REVISTA CEFAC
SPEECH, LANGUAGE, HEARING SCIENCES AND EDUCATION JOURNAL
Rev. CEFAC. 2022;24(2):e4522 https://doi.org/10.1590/1982-0216/20222424522

Original articles

Ototoxicity of an association of insecticides compounds containing dichlorvos and cypermethrin in Wistar rats

Aléxia dos Reis[1]
https://orcid.org/0000-0002-0113-6730
Suelen Pizzolatto Dalmolin[2]
https://orcid.org/0000-0002-4831-3247
Dora de Athayde Saul[1]
https://orcid.org/0000-0001-7941-5847
Márcia Salgado Machado[1]
https://orcid.org/0000-0002-8739-8976
Eliane Dallegrave[1]
https://orcid.org/0000-0001-6586-2080

[1] Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brasil.
[2] Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil.

Research support source: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Conflict of interests: Nonexistent

Received on: August 7, 2022
Accepted on: September 28, 2022

Corresponding address:
Eliane Dallegrave
Rua Sarmento Leite, 245 - Centro Histórico
CEP 90050-170 - Porto Alegre -
Rio Grande do Sul - Brazil
E-mail: elianedal@ufcspa.edu.br

ABSTRACT

Purpose: to evaluate possible ototoxicity secondary to exposure to a combination of pesticides (dichlorvos and cypermethrin based insecticides).

Methods: the Wistar rats were divided into 3 groups (12 animals per group): control (water), positive control for hearing damage (cisplatin) and experimental (exposed to dichlorvos and cypermethrin). The amplitude of distortion product otoacoustic emissions was assessed before and after the exposure. Systemic toxicity signs were also evaluated (clinical signs, weight gain and plasma cholinesterase). Wilcoxon test analyzed the post-exposure amplitudes compared to pre-exposure and Kruskal Wallis following Dunn's post hoc tests analyzed the amplitudes' variation. Normally distributed variables were evaluated by Student's t test.

Results: body weight and plasma cholinesterase values were similar comparing the pre and post exposure in the experimental group. The control group did not manifest significant amplitude reduction of otoacoustic emissions between the pre and post evaluation. In the group exposed to cisplatin there was a significant reduction in amplitudes at 12 kHz on the right ($p = 0.006$; Wilcoxon) and 4 kHz on the left ($p = 0.032$; Wilcoxon). In the group exposed to pesticides, there was a significant reduction in the right ear at 4 kHz ($p = 0.034$; Wilcoxon) and 8 kHz ($p = 0.019$; Wilcoxon) and in the left ear at 4 kHz ($p = 0.007$; Wilcoxon), 6 kHz ($p = 0.023$; Wilcoxon), 8 kHz ($p = 0.045$; Wilcoxon) and 12 kHz ($p = 0.028$; Wilcoxon).

Conclusion: there was ototoxicity in the experimental group, without a relevant systemic toxicity.

Keywords: Ototoxicity; Agrochemicals; Rats; Audiology

INTRODUCTION

Pesticides are commonly used in public health and agriculture[1] and the associations between organophosphates and pyrethroids are widely performed. However, when associated, pesticides can lead to unpredictable effects, considering the complexity of toxicological interactions[2]. In general, pesticides are correlated with more unfavorable outcomes, in relation to neurodevelopment, and their association with sensory functioning has not been completely elucidated[3].

Some studies indicate that exposure to pesticides can cause impairment in hearing[4-6] and in health, in general[7]. Nevertheless, hearing injury is often multifactorial[4,5] and may involve factors such as noise, vibration, use of antibiotics, chemotherapy, and genetic factors. It is difficult to elucidate the isolated cause of hearing damage, considering all the etiologic agents known[8].

