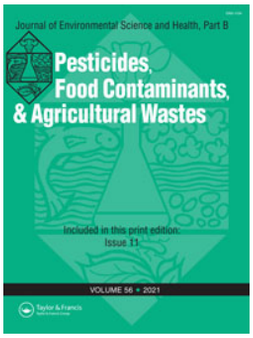

Taylor & Francis
Taylor & Francis Group

Journal of Environmental Science and Health, Part B
Pesticides, Food Contaminants, and Agricultural Wastes

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lesb20

In vivo mutagenic effects and oxidative stress parameters evaluation of cypermethrin and benzoate of emamectin and their mixtures in female mice

Wagner Bragante, Valéria Dornelles Gindri Sinhorin, Marina Mariko Sugui, Ana Paula Simões da Cunha, Weslley Bressan dos Santos & Adilson Paulo Sinhorin

To cite this article: Wagner Bragante, Valéria Dornelles Gindri Sinhorin, Marina Mariko Sugui, Ana Paula Simões da Cunha, Weslley Bressan dos Santos & Adilson Paulo Sinhorin (2022): *In vivo* mutagenic effects and oxidative stress parameters evaluation of cypermethrin and benzoate of emamectin and their mixtures in female mice, Journal of Environmental Science and Health, Part B, DOI: 10.1080/03601234.2022.2045841

To link to this article: https://doi.org/10.1080/03601234.2022.2045841

Published online: 03 Mar 2022.

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
https://doi.org/10.1080/03601234.2022.2045841

In vivo mutagenic effects and oxidative stress parameters evaluation of cypermethrin and benzoate of emamectin and their mixtures in female mice

Wagner Bragante[a], Valéria Dornelles Gindri Sinhorin[a], Marina Mariko Sugui[a], Ana Paula Simões da Cunha[a], Weslley Bressan dos Santos[b], and Adilson Paulo Sinhorin[a]

[a]Programa de Pós-graduação em Ciências Ambientais; Instituto de Ciências Naturais, Humanas e Sociais, Laboratórios Integrados de Pesquisa em Ciências Químicas (LIPEQ), Universidade Federal de Mato Grosso, Câmpus de Sinop, Brazil; [b]Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso, Câmpus de Sinop, Brazil

ABSTRACT

We evaluated the biological effects of ingestion by gavage, for 28 days, of the pesticides cypermethrin (CP) and emamectin benzoate (EB) and their mixtures in female *Swiss* mice. The groups were Control (water); CP; EB and three distinct concentrations of CP and EB mixture expressed in mg/kg/day. The biological effects were analyzed in the complete blood count and plasma (alkaline phosphatase (ALP), alanine aminotransferase (ALT) and creatinine); the biochemical parameters of oxidative stress (substances reactive to thiobarbituric acid (TBARS); reduced glutathione (GSH); catalase (CAT), superoxide dismutase (SOD) and glutathione-*S*-transferase (GST)), and bone marrow cells obtained from the femur for the micronucleus (MN) test. In the heart, there was a reduction in GSH in the groups (0.5 + 0.67 and 2.5 + 3.37), although in the brain this effect appeared for the other groups, except EB. Brain TBARS increased in CP and in the group (2.5 + 3.37) and platelets increased in the group (12.5 + 16.87). Genotoxic/mutagenic effects, showing a consistent increase dose-dependent effect on micronucleus counting for in the female mice. After 28 days of treatment, we can observe that the pesticide mixtures promoted genotoxic damage and oxidative brain damage in female mice, which can damage the health of these animals and possibly their future offspring.

ARTICLE HISTORY

KEYWORDS

Genotoxicity; oxidative stress; pesticides; platelets; redox disbalance

Introduction

From the 1960s, Brazil became part of the countries that adopted the concepts of the Green Revolution.[1]After more than fifty years, the country has become one of the largest agricultural producers on the planet, with 75,866,854 hectares cultivated,[2] as well as the world's largest consumer of pesticides, reaching in 2015 the mark of 500,000 tons of active ingredients per year.[3]

The exaggerated consumption of pesticides, combined with Brazilian policies that allow the use of several molecules prohibited in developed countries, ends up having serious impacts on the environment, society, and human health, many of them irreversible.[4]

In addition to field agriculture professionals, due to the simultaneous release of pesticides into the environment, the general population is also exposed, allowing contamination by inhalation, dermal contact, or ingestion.[5] Therefore, studies that involve the evaluation of effects such as the mutagenic, genotoxic and cytotoxic potential of pesticides ended up becoming a priority area of research.[6]

EB is an insecticide that is part of the chemical group of avermectins, framed in a mode of action 6 (MoA 6) by the classification of the Insecticide Resistance Action Committee.[7]It is an agonist of GABA (gamma-aminobutyric acid), a neurotransmitter with an inhibitory effect on the central nervous system and neuromuscular junctions,[8]and it has been reported as very toxic, with great neurotoxic potential, promoting oxidative damage in cells and genotoxicity.[9]

CP is a synthetic insecticide belonging to the group of type II pyrethroids, having a cyan group (CN) in the phenoxybenzyl portion,[10] framed to the mode of action 3 (MoA 3) by the classification of the Insecticide Resistance Action Committee.[7]This pesticide act on the sodium channels of the axons[11]and promotes genetic damage, morphological, behavioral, biochemical, oxidative stress in a wide range of species and is also considered highly toxic.[12]

Some studies demonstrated that the mixture of pesticides can potentiate the effects caused by them in non-target species[13] like human beings,[5] and to quantify such damages there are very useful exams, tests, and trials such as the blood count, the study of oxidative stress markers, and the tests to evaluate the genotoxicity.[13,14]

Oxidative stress is a condition that results from an imbalance between the amount of oxidizing and antioxidant compounds in the biochemical processes of cells, in favor of the excessive generation of free radicals or at the expense of

CONTACT Valéria Dornelles Gindri Sinhorin valeriadgindri@gmail.com Programa de Pós-graduação em Ciências Ambientais; Instituto de Ciências Naturais, Humanas e Sociais, Laboratórios Integrados de Pesquisa em Ciências Químicas (LIPEQ), Universidade Federal de Mato Grosso, Câmpus de Sinop, Brazil.

their removal speed.[15]To measure this imbalance, biomarkers for oxidative stress are investigated, such as superoxide dismutase (SOD), catalase (CAT), substances reactive to thiobarbituric acid (TBARS), reduced glutathione (GSH), and Glutathione-S-transferase (GST).[16]In addition, the micronucleus (MN) test is an important tool in toxicological genetics, a method for evaluating various types of cytogenetic damage,[17] enabling the rapid detection of damage caused to the genetic material of organisms exposed to environmental chemical contamination.[18]

Despite we can found studies that show pesticides harm on female mice,[19] most of the works involving pesticides use mainly male animals, and it is important to note that in the natural environment the females, responsible for conducting the pregnancy, are also exposed to these products. Based on that, studies their offspring realized in the state of Mato Grosso, Brazil observed that maternal exposure to pesticides is associated with a higher incidence of congenital malformations in the cities with high use of pesticides,[20,21] as well as, the presence de different classes of pesticides in breast milk human.[22]

So, based on the information set out above and knowing that simultaneous exposure to different commercial pesticides is common and that their mixture can enhance the damage caused by isolated formulations, we decide unprecedentedly to study if the exposure by 28 days could alter the blood parameters, oxidative damage, and the genotoxicity of the mixture of commercial products containing emamectin benzoate and cypermethrin, products commonly applied together in crops, in female *Swiss* mice, comparing with the effects of the same individual commercial formulations.

