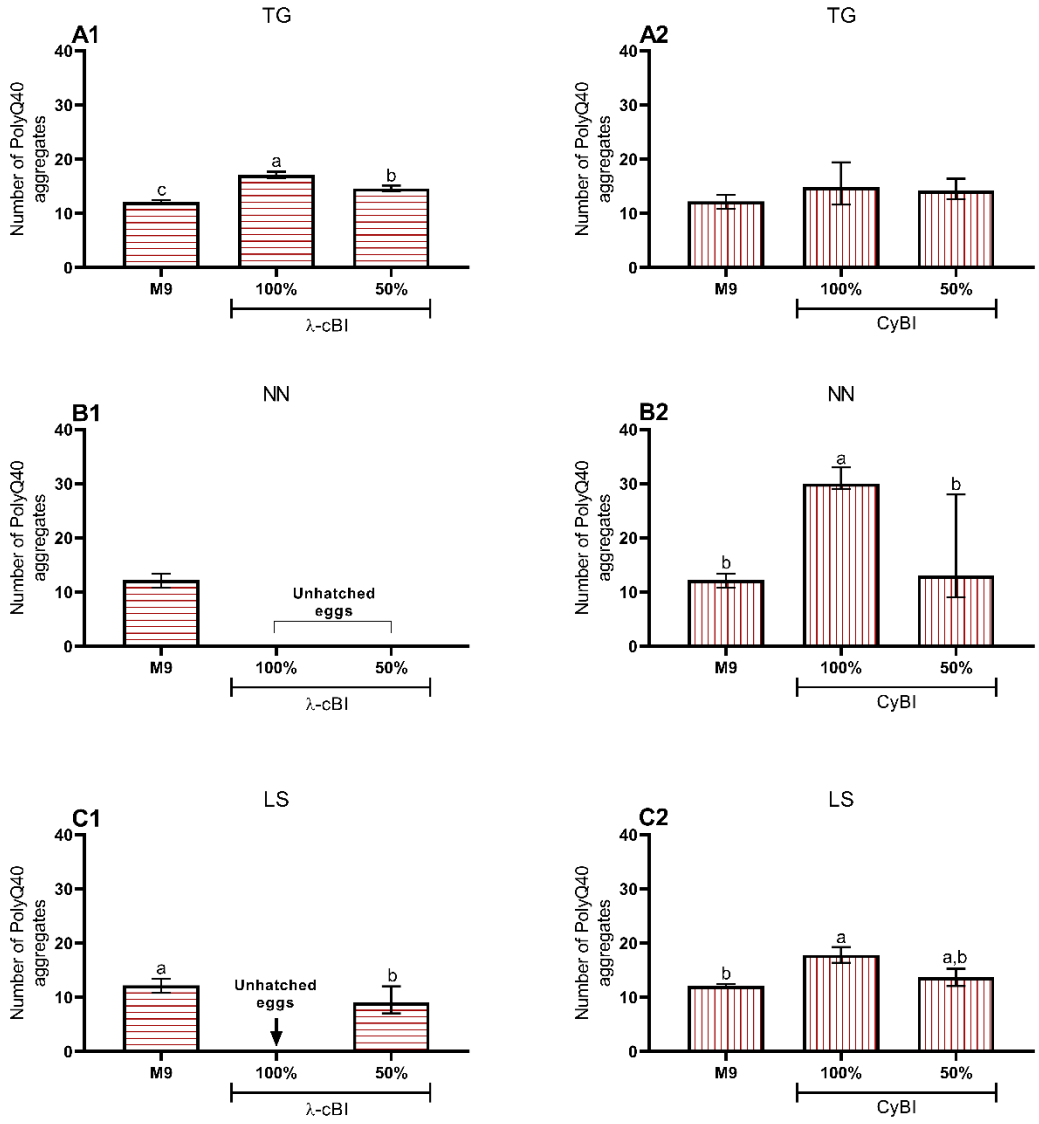

Fig. 6 – Number of PolyQ40::YFP aggregated in transgenerational (TG)(A), neonatal (NN)(B), and life span (LS)(C) exposed *C. elegans* (AM141 strain) to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI)(1) and cypermethrin (CyBI)(2). Data in the panels A2, B1, B2, and C1 are expressed as median ± interquartile range. Data in the panels A1 and C2 are expressed as mean ± SD. Different letters means statistical difference.

3.2.3. Redox status

3.2.3.1. SOD-3:GFP quantification

In the SOD-3:GFP fluorescent expression in the TG exposure way in the λ-cBI exposure (Table 2) was not observed changes in the expression of the enzyme. In the CyBI exposure the SOD-3:GFP expression was increased in the 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control group. In the NN exposure way in the CyBI exposure was no observed changes on SOD-3:GFP expression. In the LS exposure way in the λ-cBI was observed an

increase on SOD-3:GFP expression in the 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control group. In the CyBI exposure was observed an increase in the group 100% in comparison to control ($p < 0.0001$) and 50% ($p < 0.0001$).