Organophosphates (OPs), such as dichlorvos, are pesticides broadly used in agriculture as insecticides. OPs inhibit cholinesterase, causing several intoxications. Pyrethroids are compounds synthetically derived from chrysanthemum that prolong the inflow of sodium and are closely related to hypersensitivity reactions. Cypermethrin is a type II pyrethroid, which has an alpha cyan group and is more potent. Its mechanism of action involves blocking nerve conduction, persistent depolarization, reduction of the action potential amplitude and depolarization in axonal conduction. There is also a suppression of chloride channels related to the GABA receptor[9-12]. In isolation, both substances caused hearing damage in Wistar rats exposed sub-chronically by inhalation. The choice of the sub-chronic route in studies that evaluate the toxicity of pesticides is essential, since this is a frequent route of intoxication in humans and animals associated with numerous cases of chronic or sub-chronic exposure[9,10].

Considering the multifactorial nature of hearing loss, a study conducted in rats - exposed exclusively to pesticides, isolating hearing loss etiologic factors as ototoxic medications or noise - allows to characterize the hearing damage due to exposure to pesticides, as well as its mechanisms. Thus, it is relevant to study exposure to pesticides, widely used, such as organophosphates and pyrethroids, isolated and in combination. Therefore, the present study aims to evaluate the auditory effects of the association of dichlorvos and cypermethrin administered sub-chronically, via inhalation, in Wistar rats.

METHODS

The Federal University of Health Sciences of Porto Alegre Ethics Committee on Animal Use, Brazil, approved the experimental protocol under number 478/16. To reduce the risk of pain or suffering to the animals, the protocol followed the procedures established by the National Council for the Control of Animal Experimentation. The experimental protocol was based on the guidelines for sub-chronic inhalation toxicity test, number 413, proposed by the Organization for Economic Co-operation and Development[13] (OECD) for the Testing of Chemicals.

Chemical agents

An organophosphorus insecticide containing dichlorvos as active ingredient and a pyrethroid insecticide containing cypermethrin as active ingredient were associated. The pesticides were mixed with distilled water (concentration of 1.48mg/L for dichlorvos and at the concentration of 0.25mg/L for cypermethrin). The chosen concentration corresponded to 1/10 of rat inhalation LC50 (LC50 is the concentration that causes the death of 50% of animals exposed to a certain substance).

Cisplatin, an antineoplastic drug, was chosen as a positive control for hearing damage (8mg/kg intraperitoneally, once a day, during 3 days). The drug was mixed with physiological solution (10 mL of solution per kg).

Animal Model

The animals chosen for the experimental protocol were Wistar rats (*Rattus norvegicus*). The animals (36 male rats, aged 60 days, 300 ± 50g) received water and food *ad libitum*, except during exposure, in a cycle of 12h light/dark (controlled conditions of the vivarium).

Experimental

The experimental protocol was based on previous studies[9,10]. The animals included in the experimental protocol had distortion product otoacoustic emissions (DPOAE) present in the frequencies of 4, 6, 8, 10 and 12 kHz. Furthermore, an otoscopy was performed by a veterinarian and no signs of external ear pathology were found in any of the rats. Inhalation exposure chambers acoustically treated by an enclosure (avoiding noise) were coupled to ultrasonic nebulizers as an inlet stream and as an exhaust system an aspirator was coupled

to the chambers. Each chamber had 56 L of volume and accommodated 4 animals following the volume proposed by the OECD[9,13]. To determinate the sample size, a previous research[14] that evaluated rats exposed to cisplatin, an ototoxic substance, was analyzed and considered.

For 5 days (adaptation period) the animals were habituated to the instruments of auditory evaluation and to exposure chamber using only air flow (for 1h on day 1, 2h on day 2, 3h on day 3 and 4h on day 4). Water vapor was used with the air flow on day 5 for 4 hours. The animals (N=36) were allocate at random to three groups: negative control (n=12; animals exposed to inhaled water vapor during 4h, 5 times a week, for 6 weeks), positive control (n=12; animals exposed to 8mg/kg intraperitoneal cisplatin, once a day, during 3 successive days) and experimental (n=12; animals exposed to an inhaled association of the pesticides at the concentration of 1.48mg/L for dichlorvos and at the concentration of 0.25mg/L for cypermethrin, for 4h, 5 times/week, during 6 weeks).

Evaluations

Clinical signs and body weight

On exposure days the rats were assessed for body weight and clinical signs, as tremor, dyspnea, excitation, depression and piloerection.