Materials and methods

Chemicals and reagents

The commercial products used in this study were cypermethrin Nortox 250 CE® (250.0 g/L de (RS)-∝–cyano-3-phenoxybenzyl (1RS, 3RS;1RS, 3SR)-3-(2,2–dichlorovinyl)–2-2-dimethylcyclopropane carboxylate (cypermethrin), 602.5 g/L of C9 pyrolysis chain (ciclosol) - Solvesso 100 and 120.0 g/L of inert ingredients), registered with the Ministry of Agriculture, Livestock and Supply (MAPA) under No. 3101, Nortox SA, Arapongas, PR, Brazil; Proclaim 50® WG (emamectin benzoate, 50 g/kg and other ingredients 950 g/kg) registered on the MAPA under No. 29817, Syngenta Crop Protection Ltda, São Paulo, SP, Brazil; Genuxal® (cyclophosphamide, Baxter), Germany. All reagents were obtained from Sigma-Aldrich (Cotia, São Paulo, Brazil) and Merck (Darmstadt, Germany).

Animals and experimental design

Adult female *Swiss* mice, with an average age of 10 weeks and weight of 30 − 40 g, were purchased from the Central Vivarium of UFMT, Campus Cuiabá. This study was approved by the Ethics Committee on the Use of Animals under protocol number 23108.961189/2018-43. During the entire period (acclimatization for 2 weeks and experimental), the animals remained under controlled conditions of temperature (28 ± 2 °C), relative humidity (55 ± 10%), light cycle (12 hours light/dark) in a vivarium cabinet (Insight, Ribeirão Preto, São Paulo, Brazil), and receiving commercial pelleted feed and filtered water ad libitum.

The 56 animals were divided into 7 groups (n = 8 animals) treated via gavage (300 µL/a day), always at the same time, for 28 days. To choose the dose of pesticides and their mixtures, the sub-chronic contamination test model was used, with doses between the LD_{50} (lethal dose for 50% of the population), and ADI (Acceptable Daily Intake Index), both following the registration of products obtained from MAPA, because it is a common type of exposure in predatory animals, including human beings.[23–25]The dose of cyclophosphamide (CPA, 75 mg/kg) was adapted from the work of Luiz et al.[26]

The experiment followed this outline: (Control) - The animals were treated with water; (CPA) - The animals were treated with water and on the 28th day they received CPA intraperitoneally at a single dose of 75 mg/kg b.w. of commercial product/(this group was used only to the Genotoxicity test); (CP) - The animals were treated with 16.87 mg/kg b.w./day; (EB) - The animals were treated with 12.5 mg/kg b.w./day; Combination of minor doses (0.5 + 0.67) - The animals were treated with mixture of EB + CP, respective doses of 0.5 mg/kg b.w./day and 0.67 mg/kg b.w./day; Combination of intermediate doses (2.5 + 3.37) - The animals were treated with mixture of EB + CP, respective doses of 2.5 mg/kg b.w./day and 3.37 mg/kg b.w./day; Combination of high doses (12.5 + 16.87) - The animals were treated with mixture of EB + CP, respective doses of 12.5 mg/kg b.w./day and 16.87 mg/kg b.w./day.

Except for the CPA group, the other animals received on the 28th day via intraperitoneal saline solution in a volume equivalent to the applied in the group (CPA).

Analysis of biochemical, hematological, and genotoxicity parameters

Twenty-four hours after the end of the treatment period, the animals were anesthetized intraperitoneally, with Chlortamine® (ketamine, 50 mg/kg), Rompun® (xylazine, 20 mg/kg), and Acepran® (acepromazine, 20 mg/kg) with a solution containing the 3 substances prepared at the time of use. Then, a cardiac puncture was performed to obtain whole blood with heparinized syringes. After the blood was withdrawn, the animals underwent cervical dislocation and the tissues (liver, kidney, heart, and brain) and femur were removed. The samples were frozen at −85 °C in an ultra-freezer and on the day of each test, the samples were thawed and subjected to subsequent analysis.

Whole blood samples were used to perform the complete blood count (Leukocytes; Platelets; Red blood cells; Hemoglobin; Hematocrit; Media corpuscular volume (MCV), Media corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC)) and were

Table 1. Complete blood count results for red blood cell (RBC), hemoglobin, hematocrit, leukocyte, and platelets from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products.

	RBC (millions/nm^3)	Hemoglobin (g/dL)	Hematocrit (%)	Leukocyte (%/mm^3)	Platelets (%/mm^3)
CONTROL	6.87 ± 0.47	18.25 ± 2.45	53.98 ± 6.30	7467 ± 1353	223000 ± 93983
CP	7.01 ± 0.35	16.59 ± 3.83	49.25 ± 10.03	5838 ± 1405	230500 ± 142103
EB	7.17 ± 0.48	17.58 ± 2.40	52.16 ± 6.74	6417 ± 1322	158857 ± 73833
(0.5 + 0.67)	6.92 ± 0.48	17.25 ± 2.28	51.83 ± 6.77	9614 ± 2223$^{\#\ \#\#}$	505204 ± 170805$^{\#\#}$
(2.5 + 3.37)	6.86 ± 0.33	17.83 ± 2.63	53.25 ± 7.17	8400 ± 1335$^{\#}$	444357 ± 172721$^{\#\#}$
(12.5 + 16.87)	6.73 ± 0.21	17.14 ± 2.40	50.46 ± 7.14	8029 ± 1923	579600 ± 130977$^{*\#\ \#\#}$

ANOVA followed by *post hoc* Tukey test, $P < 0.001$; ($N = 8$). *Compared to control; $^{\#}$compared to CP; $^{\#\#}$compared to EB.

performed in a Clinical Analysis Laboratory. These samples were analyzed on a biochemical analyzer (XT-18000 Sysmex, Roche, Hitachi Ltd, Tokyo, Japan). This equipment uses the method of detection of electrical resistance (impedance technology) with a hydrodynamic focus. Fluorescence flow cytometry is used to measure leukocytes and to count platelets.

In the plasma samples, the activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) and creatinine dosage were analyzed using commercial kits (Labtest, Diagnostics SA, Minas Gerais, Brazil).