3.2.3.2.CTL-1.2,3:GFP quantification

CTL-1.2,3:GFP fluorescent expression in the TG exposure way in the λ-cBI contact (Table 2) decreased the expression in the group 100% in comparison to control ($p < 0.0001$) and 50% ($p < 0.0001$). In the CyBI contact the group treated with 100% increased the CTL-1.2,3:GFP expression in comparison to control ($p < 0.05$) and 50% ($p < 0.0001$). In the NN exposure way when in contact of CyBI was observed an increase in the 100% group when compared to control ($p < 0.01$) and 50% ($p < 0.001$). In the LS exposure when in contact of λ-cBI was observed an increase in the group treated with 100% in comparison to control ($p < 0.001$) and then an increase in the CTL-1.2,3:GFP expression in the 50% in comparison to 100% ($p < 0.0001$) and control ($p < 0.0001$) groups. In the CyBI contact was observed an increase in the groups 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control group.

*3.2.3.3.*GST-4:GFP quantification

GST-4:GFP fluorescent expression in the TG way of exposure in contact with λ-cBI (Table 2) was decreased in the group 100% in comparison to control ($p < 0.0001$) and 50% ($p < 0.0001$). In the contact with CyBI was observed a decrease in the 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control group. In the NN way of exposure in the contact with CyBI was observed a decrease in the group treated with 50% in comparison to control group ($p < 0.0001$). In the LS exposure way, when in contact with λ-cBI was observed a decrease in the GST-4:GFP expression in the concentration 100% in comparison to control ($p < 0.01$) and a decrease in the 50% in comparison to control group ($p < 0.0001$) and 100% ($p < 0.0001$). When in contact with CyBI was observed a decrease in the 100% in comparison to control ($p < 0.0001$) and then a decrease in the 50% in comparison to control ($p < 0.0001$) as well as with 100% ($p < 0.0001$).

3.2.3.4. HSP-16.2:GFP quantification

The HSP-16.2:GFP fluorescent expression on TG way of exposure to λ-cBI (Table 2) was increased in the group treated with 100% in comparison to control ($p < 0.0001$) and with 50% ($p < 0.0001$). In the CyBI exposure was observed an increase in the group treated with 100% ($p < 0.0001$) and 50% ($p < 0.01$) in comparison to control group. In the NN exposure way, in the worms exposed to CyBI was observed an increase in the HSP-16.2:GFP expression in the group treated with 50% from the control ($p < 0.0001$) and the concentration of 100% ($p < 0.01$), as well as the group treated with 100% then increased in comparison to control ($p < 0.0001$). In

the LS exposure way, in the worms exposed to λ-cBI was observed a decrease in the HSP-16.2:GFP expression in the concentration of 50% in comparison to control ($p < 0.0001$) and 100 ($p < 0.01$). In the worms exposed to CyBI was observed a decrease in the HSP-16.2:GFP in both groups 100% ($p < 0.0001$) and 50% ($p < 0.0001$) in comparison to control.

Table 2 – Redox status of *C. elegans* evaluated by fluorescent expression of Superoxide dismutase (SOD-3:GFP), Catalase (CTL-1,2,3:GFP), Glutathione-S-transferase (GST-4:GFP), and heat shock protein (HSP-16.2:GFP). All parameters were evaluated under three different protocol of exposure been transgenerational (TG), neonatal (NN), and life span (LS). The worms were exposed to two different pyrethroid-based insecticides been λ-cyhalothrin (λ-cBI) and cypermethrin (CyBI). Data are expressed as mean ± SD. Different lowercase bold letters mean statistical difference among the concentrations in the same insecticide and exposure protocol. All results are expressed as fluorescence intensity.

Biochemical biomarker	*Protocol of exposure*	*Dose of insecticide*	*λ-cBI*	*CyBI*
SOD-3::GFP	TG	*M9*	*22.56 ± 1.4*	*22.56 ± 1.4 b*
		100%	*22.28 ± 2.2*	*25.19 ± 2.1 a*
		50%	*23.53 ± 2.3*	*25.78 ± 1.9 a*
	NN	*M9*	*21.43 ± 1.1 a*	*21.43 ± 10.0*
		100%	*0 ± 0.0 b*	*20.71 ± 1.7*
		50%	*0 ± 0.0 b*	*23.75 ± 9.2*
	LS	*M9*	*7.31 ± 0.8 b*	*6.56 ± 2.7 b*
		100%	*14.49 ± 1.0 a*	*21.81 ± 4.8 a*
		50%	*15.82 ± 1.1 a*	*5.4 ± 2.0 b*
CTL-1,2,3::GFP	TG	*M9*	*239.40 ± 0.33 a*	*239.4 ± 0.33 b*
		100%	*219.40 ± 0.23 b*	*326.1 ± 7.4 a*
		50%	*238.40 ± 0.28 a*	*227.2 ± 0.38 b*
	NN	*M9*	*237.90 ± 0.49 a*	*23,79 ± 0.49 b*
		100%	*0.000 ± 0.0 b*	*276.9 ± 1.0 a*
		50%	*0.000 ± 0.0 b*	*228.3 ± 0.35 b*
	LS	*M9*	*179.90 ± 0.38 c*	*179.9 ± 0.38 b*
		100%	*218.10 ± 0.52 b*	*221.8 ± 0.66 a*
		50%	*341.50 ± 0.78 a*	*228.7 ± 0.75 a*
GST-4::GFP	TG	*M9*	*224.5 ± 4.3 a*	*224.5 ± 4.3 a*
		100%	*146.4 ± 4.6 b*	*146.6 ± 6.1 b*
		50%	*234.6 ± 6.5 a*	*146.2 ± 42.5 b*
	NN	*M9*	*270.2 ± 4.6 a*	*270.2 ± 4.5 a*
		100%	*0.000 ± 0.0 b*	*209.4 ± 13.6 a*
		50%	*0.000 ± 0.0 b*	*116.5 ± 4.8 b*
	LS	*M9*	*270.2 ± 4.5 a*	*270.2 ± 4.5 a*
		100%	*199.9 ± 9.4 b*	*125.3 ± 1.0 b*
		50%	*142.5 ± 3.5 c*	*89.29 ± 2.8 c*
HSP-16.2::GFP	TG	*M9*	*23.16 ± 0.3 b*	*23.16 ± 0.3 b*
		100%	*25.50 ± 0.3 a*	*25.19 ± 0.3 a*
		50%	*22.72 ± 0.3 b*	*24.41 ± 0.2 a*
	NN	*M9*	*20.92 ± 0.1 a*	*20.92 ± 0.1 c*
		100%	*0.000 ± 0.0 b*	*24.62 ± 0.4 b*