Distortion product otoacoustic emissions (DPOAEs)

The distortion product otoacoustic emissions were tested (4, 6, 8, 10 and 12 kHz) in the control and experimental groups before (pre-exposure) and after (post-exposure) the exposure period (0 and 42 days), and in the positive control immediately before the 1st administration and 24 hours after the 3rd administration of cisplatin. DPOAEs were performed without anesthetic. DPOAEs were recorded using f1 and f2 tones as acoustic stimuli at 65 and 55db sound pressure level. A child size hearing probe was inserted into the external acoustic meatus of the animal to perform the test. DPOAEs were recorded in the control and experimental groups before (pre-exposure) and after (post-exposure) the exposure period (0 and 42 days), and in the positive control previously the 1st administration and 24 hours after the 3rd administration. The DPOAEs were assessed before and after exposure and the amplitude of response was compared between groups (control, positive control and pesticides).

Euthanasia

Euthanasia was conduct 24 hours post the last exposure to substances (cisplatin, pesticides or water). Intraperitoneal sodium thiopental (40mg/kg) associated with lidocaine (10mg/ml) was previously used as anesthesia. The animals' blood was collected from the caudal vena cava and placed in an EDTA tube, centrifuged and the plasma was frozen and later analyzed to determinate the plasma cholinesterase activity. Organs (spleen, liver, heart, lungs and kidneys) were inspected for macroscopic alterations.

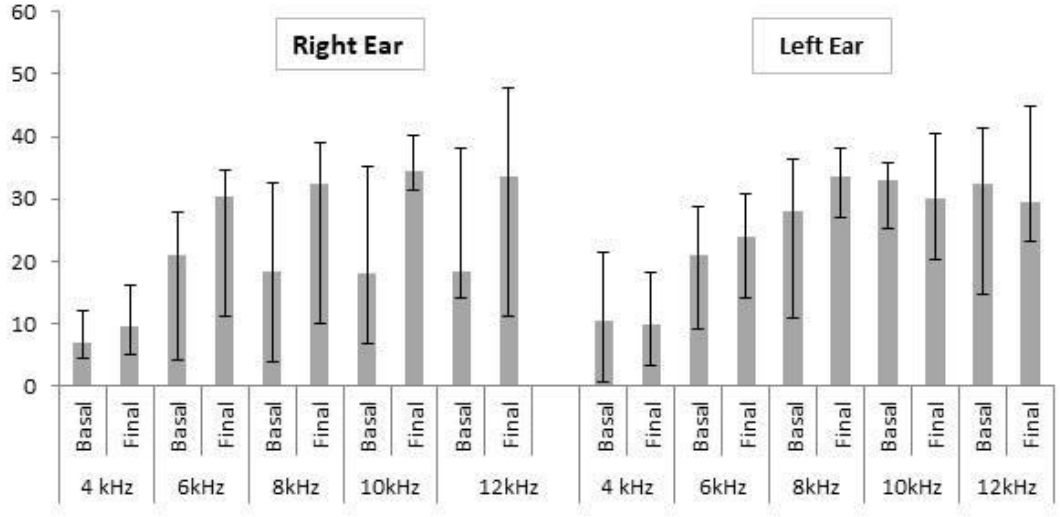
Statistical Analysis

The DPOAE were analyzed by interquartile ranges and median. Wilcoxon test analyzed the post-exposure amplitudes compared to pre-exposure. Kruskal Wallis following Dunn's post hoc tests analyzed the amplitudes' variation (final amplitude minus basal). Parametric data were presented by mean and standard error of the mean. Normally distributed variables, such as cholinesterase activity and body mass, were evaluated by Student's t test. The tests had a 95% confidence interval.