In the evaluation of oxidative stress, for lipid damage in the tissues, substances reactive to thiobarbituric acid (TBARS) were determined by measuring malondialdehyde (MDA) levels, a residue of lipid oxidation. Results were expressed in nmol MDA/mg protein, following the curve of calibration for MDA.[27]

The dosage of reduced glutathione (GSH), one of the main non-enzymatic antioxidants present in cells, based on the formation of anionic thiolate was determined at 412 nm and compared with the standard GSH curve. Data were expressed in µmol of GSH/mg protein.[28] Superoxide dismutase (SOD) activity, which catalyzes the dismutation of superoxide radicals in H_2O_2, was determined by the inhibition of adrenaline oxidation. It was measured at 480 nm, expressed in UI SOD/mg protein.[29] Catalase (CAT), an antioxidant enzyme, which decomposes H_2O_2 produced by cell activity, measured the decomposition of H_2O_2 at 240 nm. Results were expressed in µmol H_2O_2/min/mg protein.[30] Glutathione-S-transferase (GST) activity, belonging to a family of enzymes that has the function of protecting cellular macromolecules from the attack of reactive electrophiles and catalyzing the conjugation of GSH with a wide variety of exogenous and endogenous compounds, was measured based on the formation of GS-DNB adduct and expressed in µmol GS-DNB/min/mg protein.[31]The protein content of the tissues was determined according to Bradford[32] using bovine serum albumin as a standard for the elaboration of the standard curve. These samples were read at 595 nm.

To assess genotoxicity, the micronucleus (MN) test was performed, removing cells from the bone marrow of the femur, according to MacGregor et al.[33]

A formula was used to verify the percent harm reduction as the mean frequency decrease of Micronucleated cells using the formula:[34]

$$(\%)\ \text{reduction} = \frac{(\text{frequency of MN in A} - \text{frequency of MN in B}) \times 100}{(\text{frequency of MN in A} - \text{frequency of MN in C})}$$

Where A corresponds to the positive control group; B the group of analysis (a group that was observed the reduction of micronuclei) and C the negative control group.

Statistical analysis

Biochemical data were submitted to normality and homogeneity of variances test. When the results didn't present normal distribution or homogeneous variances, we realized a non-parametric test (Kruskal-Wallis, *post hoc* Dunn's test) to verify the differences between the experimental groups. On the other hand, if the data were parametric, we submitted to one-way analysis of variance - ANOVA followed by *post hoc* Tukey test.

The data were presented as mean ± standard deviation (SD) and/or median and interquartile range. The frequency of micronucleated cells in the different experimental groups was assessed using the chi-square test.[35] A probability level of $P < 0.05$ was considered significant.

Results

Complete blood count

As shown in Table 1, no significant differences were found between treatments for red blood cell (RBC) count, hemoglobin, hematocrit, as well as for MCV, MCH, MCHC (data not shown). In the leukocyte count, it was observed that the results in the mixture of commercial products (0.5 + 0.67) and (2.5 + 3.37) were higher than CP and (0.5 + 0.67) were higher than EB, not differing from the control.

In the platelet count, we found that the treatment with the mixture in the highest doses showed results significantly higher than the control, CP, and EB. The treatments with minor and intermediate doses had values significantly higher than the commercial product alone (EB).

Results of the biochemical analysis of plasma

According to plasma analysis, in Figure 1, no treatment showed significant differences in ALP and creatinine. Regarding ALT activity, the combination of high doses presented significant reduction when compared to the combination of minor doses.

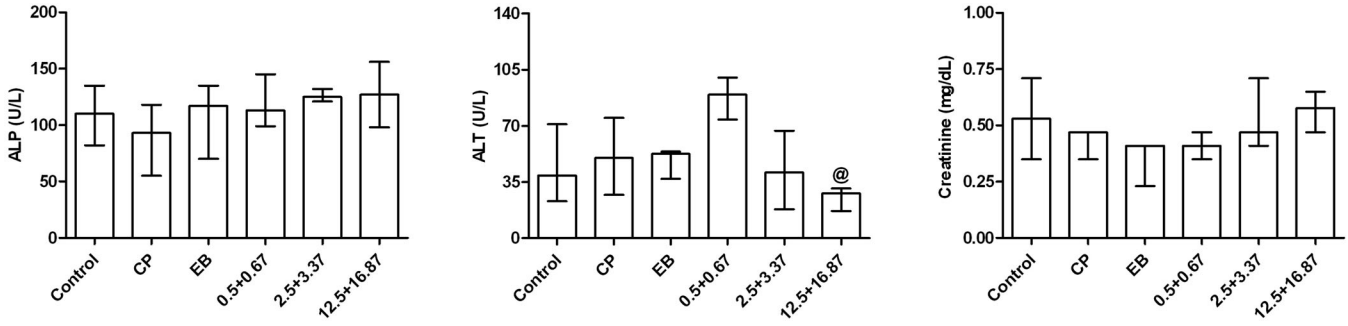

Figure 1. Results of the plasma biochemical analysis from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products. ALP (alkaline phosphatase); ALT (alanine aminotransferase); Creatinine. Kruskal-Wallis analysis followed by *post hoc* Dunn's test; $P < 0.05$; ($N = 8$). @Compared to (0.5 + 0.67).

Results of the analysis of oxidative stress of renal tissue

Data obtained from kidney analysis presented no significant differences between the treatments for catalase, GSH, and TBARS (Fig. 2).

Results of the analysis of oxidative stress in cardiac tissue

In the results of the analysis of cardiac tissues, as shown in Figure 3, catalase activity and TBARS levels were not altered with the different treatments. The concentration of GSH obtained in the (0.5 + 0.67) and (2.5 + 3.37) combinations showed reduced values when compared to the control group and also (2.5 + 3.37) presented reduction when compared to CP.

Results of the analysis of oxidative stress in liver tissue

SOD, GST, and GSH didn't present significant differences between treatments. On the other hand, the CAT activity showed an increase in the (12.5 + 16.87) group when compared to CP and (0.5 + 0.67), but for TBARS we observed that the same group presented a reduction in their levels (Fig. 4).

Results of the oxidative stress analysis in brain tissue

Regarding CAT activity, there were no significant differences between treatments. For GSH, the results obtained in the analysis show that the CP group and all treatments containing the pesticide mixtures had significantly lower values than the control and the (2.5 + 3.37) and (12.5 + 16.87) groups showed reduction when compared to EB. For the TBARS levels, we observed that the CP and the mixture (2.5 + 3.37) showed increased values than the control and that the treatments (2.5 + 3.37) and (12.5 + 16.87) increased TBARS levels when compared to (0.5 + 0.67). Also, there was a reduction in TBARS levels of (0.5 + 0.67) group when compared to CP, as shown in Figure 5.

Micronucleus (MN) test results

To evaluate the mutagenic activity of pesticides alone or in mixtures, the frequency of micronucleated polychromatic erythrocytes was observed in Table 2. There was a significant increase in micronuclei in the groups treated with the pesticide mixture (EB + CP), being an increased dose-dependent, when compared to the control group and with the products alone (CP and EB) for intermediate and high doses.

Discussion

This investigation studied the effects of EB and CP pesticides and their mixture at different doses in female mice treated for 28 days. There are reports in the literature of studies involving analyzes of blood parameters and oxidative stress in the male rats and mice using these pesticides.[36–38]However, our work provides information regarding the use of the mixture of these pesticides comparing their effects also with each alone and their possible harmful effects in female mice, once since they can also be affected by these substances and few studies investigate females.