	50%	*0.000 ± 0.0 b*	*27.71 ± 0.4 a*
	M9	*28.70 ± 0.4 a*	*29.20 ± 0.5 a*
LS	*100%*	*28.13 ± 1.2 a*	*24.09 ± 1.1 b*
	50%	*27.11 ± 1.7 b*	*20.85 ± 0.2 b*

4. Discussion

Here we evaluated the toxic effects of λ-cihalothrin- (λ –cBI) and cypermethrin- (CyBI) based insecticide in transgenerational (TG), neonatal (NN), and life span (LS) protocols in *C. elegans* model organisms. The effect of TG and LS seems to be similar in many analyzed parameters such as body bends and pharyngeal pumping concluding that they reduced the health spam of the worms more that the NN exposure. Although, the mechanism involved in these changes do not seems to be similar due to distinct formulations.

We observed a reduction in the body bends in the TG and pharyngeal pumping in the TG and LS. Body bends are the locomotor movements in *C. elegans*. The amplitude of these movements is controlled by PVD (nociceptive neurons) and DVA (interneuron with cell body in dorsal rectal ganglion) neurons (Supplementary figure 3) (Schafer, 2015). These neurons are presynaptic to both the forward and backing interneurons, and provide input to both the anterior and posterior touch circuits in *C. elegans* (Albeg et al., 2011). Many receptors are expressed in these neurons for example the mechanosensitive transient receptor potential (TRPN) channel which is predicted to enable calcium channel activity and to be involved in calcium ion transmembrane transport (Nekimken, 2019). So, the reduction of the body bends rate on LS might be related with deficient signaling in the aforementioned neurons. Pyrethroids have effect on ionic channels by increasing the time open for this reason can affect the signaling in the PVD and DVA channels changing the body bends. *C. elegans* does not have sodium ionic channels as vertebrates (Bargmann, 1998), the mechanism of action of pyrethroids are linked with the opening of sodium channels. Thus, *C. elegans* do not have the classic effect of contamination by pyrethroids like the two substances tested (Casida, 2009). In *C. elegans* the sodium-channels are replaced for calcium and potassium channels. Even so, reported changes were observed, as it can act on calcium and potassium channels present in neurons. In other studies, carried out with *C. elegans* were observed that pyrethroids are linked with down-regulation of the voltage-gated calcium channels (VGCC). This modulation is due to an clear effect in the expression of α1-subunit of VGCC (Zeng et al., 2017).

Body bends, and pharyngeal pumping alterations in *C. elegans* are linked with exogenous and/or endogenous alterations (Kaplan and HoRVITZ, 1993). Exogenous changes in the behavior can be due to the food quality, medium temperature, odorants and many others.

However, all these changes are induced due to central responses that configures the cognition and health spam of the worm. Problems in the central response are linked with physiological alterations and might leads to the individual death (Stergiou and Hengartner, 2004). In this way, many endogenous changes are linked with nervous system such as cholinergic signaling.

Here, the enzyme acetylcholinesterase (AChE) decreases specially in the TG probably actuate as the mechanism for the underlying the behavioral changes observed. When AChE is inactivated or even reduced, the neurotransmitter acetylcholine (Ach) remains for a longer time in the synaptic cleft and cause an hiperexcitation of the central and peripheral nervous systems changing the behavior, physiology as well as the quality of life leading to death as reviewed by Tamagno et al., (2022). Cholinergic signaling is responsible mainly for cognition and movement. The neurotransmitter ACh agonize muscarinic and nicotinic receptors in the central and peripheral nervous systems (Kaizer et al., 2008). Once liberated in the synaptic cleft, ACh remains exciting the postsynaptic termination until the enzyme AChE clivate the ACh in acetate and choline. Many pesticides have the cholinergic signaling as mechanism of action such as organophosphates. Although, pyrethroids do not have the cholinergic signaling as target mechanism but can affect AChE (Thakur and Pomeroy-Black, 2019).