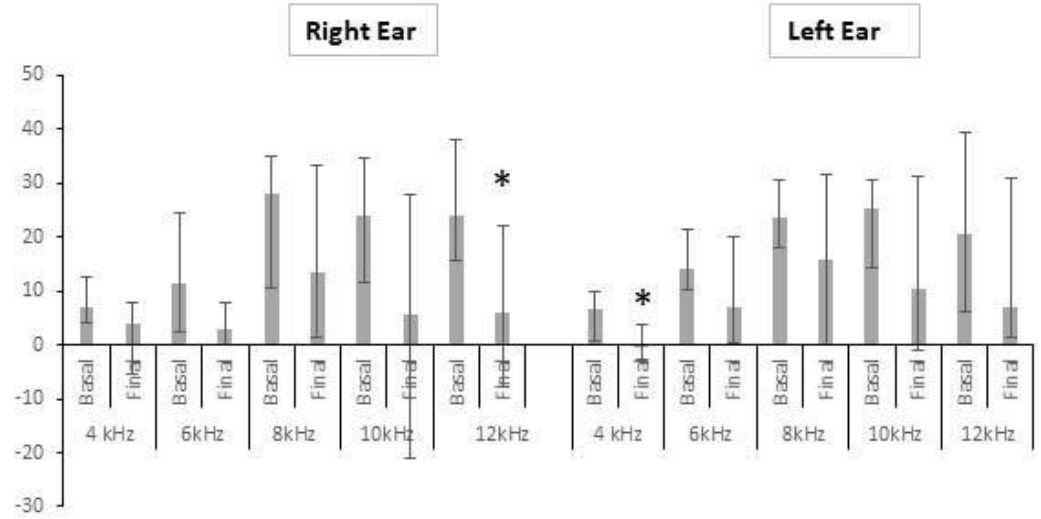
RESULTS

Distortion product otoacoustic emissions

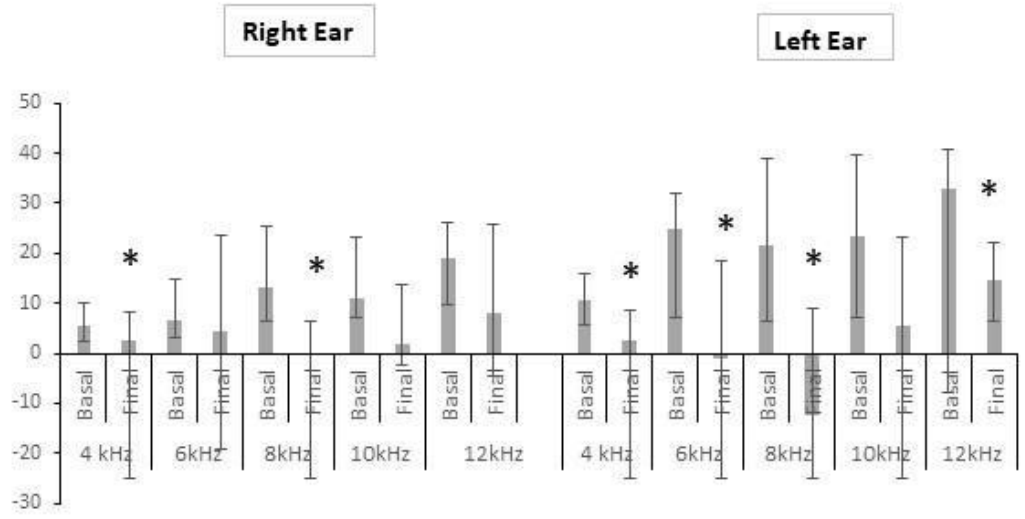
Regarding the pre- and post-exposure amplitudes, the Wilcoxon test (Figure 1) did not demonstrate a significant reduction for the frequencies analyzed in the control group, confirming the robustness of the model. For the same test, in the group exposed to cisplatin there was a significant reduction in amplitudes pre and post exposure at 12 kHz on the right ($p = 0.006$; Wilcoxon) and 4 kHz on the left ($p = 0.032$; Wilcoxon). In the group exposed to pesticides, there was a significant reduction in amplitudes pre and post exposure in the right ear at 4 kHz ($p = 0.034$; Wilcoxon) and 8 kHz ($p = 0.019$; Wilcoxon); in the left ear, there was a significant reduction at 4 kHz ($p = 0.007$; Wilcoxon), at 6 kHz ($p = 0.023$; Wilcoxon), at 8 kHz ($p = 0.045$; Wilcoxon) and at 12 kHz ($p = 0.028$; Wilcoxon).