Pesticides and their mixtures did not cause changes in blood parameters (red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC, and leukocytes), which was already expected according to Anvisa,[39] as they are among the parameters used for the commercial release of pesticides and similarly to studies realized by Aroonvilairat et al.[40]On the other hand, our data diverge from other studies that also tested mixtures containing deltamethrin and cadmium in mice males,[41]and El-Sheikh and Galal,[37] which studied the toxic effects of sub-chronic exposure of male albino rats to emamectin benzoate for 28 days. Differently, platelets increased in the group with the highest dose of the mixture according to Aroonvilairat et al.[40], which also studied a mixture of pesticides. In our study, we can observe that among the hematological parameters evaluated, platelets were the ones that suffered the most changes in the face of different treatments, although leukocytes also showed changes compared to pesticides alone. Such outcome variations may be due to pesticide and mixture used, doses administered, treatment time, and the animal model used in the investigation.

Pesticides used in this study for 28-days did not cause kidney and liver damage, like creatinine and ALP and ALT enzymes did not change, similar to results demonstrated by

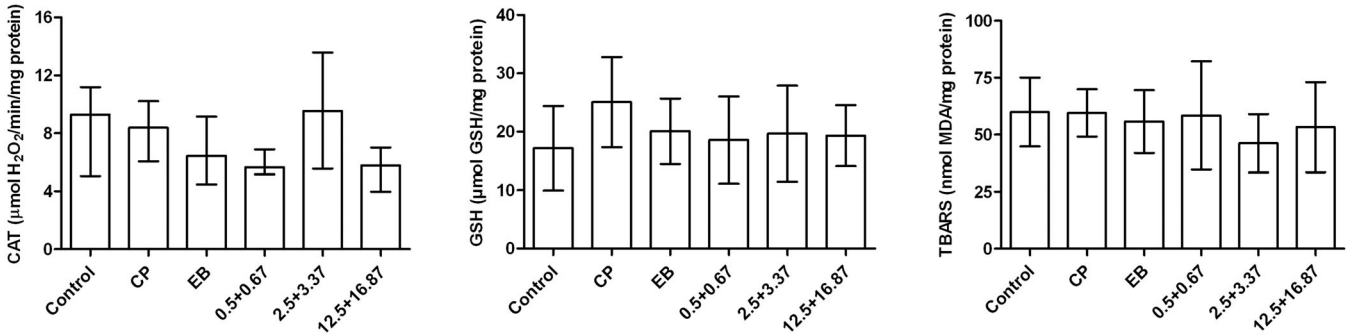

Figure 2. Results of the analysis in renal tissue from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products. CAT (Catalase); GSH (reduced glutathione; ANOVA followed by *post hoc* Tukey test); TBARS (thiobarbituric acid reactive substances). CAT and TBARS (Kruskal-Wallis followed by *post hoc* Dunn's test); $P > 0.05$ ($N = 8$).

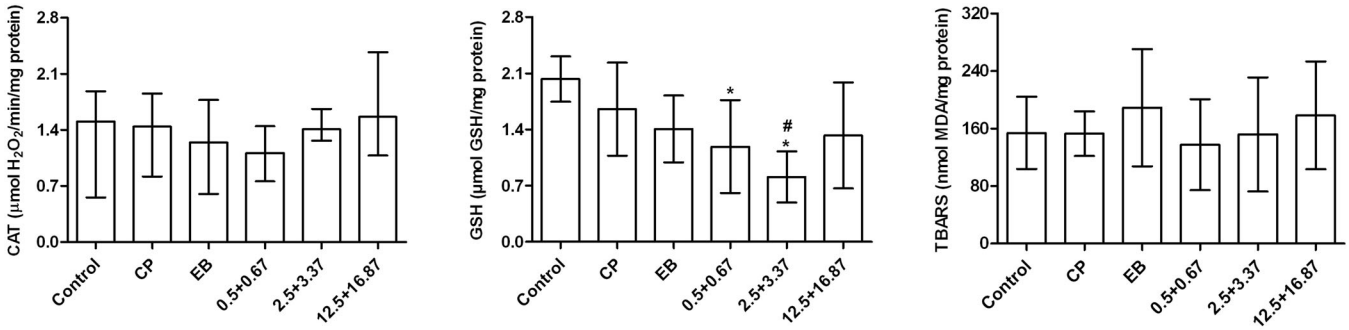

Figure 3. Results of the analysis in cardiac tissue from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products. CAT (Catalase); GSH (reduced glutathione); TBARS (thiobarbituric acid reactive substances; ANOVA followed by *post hoc* Tukey test). Kruskal-Wallis followed by *post hoc* Dunn's test; $P < 0.05$; ($N = 8$). *Compared to to control; #compared to CP.