Another result that migth be related to the AChE alterations is the pharyngeal pumping, that was increased in the TG and reduced in the LS. This observation suggests that in the TG the affected mechanism by pyrethroids that is transferred to the progeny, is the AChE decreased activity. With AChE reduction is expected that the pharyngeal pumping increase the rate due to the increased excitability of the nervous system (Tamagno et al., 2021). This increased pharyngeal pumping rate is observed just in the TG due to transgenerational effects of the compounds. In the LS a decrease on pharyngeal pumping is observed what is related with senile aging and chronic exposure to toxicants (Tamagno et al., 2022b), highlighting the chronic effect of the pyrethroids.

As aforementioned, the behavioral repertoire is related to exogenous and endogenous changes. The agglomeration is a good example of this, and can than underline the discussion above. Social feeding is related to cognition and several neurotransmitters are involved in its performance (Dallière et al., 2017). In the TG, AChE was reduced and as a result of it the social perception and feeding behavior was decreased in both λ-cBI and CyBI characterizing a central effect on cognition of the worm. While in the LS, the social behavior was largely decreased in both, we hypothesized that it can be due to two interactions: first due to body bends decrease that decrease the motility and consequently the aggregation of the worms, and in second the olfactory deficiency. For feeding, the chemosensory regulation of locomotion is critical

(Milward et al., 2011). The gene *npr-1* is linked to the modulation of the social behavior by acting on several neural circuits (Coates and De Bono, 2002). In addition, social behavior depends on nociceptive neurons that signal through chemoreceptors. These neurons apparently detect repulsive signals produced by bacteria inducing aggregation (de Bono et al., 2002). To finding food the worms use neurons of the olfactory bulb to recognize the environment and the food, for this is necessary activate ionotropic channels for begin the neurotransmission to other neurotransmitters before beginning to eat. Considering that in the LS the worms were kept whole life in a medium with both λ-cBI or CyBI that affect the ionotropic channels is expected them become to be deficient and affect the physiology of the olfactory bulb resulting in feeding alterations.

In conclusion, even though the behavioral changes observed in TG and LS were similar, we highlight here two different mechanisms responsible for the behavioral changes observed in each of the protocols. While in TG the worm does not have contact with the compound throughout its life, it is hypothesized that the observed behavioral alteration is due to central alterations initiated in the embryological period. A decreased modulation on the enzyme acetylcholinesterase appears to be behind the behavioral changes. In this context few studies are found in the literature that highlight the clear mechanism for AChE transgenerational down-regulation in *C. elegans*. On the other hand, what was observed in the LS protocol did not properly alter AChE activity, but similarly changed the behaviors, which seems to be involved in a permanent opening of the DVA and PVD VGCC, typical of a chronic effect of the substances tested.

Another important observation of our work is due to PolyQ40::YFP aggregates in mutant worms that were increased in the λ-cBI on TG and CyBI on LS and NN. PolyQ40::YFP aggregates in *C. elegans* are long glutamine expressions that create a tangled form of the protein in the muscle cells affecting the motility as well as the quality of life. In humans, PolyQ40::YFP aggregates are present on Huntington's Disease (HD). HD is a neurodegenerative and hereditary disease that affects the central nervous system, causing changes in movement, behavior, and cognitive ability (Markaki and Tavernarakis, 2020). Many toxic compounds can affect the transcription of a gene, and in this study, we observe that even the λ-cBI and CyBI can increase the expression of the PolyQ40::YFP aggregates. The environmental effect in a gene transcription can be exerted before the transcription process in the up-regulation of a gene transcription or, in hereditary cases, interact with pre- or post-transcriptional preparing protein process (Schott et al., 2014). In this second one, interact with folding, transport, storage, and solubilization of different proteins. The expanded glutamines supposedly impair the function

of mitochondria, chaperones and the ubiquitin proteasome system, that is, disrupt the proteostasis in neuronal cells (Peixoto et al., 2016; Weber et al., 2014). Environment toxicants might increase the prevalence of hereditary conditions due to interact with the post-transcriptional process.

Cellular stress response is important for reestablishment of the homeostatic status in the cell. Stress protein such as heat shock proteins (HSPs) are linked with cellular stress avoidance or protection against heat or toxic stress. Here the HSP-16.2::GFP was increased in the worms exposed to λ-cBI on TG and CyBI on TG and NN. The lipophilic nature of these compounds easily allows it to pass through the plasma membrane and alter vital cellular function before interacting with cellular proteins, denaturing them and triggering stress protein induction (Shashikumar and Rajini, 2010). It has been reported that HSPs induction by certain toxicants is generally correlated with early cytotoxic events and is a secondary consequence of damages that affect cellular integrity (Gupta et al., 2005). This increase highlights the stress response in a TG way. In the LS exposure the HSP-16.2::GFP was decreased, this observed effect can be harmful to the organism oxidative scavenger response. In a study carried out with cypermethrin in *C. elegans*, after a long-time exposure the HSP-16.2::GFP were reduced (Shashikumar and Rajini, 2010), it might explain our finds.