A. Control; P>0.05; Wilcoxon



B. Positive Control for Hearing Damage (Cisplatin); *P<0.05; Wilcoxon



C. Pesticides Association; *P<0.05; Wilcoxon

Figure 1. Median and interquartile ranges of DPOAE amplitudes pre (basal) and post (final) exposure to pesticides, cisplatin (positive control for hearing damage) and water (control) – Wilcoxon test.

According to the variation in amplitudes (final assessment minus initial assessment - Figure 2), in the association group there was a significant decrease in the frequencies 8 kHz, 10 kHz and 12 kHz on the right and 6 and 8 kHz on the left ear ($p < 0.05$, Kruskal Wallis). Table 1 shows which groups were different from each other in frequencies analyzed according to Dunn's Post Hoc test.

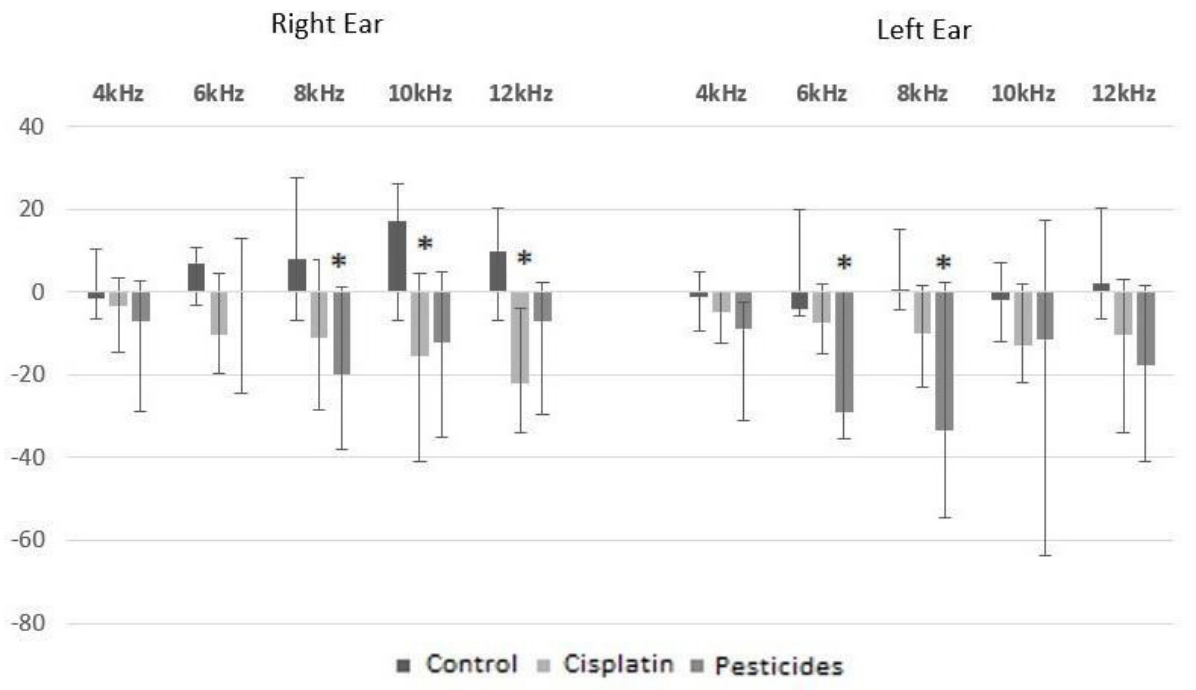


Figure 2. Variation of the amplitudes (medians and interquartile ranges) between the pre and post-exposure period to control (water), positive control (cisplatin) and pesticides.