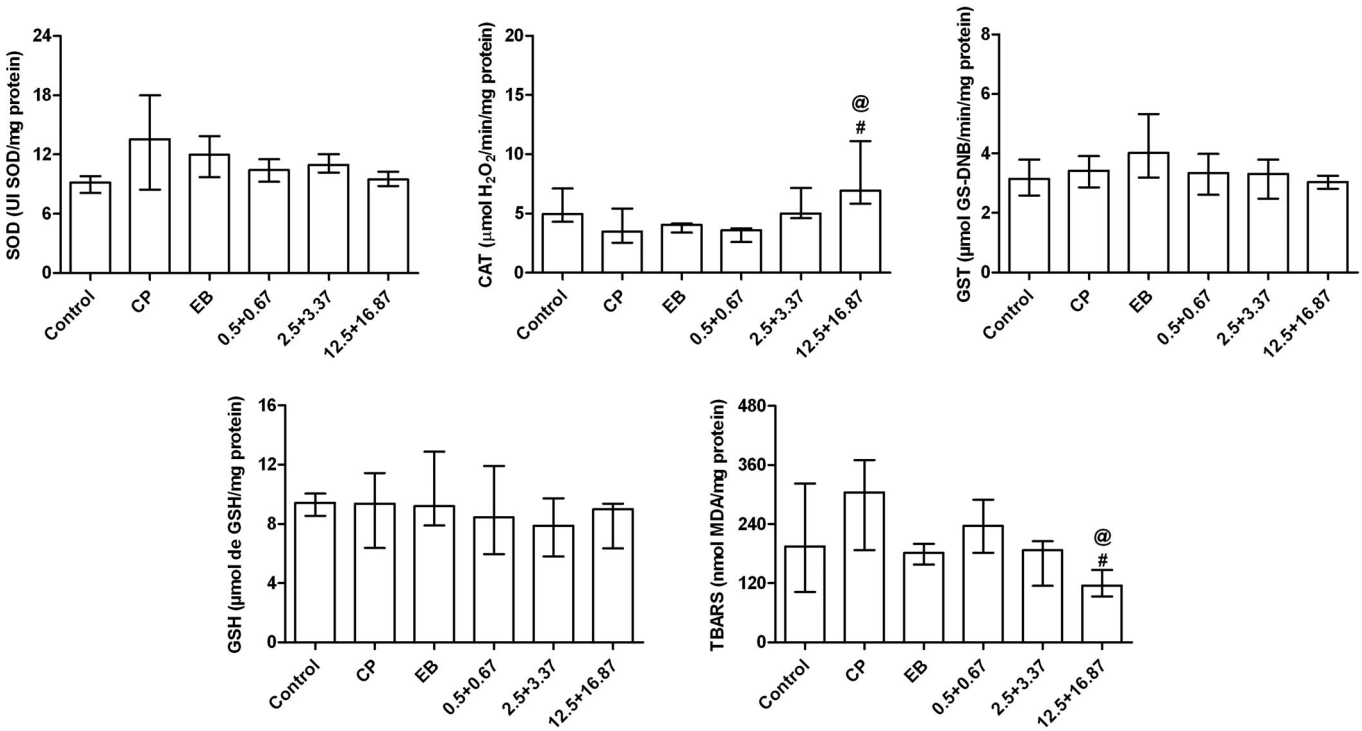

Figure 4. Results of the analysis in liver tissue from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products. SOD (Superoxide dismutase); CAT (Catalase); GST (Glutathione-s-transferase); GSH (reduced glutathione); TBARS (thiobarbituric acid reactive substances). Kruskal-Wallis followed by *post hoc* Dunn's test; $P < 0.05$; ($N = 8$). #Compared to CP, @compared to 0.5 + 0.67.

Aroonvilairat et al.[40] In the same way, there were no changes in the redox status parameters in the liver and kidney tissue, although we can only observe that the combination of high doses showed opposite results for CAT and TBARS when compared to the combination of minor doses. Since catalase is an antioxidant that acts by degrading

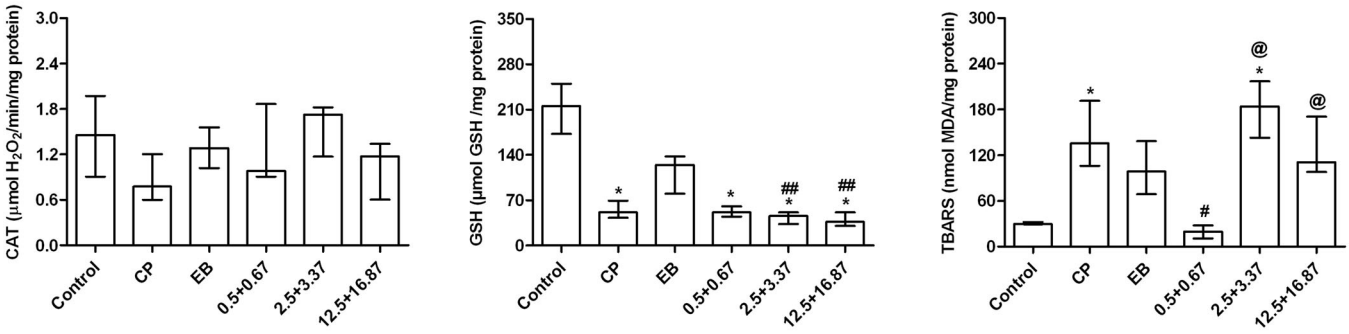

Figure 5. Results of the analysis in brain tissue from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) plus cypermethrin (CP), and same individual commercial products. CAT (Catalase); GSH (reduced glutathione); TBARS (substances reactive to thiobarbituric acid). Kruskal-Wallis followed by *post hoc* Dunn's test; $P < 0.0001$; ($N = 8$). *Compared to control, #compared to CP; ##compared to EB; @compared to 0.5 + 0.67.

Table 2. Frequency of micronucleated polychromatic erythrocytes (MN) of bone marrow cells from female mice exposed to a mixture of commercial products containing emamectin benzoate (EB) and cypermethrin (CP), and the same individual commercial products.

Treatments	Analized cells	MN
Control	8000	168
CPA	8000	358**
CP	8000	177
EB	8000	183
0.5 + 0.67	8000	206*
2.5 + 3.37	8000	223*# ##
12.5 + 16.87	8000	390*# ##

Chi-square test, $P < 0.01$ and $P < 0.05$ (0.5 + 0.67); ($N = 8$). *Compared to control; ** compared to control; #compared to CP; ##compared to EB.

hydrogen peroxide, less of this compound will be available for the formation of TBARS, which may justify the observed readings. On the other hand, literature data using males as an experimental model demonstrated hepatic biochemical changes, as well as different doses and treatment time,[42,43] and studies made by Manzoor et al.,[44] who demonstrated the effects of cypermethrin on nephrotoxicity in mice, and El-Ballal et al.,[45] after to evaluate hepatorenal toxicity of the mixture of fipronil and emamectin benzoate in rats.

In brain tissue, we found no significant differences between treatments for catalase activity. On the other hand, the GSH concentration, a major non-enzymatic antioxidant, decreased for practically all treatments and even in cardiac tissue with combinations of lower and intermediate doses. The decrease in the concentration of GSH may indicate a high level of oxidative stress, which is very similar to that found by El-Demerdash,[46] who evaluated the oxidative stress in male rat brains exposed to a combination of organophosphate and pyrethroids. On the other hand, TBARS levels increased for CP and intermediate combination of EB plus CP. These results confirm, for example, the toxic effects of cypermethrin on this tissue, as was found by Mezni et al.[47] and Ali et al.[48] In the same way, Nasr et al.[49] and Noshy and Azouz[50] observed effects similar to ours using a combination of chlorpyrifos plus abamectin and emamectin benzoate, respectively. Cypermethrin can cross the blood-brain barrier to exert neurotoxicity[48] and the lipophilicity of emamectin benzoate makes it easy to penetrate cell membranes and produce considerable toxicity in humans and animals.[36] Considering that the toxicity of these pesticides implicates by increased production of reactive oxygen species and promotes oxidative stress,[49]our results demonstrate that the brain was more responsive tissue to the treatments, which may explain the effects found in this study caused by pesticides and their combinations.

Regarding MN test, we observed a significant and growing increase in micronuclei in the groups treated with the different combination of pesticide when compared to the control and in the highest dose when compared with the isolated products, which may be related to the increase in the number of platelets found in the results of blood count analyzes. As thrombocytosis is common in acute and chronic inflammation[51], maybe the dose of the mixture causes inflammation to increase the platelet count demonstrating there was hematopoietic toxicity.

The groups treated with the isolated products (CP and EB), under the conditions performed, do not suggest mutagenic potential with a significant increase in micronuclei. On the other hand, some studies have demonstrated micronucleus induction and DNA damage *in vivo* and *in vitro* models using cypermethrin.[52–54]

Regarding the results observed in the treatments with the mixture of pesticides, in the literature, there are few studies on the genotoxicity from the combination of these two chemically different groups of used compounds, although commercially formulated mixtures of insecticides are widely used in agriculture. In this context, Zhang et al.[55]investigated the toxic effect of the mixture of β-cypermethrin and emamectin benzoate on the reproductive health of male mice and found that the rate of apoptosis of murine testicular cells increased and that DNA damage occurred with a prolonged duration of exposure.