Another mechanism for cellular toxicity avoidance is the enzymatic antioxidant system that act to help in the free radicals scavenger. The antioxidant enzymes help to reduce toxic and reactive molecules into less toxic and eliminate them. The enzymes superoxide dismutase (SOD) and catalase (CTL) are enzymes linked to the first defense line in the organism (dos Santos Carvalho et al., 2012). In GFP expression of antioxidant enzymes to pesticides were highlighted that pyrethroids have harmful effect on *C. elegans* antioxidant enzymes (Ruan et al., 2009; Shashikumar and Rajini, 2010). SOD reacts with the superoxide anion (O_2^-) reducing it to hydrogen peroxide (H_2O_2) (Chang et al., 2020). In this way, the H_2O_2 is reduced into water (H_2O) and oxygen (O_2) by the CTL enzyme (Tamagno et al., 2021). In our study the CyBI increased the SOD-3::GFP on TG and LS as well as the λ-cBI increased in the LS. This could be probably due to the ability of these pesticides to induce an over-production of O_2^- in the tissue of the exposed worms that stimulates the activity of SOD to catalyze O_2^- into H_2O_2 (Chatterjee et al., 2021; Nataraj et al., 2017). This might be related with the observed increase on SOD-3::GFP expression. After the increased SOD, was observed an increase in the CTL-1,2-3::GFP in same groups. The reason for CTL-1,2-3::GFP increase is due to its subtract increase (H_2O_2) formed by SOD. So, both CTL and SOD work in a tandem manner, with the increasing activity of SOD, CTL also increases. In the present study, all pesticides induced a

significant increase in CTL-1,2-3::GFP expression probably due to its role in the neutralization of the ROS generated by the pesticides (Bertrand et al., 2016; Chang et al., 2020; Chatterjee et al., 2021).

In the second line of antioxidant defenses, the enzyme Glutathione-S-transferase (GST), acts inserting glutathione groups in toxic compounds in order to transform these compounds more hydrophilic and eliminate them (Shou-Min, 2012). Here we evaluated the GST-4::GFP expression, and both compounds CyBI and λ-cBI in all three protocols of exposure (TG, NN, and LS) reduced the GST activity. GST-4::GFP reduced is related with cellular loss of cellular ability of fighting against toxic compounds.

At least, the NN protocol in the λ-cBI was not able to be evaluated due to non-hatching of eggs. In this protocol, the worms were exposed right after synchronizations and were kept in contact with the solution still reach the total hatching of eggs. Probably the effect of λ-cBI in eggs avoided the hatching due to low quality of the outside environment or causing the embryo death. This is dangerous for the specie once that the compound is largely used in crop fields where *C. elegans* live freely. In the other hand, the NN protocol in the CyBI, the body bends were not changed in comparison to control. The pharyngeal pumping was decreased in the 50% as well as the social feeding were decreased in the 100% and the borderline behavior had no changes. AChE activity was largely decreased in the NN CyBI which is the key for the behavioral observed alterations. In addition, taken together, the aforementioned changes on PolyQ40::YFP, HSP-16.2::GFP, CTL-1,2,3::GFP and GST-4::GFP characterizes a persistent effect of these compounds in the worm's life.

The last comment about our work is the relevance for the SDGs of UN that talk about agricultural efficiency so that we can produce food, and energy crops, in a less resource-intensive way. Looking for the results presented here, become more necessary to ensure food and nutritional security, including advances in genetics and technologies that allow the sustainable intensification of crops, livestock, and fish production to help meet demand as efficiently as possible using even fewer agrichemicals that interfere in non-target animals.

5. Conclusion

We concluded that the exposure to λ-cBI as well as CyBI is linked with transgenerational and persistent deficient cholinergic signaling as well as a sign for chronic nervous damage. All these changes are linked with behavioral alterations and PolyQ40::YPF overexpression increasing the incidence of HD in heditary predisposed individuals. In addition,

both compounds change the oxidative homeostasis due to overproduction of free radicals changing enzymes from the first and second antioxidant defense line. Altogether the results lead to conclude that even non-target animals might suffer physiological changes that can compromise the survival of species and lead to an ecological imbalance.

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Conflict of interest

The authors declare that they have no competing interests.

Authors contributions

W A Tamagno: Conceptualization; Data curation; Formal analysis; Writing –original draft; Investigation; Project. **C Alves:** Methodology, Writing –review & editing; Data curation; Formal analysis. **A Pompermaier:** Methodology, Writing –review & editing; Data curation; Formal analysis. **A P Vanin:** Methodology. **Leonardo Barcellos:** Supervision; Writing–original draft; Writing–review& editing; Funding acquisition.

Ethical approval

This work was not carried out with vertebrate animals. *Caenorhabditis elegans* is a soil nematode and does not need any ethical approval to be studied.

Patient consent to participate

Not applicable.