Table 1. Kruskal-Wallis and Dunn's post hoc tests results

Ear	Frequency	Kruskal-Wallis	Dunn's Post Hoc		
			Control x Pesticides	Control x Cisplatin	Cisplatin x Pesticides
Right	4kHz	p=0.158			
	6kHz	p=0.107			
	8kHz	p=0.014*	p=0.012*		
	10kHz	p=0.024*		p=0.034*	
	12kHz	p=0.011*		p=0.008*	
Left	4kHz	p=0.080			
	6kHz	p=0.049*	p=0.045*		
	8kHz	p=0.009*	p=0.007*		
	10kHz	p=0.370			
	12kHz	p=0.075			

Kruskal-Wallis test; *p<0,05
Dunn's Post Hoc test; *p<0,05

Systemic toxicity signs

No statistically significant change occurred in plasma cholinesterase and body mass gain in the association group. Transient clinical signs (during the exposure period) were observed, including piloerection, reduced activity, dyspnea and pruritus. In euthanasia, there were no differences in the relative mass of the organs or macroscopic changes.

DISCUSSION

The control and positive control groups behaved as expected, once at the control (water) group there was no reduction and the positive control (cisplatin) showed reduction between their final DPOAEs amplitude values compared to the initial one, which corroborates the consistency of the experimental protocol. Thus, the difference (final minus initial) presented by the pesticides' association group indicates that there is an alteration in DPOAEs amplitude values due to sub-chronic inhalation exposure to dichlorvos and cypermethrin association.

Previous studies[9,10] carried out with the active ingredients alone, using the same protocol, resulted in decreased amplitudes in exposure to dichlorvos (8 kHz and 10 kHz bilaterally) and cypermethrin (8, 10 and 12 kHz bilaterally and 4 and 6 kHz on the right). In the present study, the association of both was able to decrease the amplitudes of DPOAEs between pre and post exposure at 4 kHz and 8 kHz on the right and 4, 6, 8 and 12 kHz on the left.

The similarity between the amplitude variations (final minus initial) between the association group and the cisplatin group ($p < 0.05$, Kruskal Wallis) also indicates that the pesticides tested in association are comparable to the ototoxic drug (positive control). Regarding this variation, the decrease in the association group compared to the control at 8 kHz on the right and 6 and 8 kHz on the left ear ($p < 0.05$, Kruskal Wallis; $p < 0.05$, Dunn's post hoc), and its similarity with the positive control group is congruent with previous studies that evaluated DPOAEs before and after exposure to dichlorvos[9] and cypermethrin[10] alone in Wistar rats. The exposure to cypermethrin generated a reduction in the amplitude variation in 10 and 12 kHz on the right and in 8, 10 and 12 kHz on the left. Dichlorvos caused a reduction in the amplitude variation in 10 kHz on the right and 8kHz and 10 kHz on the left. Such results indicate hearing damage secondary to exposure to ototoxic agents, since they occur at high frequencies and were pointed out after assessment of DPOAEs, an assessment sensitive to cochlea's hair cells damage.

Regarding the ototoxic results manifested without significant alterations in the clinical signs, as well as no reduction in the plasmatic cholinesterase activity, representing absence of systemic toxicity, these findings are similar to the ones evidenced in previous studies using the same model[9,10]. The concentrations of pesticides in those studies, as well as in the present study, corresponded to 1/10 of the inhaled LC50 for rats, which represents that even at low concentrations there was damage to the DPOAEs, without clinical signs indicative of toxicity or even changes in plasma cholinesterase. A normal range of plasma cholinesterase levels was also found in workers chronically exposed to organophosphates, suggesting relative resistance of higher nervous system functions to mild chronic pesticide exposure[15].

Although studies in humans have already suggested the correlation between hearing damage and exposure to pesticides[16,17], most research have limitations, once human beings may be exposed to more than a factor that contributes to this outcome, such as noise, vibration and use of ototoxic drugs. The exact mechanisms of cochlear function damage secondary to exposure to pesticides are not yet well established, however, similar changes induced by medications indicate that oxidative stress might play a role in this issue and in toxicity in humans and animals in general[18,19]. The sensorineural hearing loss mechanism associated with cisplatin and aminoglycosides occurs due to damage to the outer hair cells, and specific aminoglycosides, such as gentamicin, can still damage the vestibular system. In this case, oxidative stress would occur in the inner ear followed by cell damage[20]. Lee *et al.* (2016)[21], in research that evaluated the effect of a catalase inhibitor as a protector against ototoxicity in mice, found out that apparently the ototoxic effects of cobalt would be related to the generation of reactive oxygen species (ROS) and the production, in the auditory system, of pro-inflammatory cytokines. Still, Jiang *et al*. (2016) also report, in their research that investigated ototoxicity by inducing cell death by aminoglycoside, the contribution of the generation of ROS in ototoxicity[22].