Other studies have already evaluated the effects caused by exposure to the pesticide mixture, observing genotoxic damage induced by combination containing cypermethrin,[56,57] such as Chauhan et al.,[58] which evaluated the cytogenetic effects of the mixture of cypermethrin and quinalphos (organophosphate) in mice by testing the micronucleus and chromosomal aberration in bone marrow cells and observed a synergistic effect of genotoxicity of the dose-dependent mixture. Besides, Chauhan et al.[59] observed genotoxicity of the mixture of cypermethrin and chlorpyrifos (organophosphate) in *Swiss* mice exposed for 60 days, orally, in dose-dependent induction of micronuclei and chromosomal aberrations.

Regarding the toxicity of combination with cypermethrin and emamectin benzoate, Khan et al.[60] evaluated the

synergistic interaction in the toxic potential of different mixtures of insecticides on *Musca domestica* L. observing an increased effect between the mixtures of these two pesticides. Thus, these toxicity and genotoxicity analyzes are of paramount importance due to the scarcity of studies that analyze the exposure of populations to several pesticides simultaneously, which currently corresponds to the most frequent reality.[61]

Besides, the exacerbated use of pesticides in crops associated with the exposure of people to these contaminants, whether through inhalation, dermal contact, or ingestion,[5] ends up reflecting in situations such as those seen by a higher incidence of congenital malformations,[19,20] the presence of them in breast milk human,[21] and cancer.[62,63]

Although a chemical analysis was not performed to investigate whether there is any interaction between these pesticides, this study contributed to investigating whether female mice exposed for 28 days are affected by the toxicity of the products alone or in mixtures, as is often used in agricultural practice.

Conclusion

We can conclude that after 28 days of treatment with the different combinations of CP and EB, we could see changes, mainly in platelets and status redox in the animals' brain and the mutagenicity can be observed in a dose-dependent manner for the mixtures and their joint action proved to be more effective than the products alone when applied under the experimental conditions in female mice.

Acknowledgments

The authors acknowledge Universidade Federal de Mato Grosso for logistic support, Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT) for granting the scholarships to A. P. S. C. and W.B.S.

Conflict of interest: The authors declare that there is no conflict of interest.

ORCID

Valéria Dornelles Gindri Sinhorin http://orcid.org/0000-0002-5070-0043

Data availability statement

The data that support the findings of this study are available from the corresponding author, V. D. G. Sinhorin, upon reasonable request.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

[1] Weihs, M.; Sayago, D.; Tourrand, e. J.-F. Dinâmica da fronteira agrícola do Mato Grosso e implicações para a saúde. *Estud. av.* **2017**, *31*, 323–338. DOI: 10.1590/s0103-40142017.31890024.

[2] IBGE. Instituto Brasileiro de Geografia e Estatística. LevantamentoSistemático da ProduçãoAgrícola, Janeiro **2020**. https://sidra.ibge.gov.br/tabela/1618#resultado.

[3] Bombardi, L. M. *Geografia do uso de agrotóxicos no Brasil e conexões com a União Europeia*. FFLCH-USP, **2017**.

[4] Carneiro, F. F. W.; Rigotto, R. M.; Augusto, L. G. S.; Rizzolo, A.; Faria, N. . X.; Alexandre, V. P.; Friedrich, K.; Mello, M. S. C. *Dossiê ABRASCO – Um alerta sobre os impactos dos agrotóxicos na saúde*; Rio de Janeiro: RJ, **2012**, p. 88.

[5] Reffstrup, T. K.; Larsen, J. C.; Meyer, O. Risk Assessment of mixtures of pesticides. Current approaches and future strategies. *Regul. Toxicol. Pharmacol.* **2010**, *56*, 174–192.

[6] Bagri, P.; Kumar, V.; Sikka, A. K. Assessment of Imidacloprid-Induced Mutagenic Effects in Somatic Cells of *Swiss* Albino Male Mice. *Drug Chem. Toxicol.* **2016**, *39*, 412–417.

[7] IRAC. Insect Resistance Action Conceal. *IRAC Mode of Action Classification Scheme* **2020**, *9*, 4.

[8] Marques, L. Mecanismo de ação e status do inseticida benzoato de emamectina no Brasil. Disponível em. **2018**. https://elevagro.com/materiais-didaticos/mecanismo-de-acao-e-status-do-inseticida-benzoato-de-emamectina-no-brasil/ (accessed Nov 15, 2020).

[9] Zhang, Z.; Zhao, X.; Qin, X. Potential Genotoxic and Cytotoxicity of Emamectin Benzoate in Human Normal Liver Cells. *Oncotarget* **2017**, *8*, 82185–82195.

[10] Assayed, M. E.; Khalaf, A. A.; Salem, H. A. Protective Effects of Garlic Extract LLK and Vitamin C Against *In Vivo* Cypermethrin-Induced Cytogenetic Damage in Rat Bone Marrow. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2010**, *702*, 1–7. DOI: 10.1016/j.mrgentox.2010.02.020.

[11] Bradberry, S. M.; Cage, S. A.; Proudfoot, A. T.; Vale, J. A. Poisoning Due to pyrethroids. *Toxicol Rev.* **2005**, *24*, 93–106. DOI: 10.2165/00139709-200524020-00003.

[12] Patel, S.; Pandey, A. K.; Bajpayee, M.; Parmar, D.; Dhawan, A. Cypermethrin-Induced DNA Damage in Organs and Tissues of the Mouse: Evidence from the Comet Assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **2006**, *607*, 176–183. DOI: 10.1016/j.mrgentox.2006.04.010.

[13] Godoy, B. R. B.; Conte, A. M.; Govoni, B.; Boeira, J. M. Evaluation of Micronuclei and Other Nuclear Alterations in Oral Mucosa Exfoliated Cells of Individuals Directly and Indirectly Exposed to Pesticides. *Bjd.* **2019**, *5*, 23889–23906. DOI: 10.34117/bjdv5n11-086.

[14] Barreiros, A. L.; David, J. M.; David, J. P. Estresse Oxidativo: relação Entre Geração de espécies reativas e defesa do organismo. *Quím. Nova* **2006**, *29*, 113–123. DOI: 10.1590/S0100-40422006000100021.

[15] Silva, C. T. D.; Jasiulionis, M. G. Relação Entre Estresse Oxidativo, Alterações Epigenéticas e Câncer. *Cienc. Cult.* **2014**, *66*, 38–42. DOI: 10.21800/S0009-67252014000100015.

[16] Rajput, S.; Zhang, C.; Feng, Y.; Wei, X.; Khalil, M.; Rajput, I.; Baloch, D.; Shaukat, A.; Rajput, N.; Qamar, H.; et al. Proanthocyanidins Alleviates aflatoxinB1-Induced Oxidative Stress and Apoptosis Through Mitochondrial Pathway in the Bursa of Fabricius of Broilers. *Toxins* **2019**, *11*, 157. DOI: 10.3390/toxins11030157.

[17] Camacho, A. C.; Baptista, A.; Marques, M. J.; Campos, M. M. Função Plaquetária-Métodos Laboratoriais e Aplicações Clínicas. *Boletim da Sociedade Portuguesa de Hemorreologia e Microcirculação* **2018**, *33*, 5–12.