Permission to reproduce material prim other sources

Not applicable.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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5. DISCUSSÃO CONJUNTA

Ao considerarmos os três artigos apresentados envolvendo a exposição transgeracional, neonatal e ao longo da vida à piretroides utilizados de maneira doméstica e na agropecuária, podemos observar que mesmo em doses baixas verificamos neurotoxicidade.

Os principais achados estão relacionados com a exposição transgeneracional, principalmente a praletrina e transflutrina, que mostraram um efeito deletério durante o período uterino-embriológico de *C. elegans*. As alterações comportamentais, bioquímicas e, principalmente, aumento da expressão de PolyQ40::YFP verificados nos vermes progenitores expostos, também estavam presentes nos seus descendentes quando alcançaram o estágio adulto. Efeito similar ao observado em indivíduos que foram expostos durante toda a vida.

Ainda, mesmo que a AChE não seja um mecanismo alvo clássico dos piretroides, a enzima foi amplamente afetada, principalmente em exposições transgeracionais. O retardo na expressão de proteínas de estresse térmico como as HSP::GFP pode reduzir a eficiência dos mecanismos relacionados ao controle do estresse oxidativo. Nossos resultados sugerem que o aumento do uso de inseticidas domésticos pode exercer um efeito causal de patologias de etiologia desconhecida como a doença de Huntington.

Diferentemente dos inseticidas domésticos, os de uso agropecuário além de afetarem a nível trangeracional e em exposições ao longo da vida. O composto λ-cialotrina impediu que os ovos de *C. elegans* eclodissem em exposições neonatal, resultado bastante curioso, pois nenhum dos outros compostos testados, nem mesmo aquele com associação de organofosforados (cipermetrina), foi capaz de impedir a eclosão dos ovos. A película dos ovos, bem como o revestimento da cutícula dos vermes, impede que muitos compostos causem toxicidade neste período, mas de alguma forma a λ-cialotrina interrompeu o desenvolvimento e eclosão dos vermes nesta exposição, para tal novos estudos com doses ainda mais baixas são necessários para compreender o efeito persistente de exposições neonatal. Podemos concluir que a exposição ao λ-cialotrina, bem como ao cipermetrina, estão ligadas à sinalização colinérgica deficiente transgeracional e persistente, bem como um sinal de dano nervoso crônico.

6. CONSIDERAÇÕES FINAIS

Por fim, salientamos que o o uso incorreto, bem como o descarte incorreto destes compostos, pode afetar organismos não-alvo. Esse efeito, indica que o descarte incorreto destes compostos ou até mesmo pela aplicação, pode alterar a biota do solo e permanecendo no ambiente por um longo período, sendo assim muito perigoso para muitos organismos, podendo levar a um desequilíbrio ecológico. Neste trabalho observamos que os inseticidas piretroides exercem um possível sinal do aumento da expressão de proteínas relacionadas com a doença de Huntington em *C. elegans*. Como perspectivas, pretende-se compreender o efeito destes compostos em peixe-zebra (*Danio rerio*), para elucidar principalmente o efeito toxicológico no sistema nervoso e o impacto no desenvolvimento e comportamento social em modelos vertebrados. O peixe-zebra é uma espécie sociável e por conta disso, compreender os mecanismos que afetem este comportamento pode nos dar uma visão pertinente dos potenciais riscos destes compostos no que tange a preservação de espécies, mas também à nível translacional, trazendo os possíveis efeitos comportamentais e fisiológicos observados no peixe para explicar patologias humanas ainda desconhecidas.

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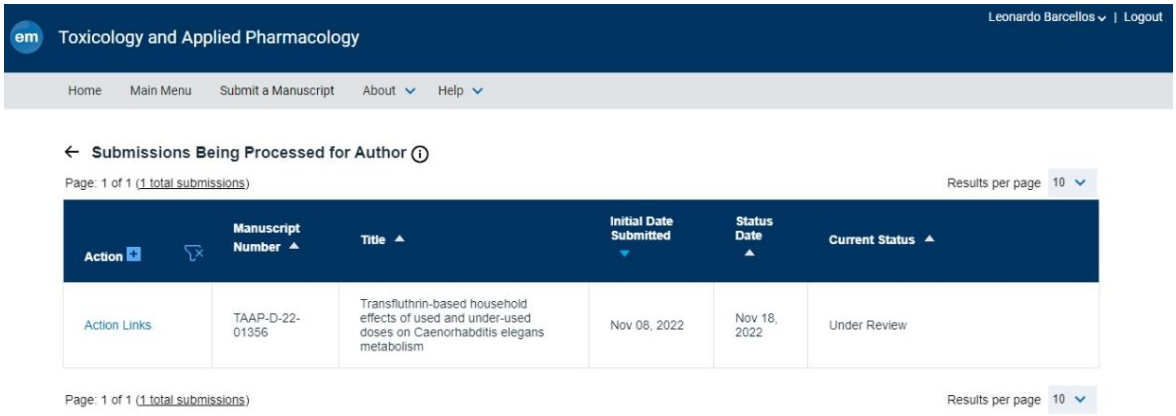
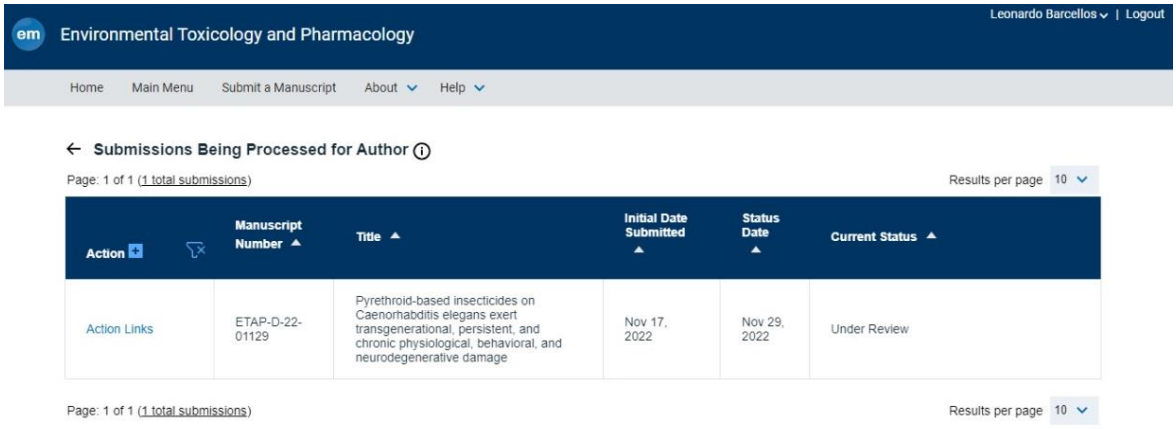
9. ANEXO