Kim *et al*. (2017)[23] evaluated the role of the autophagosome marker LC3-II, related to the induction of damage in vivo and in vitro. These authors suggest that autophagic flux is a possible mechanism for gentamicin-induced ototoxicity. In this case, the damage could be related to the accumulation of autophagosomes, due to impaired autophagy. Choi *et al*. (2019)[24]

investigated the relationship between cisplatin-induced hearing loss and receptor-interacting protein kinase (RIP) 3-dependent necroptosis in vivo and in vitro, concluding that RIP3-dependent necroptosis was highly expressed in cisplatin-induced ototoxicity. The authors noted that the Corti organ and the spiral ganglion neurons were sites particularly susceptible to the process of necroptosis.

There may be some potential limitations in this study. Although hearing damage caused by pesticides may be similar to the ones caused by other ototoxic substances (sensory-neural and damaging to the hair cells of the cochlea, frequently causing a decrease in the amplitude of otoacoustic emissions), the lack of previous studies analyzing specifically the mechanisms that cause such damage after exposure to pesticides make this aspect unclear. Further studies need to be conducted in order to clarify the decrease in cochlear function related specifically to this type of exposure. Such studies could associate electrophysiological results with morphological data of structures such as hair cells, which could better elucidate the mechanism of hearing injury by pesticides. Understanding these mechanisms is a major matter for the development of studies that evaluates substances with otoprotective activity.

CONCLUSION

Considering the ototoxicity secondary to sub-chronic inhalation exposure to the association of compounds containing cypermethrin and dichlorvos, even without relevant systemic toxicity, the importance of raising awareness of individuals occupationally exposed to pesticides, even at low concentrations, about the importance of performing their work activities with the use of personal protective equipment, is highlighted. Furthermore, measures seem to be important to regulate audiometric control in workers exposed to pesticides and not only in those exposed to noise, since strong evidence points to the risk of ototoxicity secondary to exposure to this type of substance.

ACKNOWLEDGMENTS

This study was financed, in part, by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

REFERENCES

1. Zeigelboim BS, Malisky JS, Rosa MR, Lacerda ABM, Alcaraz PS, Fonseca VR. The importance of otoneurological evaluation in Brazilian workers exposed to pesticides: a preliminary study. Int. Arch. Otorhinolaryngol. 2019;23(4):389-95.
2. Hernández AF, Gil F, Lacasaña M. Toxicological interactions of pesticide mixtures: an update. Arch Toxicol. 2017;91(10):3211-23.
3. Sturza J, Silver MK, Xu L, Li M, Mai X, Xia Y et al. Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9months in a cohort study of Chinese infants. Environ Int. 2016;92-93:478-85.
4. Kós MI, Hoshino AC, Asmus CIF, Mendonça R, Meyer A. Efeitos da exposição a agrotóxicos sobre o sistema auditivo periférico e central: uma revisão sistemática. Cad Saúde Pública. 2013;29(8):1491-1506.
5. Alcarás PAS, Larcerda ABM, Marques JM. Study of Evoked Otoacoustic Emissions and suppression effect on workers exposed to pesticides and noise. CoDAS. 2013;25(6):527-33.
6. Teixeira CF, Augusto LGS, Morata TC. Saúde auditiva de trabalhadores expostos a ruído e inseticidas. Rev Saúde Pública. 2003;37(4):417-23.
7. Cazé AMB, Lacerda ABM, Lüders D, Conto J, Marques J, Leroux T. Perception of the quality of life of tobacco growers exposed to pesticides: emphasis on health, hearing, and working conditions. Int. Arch. Otorhinolaryngol. 2019;23(1):50-9.
8. Reis AD, Dalmolin SP, Dallegrave E. Animal models for hearing evaluations: a literature review. Rev. CEFAC. 2017;19(3):417-28.
9. Reis AD, Cunha EO, CostaValle MT, Machado MS, Dallegrave E. Effects of subchronic inhalation exposure to an organophosphorus insecticide compound containing dichlorvos on wistar rats' otoacoustic emissions. Braz J Otorhinolaryngol. 2022;88(1):28-35.
10. Cunha EO, Reis AD, Macedo MB, Machado MS, Dallegrave E. Ototoxicity of cypermethrin in Wistar rats. Braz J Otorhinolaryngol. 2019;86(5):587-92.
11. Filho ADA, Souza SD. Anticolinesterásicos. In: Filho ADA, Campolina D, Dias MB, editors. Toxicologia na prática clínica. 2nd ed. Belo Horizonte: Folium; 2013. p. 89-95.