[18] Valentin-Severin, I.; Hegarat, L.; Lhuguenot, J. C.; Le Bon, A. M.; Chagnon, M. C. Use of HepG2 Cellline for Direct or Indirect Mutagens Screening: Comparative Investigation Between Comet and Micronucleus Assays. *Mutation Research/*

Genetic Toxicology and Environmental Mutagenesis **2003**, *536*, 79–90. DOI: 10.1016/S1383-5718(03)00031-7.

[19] Zhou, Y. J.; Huang, H. R.; Zhou, J.; Wang, L. Q. Beta-Cypermethrin Exposure Affects Female Reproduction by Enhancing Oxidative Stress in Mice Uterine Tissue. *Regul. Toxicol. Pharm.* **2018**, *98*, 284–290. DOI: 10.1016/j.yrtph.2018.08.011.

[20] Oliveira, N. P.; Moi, G. P.; Atanaka-Santos, M.; Silva, A. M. C.; Pignati, W. A. Congenital Defects in the Cities with High Use of Pesticides in the State of Mato Grosso, Brazil. *Ciênc. saúde Coletiva.* **2014**, *19*, 4123–4130. DOI: 10.1590/1413-812320141910.08512014.

[21] Ueker, M. E.; Silva, V. M.; Moi, G. P.; Pignati, W. A.; Mattos, I. E.; Silva, A. M. C. Parenteral Exposure to Pesticides and Occurence of Congenital Malformations: Hospital-Based Case–Control Study. *BMC Pediatr.* **2016**, *16*, 125–127.

[22] Palma, D. C. A.; Lourencetti, C.; Uecker, M. E.; Mello, P. R. B.; Pignati, W. A.; Dores, E. F. G. C. Simultaneous Determination of Different Classes of Pesticides in Breast Milk by Solid-Phase Dispersion and GC/ECD. *Journal of Brazilian Chemical Society* **2014**, *25*, 1419–1430.

[23] Adapar. Agência de Defesa Agropecuária do Paraná Proclaim 50, Bula **2017**, http://www.adapar.pr.gov.br/arquivos/File/defis/DFI/Bulas/Inseticidas/25PROCLAIM50.pdf (accessed Feb 15, 2020).

[24] Aenda. Associação Brasileira de Defensivos Pós-Patente. http://www.aenda.org.br/wp-content/uploads/2020/08/cir0879-anexo_cp-885_Benzoato-de-emamectina.pdf (accessed Feb 20, 2020).

[25] Cestari, M. M. Genotoxicity and mutagenicity. *Pollution and Fish Health in Tropical Ecosystems* **2013**, 132163.

[26] Luiz, T. C.; Cunha, A. P. S.; Aguiar, D. H.; Sugui, M. M.; Bicudo, R. C.; Sinhorin, A. P.; Sinhorin, V. D. G. Antioxidant Potential of *Carica papaya* Linn (Caricaceae) Leaf Extract in Mice with Cyclophosphamide Induced Oxidative Stress. *Sci. Med.* **2020**, *30*, e34702. DOI: 10.15448/1980-6108.2020.1.34702.

[27] Buege, J. A.; Aust, S. D. Microsomal Lipid Peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310. [Database] DOI: 10.1016/s0076-6879(78)52032-6.

[28] Sedlak, J.; Lindsay, R. H. Estimation of Total, Protein-Bound, and Nonprotein Sulfhydryl Groups in Tissue with Ellman's Reagent. *Anal. Biochem.* **1968**, *25*, 192–205.

[29] Misra, H. P.; Fridovich, I. The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *J. Biol. Chem.* **1972**, *247*, 3170–3175. DOI: 10.1016/S0021-9258(19)45228-9.

[30] Nelson, D. P.; Kiesow, L. A. Enthalpy of Decomposition of Hydrogen Peroxide by Catalase at 25 °C (with Molar Extinction Coefficients of H_2O_2 Solutions in the UV). *Anal. Biochem.* **1972**, *49*, 474–478. DOI: 10.1016/0003-2697(72)90451-4.

[31] Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione S-Transferase, the First Enzymatic Step in Mercapturic Acid Formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139. DOI: 10.1016/S0021-9258(19)42083-8.

[32] Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. DOI: 10.1016/0003-2697(76)90527-3.

[33] MacGregor, J. T.; Heddle, J. A.; Hite, M.; Margolin, B. H.; Ramel, C.; Salamone, M. F.; Tice, R. R.; Wild, D. Guidelines for the Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes. *Mutat Res. Genet. Toxicol.* **1987**, *189*, 103–112. DOI: 10.1016/0165-1218(87)90016-4.

[34] Waters, M. D.; Brady, A. L.; Stack, H. F.; Brockman, H. E. Antimutagenicity Profiles for Some Model Compounds. *Mutat. Res. Rev. Genet. Toxicol.* **1990**, *238*, 57–85. DOI: 10.1016/0165-1110(90)90039-E.

[35] Pereira, C. A. B. Teste Estatístico Para Comparar Proporções em Problemas de Citogenética. Mutagênese, Carcinogênese e Teratogênese: Métodos e Critérios de Avaliação. *Sociedade Brasileira de Genética* **1991**, 113–121.

[36] Abou-Zeid, S. M.; AbuBakr, H. O.; Mohamed, M. A.; El-Bahrawy, A. Ameliorative Effect of Pumpkin Seed Oil Against Emamectin Induced Toxicity in Mice. *Biomed. Pharmacother.* **2018**, *98*, 242–251.

[37] El-Sheikh, E. S. A.; Galal, A. A. Toxic Effects of Sub-Chronic Exposure of Male Albino Rats to Emamectin Benzoate and Possible Ameliorative Role of *Foeniculum vulgare* Essential Oil. *Environ. Toxicol. Pharmacol.* **2015**, *39*, 1177–1188.

[38] Sankar, P.; Telang, A. G.; Manimaran, A. Protective Effect of Curcumin on Cypermethrin-Induced Oxidative Stress in Wistar Rats. *Exp. Toxicol. Pathol.* **2012**, *64*, 487–493.

[39] Anvisa. Índice monográfico, **2019**. http://portal.anvisa.gov.br/ (accessed Jan 12, 2020).

[40] Aroonvilairat, S.; Tangjarukij, C.; Sornprachum, T.; Chaisuriya, P.; Siwadune, T.; Ratanabanangkoon, K. Effects of Topical Exposure to a Mixture of Chlorpyrifos, Cypermethrin and Captanon the Hematological and Immunological Systems in Male Wistar Rats. *Environ. Toxicol. Pharmacol.* **2018**, *59*, 53–60. DOI: 10.1016/j.etap.2018.02.010.

[41] Rehman, H.; Aziz, A. T.; Saggu, S.; VanWert, A. L.; Zidan, N.; Saggu, S. Additive Toxic Effect of Deltamethrin and Cadmium on Hepatic, Hematological, and Immunological Parameters in Mice. *Toxicol. Ind. Health.* **2017**, *33*, 495–502.

[42] Dawoud, S. F.; Al-Akra, T. M.; Zedan, A. M. Hepatoprotective Effects of Chitosan and Chitosan Nanoparticles Against Biochemical, Genetic, and Histological Disorders Induced by the Toxicity of Emamectin Benzoate. *Rep. Biochem. Mol. Biol.* **2021**, *10*, 506–514.