ANEXO 1 – Comprovação de submissão do artigo que compõe o capítulo II à revista científica *Toxicology and Applyed Pharmacology* intitulado: *Transfluthrin-based household effects of used and under-used doses on Caenorhabditis elegans metabolism.*

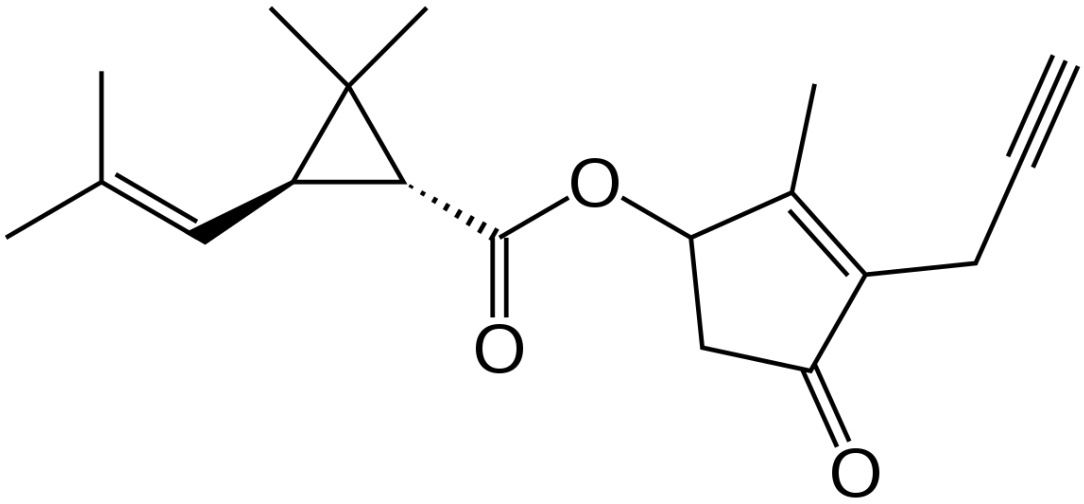


ANEXO 2 – Comprovação de submissão do artigo que compõe o capítulo III à revista científica *Environmental Toxicology and Pharmacology* intitulado: *Pyrethroid-based*

insecticide on Caenorhabditis elegans exert transgenerational, persistent, and chronic physiological, behavioral, and neurodegenerative damage.