12. Wakeling EN, Neal AP, Atchison WD. Pyrethroids and their effects on ion channels. In: Soundararajan R, editor. Pesticides - advances in chemical and botanical pesticides [Internet]. London: IntechOpen. 2012. Available from: https://www.intechopen.com/chapters/37969 doi: 10.5772/50330.
13. OECD. Test No. 413: subchronic inhalation toxicity: 90-day study, OECD guidelines for the testing of chemicals, Section 4. 2009. OECD Publishing, Paris.
14. De Freitas MR, Figueiredo AA, Brito GAC, Leitão RFC, Junior JVC, Junior RMG et al. The role of apoptosis in cisplatin-induced ototoxicity in rats. Braz J Otorhinolaryngol. 2009;75(5):745-52.
15. Rodnitzky RL, Levin HS, Mick DL. Occupational exposure to organophosphate pesticides. Archives of Environmental Health: An International Journal. 1975;30:2:98-103.
16. Sena TRR, Dourado SSF, Lima LV, Antoniolli ÂR. The hearing of rural workers exposed to noise and pesticides. Noise Health. 2018;20(92):23-6.
17. Tomiazzi JS, Pereira DR, Judai MA, Antunes PA, Favareto APA. Performance of machine-learning algorithms to pattern recognition and classification of hearing impairment in Brazilian farmers exposed to pesticide and/or cigarette smoke. Environ Sci Pollut Res Int. 2019;26(7):6481-91.
18. Medithi S, Jonnalagadda PR, Jee B. Predominant role of antioxidants in ameliorating the oxidative stress induced by pesticides. Arch Environ Occup Health. 2021;76(2):61-74.
19. How V, Hashim Z, Ismail P, Omar D, Said SMD, Tamrin SBM. Characterization of risk factors for DNA damage among paddy farm worker exposed to mixtures of organophosphates. Arch Environ Occup Health. 2015;70(2):102-90.
20. Schacht J, Talaska AE, Rybak LP. Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention. Anat Rec (Hoboken). 2012;295(11):1837-50.
21. Lee JN, Kim SG, Lim JY, Dutta RK, Kim SJ, Choe SK et al. 3-Aminotriazole protects from CoCl2-induced ototoxicity by inhibiting the generation of reactive oxygen species and proinflammatory cytokines in mice. Arch Toxicol. 2016;90(4):781-91.
22. Jiang P, Ray A, Rybak LP, Brenner MJ. Role of STAT1 and oxidative stress in gentamicin-induced hair cell death in organ of corti. Otol Neurotol. 2016;37(9):1449-56.
23. Kim YJ, Tian C, Kim J, Shin B, Choo OS, Kim YS et al. Autophagic flux, a possible mechanism for delayed gentamicin-induced ototoxicity. Sci Rep. 2017;7:41356.
24. Choi MJ, Kang H, Lee YY, Choo OS, Jang JH, Park SH et al. Cisplatin-induced ototoxicity in rats is driven by RIP3-dependent necroptosis. Cells. 2019;8(5):409.