[43] Temiz, Ö. Biopesticide Emamectin Benzoate in the Liver of Male Mice: Evaluation of Oxidative Toxicity with Stress Protein, DNA Oxidation, and Apoptosis Biomarkers. *Environ. Sci. Pollut. Res. Int.* **2020**, *27*, 23199–23205. DOI: 10.1007/s11356-020-08923-w.

[44] Manzoor, S.; Mehboob, K.; Naveed, A. K. Comparison of Protective Effect of Green Tea and Vitamin C Against Cypermethrin Induce Nephrotoxicity in Mice. *J. Ayub Med. Coll. Abbottabad* **2016**, *28*, 241–244.

[45] El-Ballal, S. S.; Amer, H. A.; Tahoun, E. A.; El-Borai, N. B.; Zahra, M. A. A. Bee Pollen Alleviates Fipronil and Emamectin Benzoate Induced-Hepatorenal Toxicity in Rats. *Assiut Veterinary Medical Journal* **2019**, *65*, 164–173.

[46] El-Demerdash, F. M. Lipid Peroxidation, Oxidative Stress and Acetylcholinesterase in Rat Brain Exposed to Organophosphate and Pyrethroid Insecticides. *Food Chem. Toxicol.* **2011**, *49*, 1346–1352. DOI: 10.1016/j.fct.2011.03.018.

[47] Mezni, A.; Mhadhbi, L.; Khazri, A.; Sellami, B.; Dellali, M.; Mahmoudi, E.; Beyrem, H. The Protective Effect of *Hibiscus sabdariffa* Calyxes Extract Against Cypermethrin Induced Oxidative Stress in Mice. *Pestic. Biochem. Physiol.* **2020**, *165*, 104463.

[48] Ali, H. F. H.; El-Sayed, N. M.; Khodeer, D. M.; Ahmed, A. A. M.; Hanna, P. A.; Moustafa, Y. M. A. Nano Seleniumameliorates Oxidative Stress and Inflammatory Response Associated with Cypermethrin-Inducedneurotoxicity in Rats. *Ecotoxicol. Environ. Saf.* **2020**, *195*, 110479.

[49] Nasr, H. M.; El-Demerdash, F. M.; El-Nagar, W. A. Neuro and Renal Toxicity Induced by Chlorpyrifos and Abamectin in Rats. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 1852–1859.

[50] Noshy, P. A.; Rehab, A.; Azouz, R. A. Neuroprotective Effect of Hesperidin Against Emamectin Benzoate-Induced Neurobehavi Oral Toxicity in Rats. *Neurotoxicol. Teratol.* **2021**, *86*, 106981. DOI: 10.1016/j.ntt.2021.106981.

[51] Sulai, N. H.; Tefferi, A. Why Does My Patient Have Thrombocytosis? *Hematology/Oncology Clinics of North America* **2012**, *26*, 285–301. DOI: 10.1016/j.hoc.2012.01.003.

[52] Bakhitova, L. M.; Pashin, I. Cypermethrin Induction of Micronuclei in Mammalian Somatic Cells. *Tsitol. Genet.* **1988**, *22*, 32–35.

[53] Kocaman, A. Y.; Topaktaş, M. Genotoxic Effects of a Particular Mixture of Acetamiprid and α-Cypermethrin on Chromosome

Aberration, Sister Chromatid Exchange, and Micronucleus Formation in Human Peripheral Blood Lymphocytes. *Environ. Toxicol. Int. J.* **2010**, *25*, 157–168.

[54] Suman, G.; Naravaneni, R.; Jamil, K. In Vitro Cytogenetic Studies of Cypermethrinon Human Lymphocytes. *ndian J. Exp. Biol. I* **2006**, *44*, 233–239.

[55] Zhang, Y.; Kong, C.; Chi, H.; Li, J.; Xing, J.; Wang, F.; Shao, L.; Zhai, Q. Effect of a Beta-Cypermethrin and Emamectin Benzoate Pesticide Mixture on Reproductive Toxicity in Male Mice in a Greenhouse Environment. *Toxicol. Mech. Methods* **2020**, *30*, 100–106. DOI: 10.1080/15376516.2019.1669241.

[56] Muchut, S.; Simoniello, M. F.; Scagnetti, J.; Poletta, G. L.; Kleinsorge, E. C. Evaluación de genotoxicidaden linfocitos humanos expuestos a mezclas de iocidas mediante electroforesis en gel de células individuales (Ensayocometa). *FABICIB* **2011**, *15*, 108–118. DOI: 10.14409/fabicib.v15i1.885.

[57] Rupa, D. S.; Reddy, P. P.; Reddi, O. S. Frequencies of Chromosomal Aberrations in Smokers Exposed to Pesticides in Cotton Fields. *Mutat. Res. Genet. Toxicol.* **1989**, *222*, 37–41. DOI: 10.1016/0165-1218(89)90033-5.

[58] Chauhan, L. K. S.; Chandra, S.; Saxena, P. N.; Gupta, S. K. *In Vivo* Cytogenetic Effects of a Commercially Formulated Mixture of Cypermethrin and Quinalphos in Mice. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2005**, *587*, 120–125. DOI: 10.1016/j.mrgentox.2005.08.004.

[59] Chauhan, L. K.; Varshney, M.; Pandey, V.; Sharma, P.; Verma, V. K.; Kumar, P.; Goel, S. K. ROS-Dependent Genotoxicity, Cell Cycle Perturbations and Apoptosis in Mouse Bone Marrow Cells Exposed to Formulated Mixture of Cypermethrin and Chlorpyrifos. *Mutage.* **2016**, *31*, 635–642. DOI: 10.1093/mutage/gew031.

[60] Khan, H. A. A.; Akram, W.; Shad, S. A.; Lee, J. J. Insecticide Mixtures Could Enhance the Toxicity of Insecticides in a Resistant Dairy Population of *Musca domestica* L. *PLoS One* **2013**, *8*, e60929. DOI: 10.1371/journal.pone.0060929.

[61] Figueiredo, G. M.; Trape, A. Z.; Alonzo, H. A. Exposição a múltiplos agrotóxicos e prováveis efeitos a longo prazo à saúde: estudo transversal em amostra de 370 trabalhadores rurais de Campinas (SP). *Rev. Bras. Medicina Trab.* **2011**, *9*, 1–9.

[62] Pereira, M. S.; Angeoletto, F. Geografia Médica e Agronegócio: evolução Espaço Temporal Dos Cânceres do estômago, esófago, e pâncreas no estado de Mato Grosso a partir da década de 1990. *Rev. Espaço Acad.* **2016**, *179*, 86–97.

[63] Maggioni, D. A.; Signorini, M. L.; Michlig, N.; Repetti, M. R.; Sigrist, M. E.; Beldomenico, H. R. Comprehensive Estimate of the Theoretical Maximum Daily Intake of Pesticide Residues for Chronic Dietary Risk Assessment in Argentina. *J. Environ. Sci. Health Part B* **2017**, *52*, 256–266. DOI: 10.1080/03601234.2016.1272997.