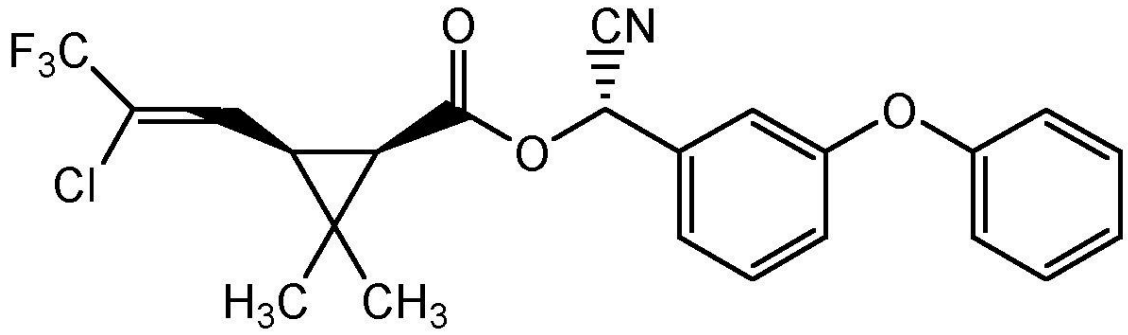


ANEXO 3 – Figura suplementar 1 – CAPÍTULO 1



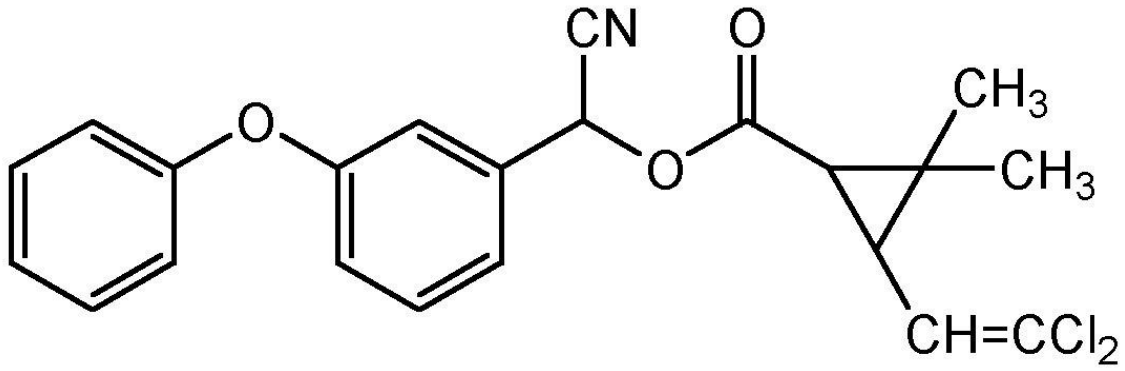
Supplementary figure 9 - Chemical structure of prallethrin ((S)-2-methyl-4-oxo-3-prop-2-ynylcyclopent-2-enyl(1R)-cis-trans-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropa-nencarboxylate).

ANEXO 4 – Figura suplementar 1 – CAPÍTULO 3



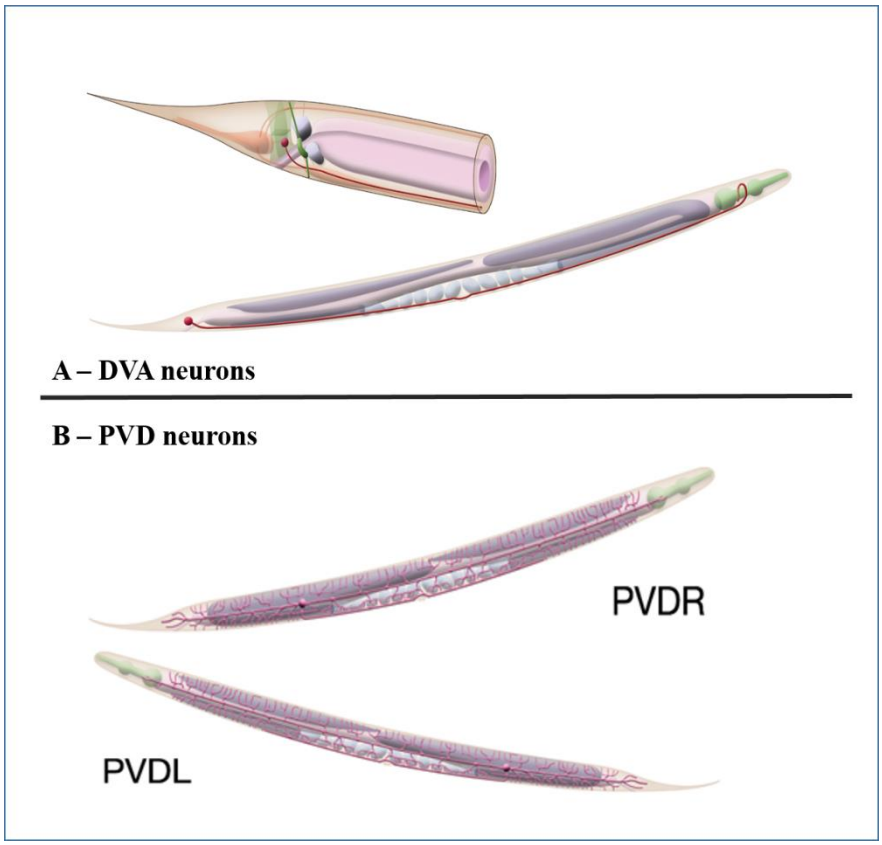
Supplementary figure 10 – L-cyhalothrin chemical structural form (CAS n° 91465-08-6). Available on Chem Service INC – Analytical Standards and Certified Reference Standards (https://www.chemservice.com/lambda-cyhalothrin-n-12307-100mg.html).

ANEXO 5 – Figura suplementar 2 – CAPÍTULO 3



Supplementary figure 11 - Cypermethrin chemical structural form (CAS n° 52315-07-8). Available on Chem Service INC – Analytical Standards and Certified Reference Standards (https://www.chemservice.com/cypermethrin-n-11545-100mg.html).

ANEXO 6 – Figura suplementar 3 – CAPÍTULO 3



Supplementary figure 12 – DVA (A) and PVD (B) neurons circuits of C. elegans. Images available on https://www.wormatlas.org/neurons/Individual%20Neurons/DVAframeset.html.