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Original Article

Adverse effects of pesticides on central auditory functions in tobacco growers

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Abstract

Objective: To investigate the effects of exposure to pesticides on the central auditory functions (CAF) of Brazilian tobacco growers. *Design*: This was a cross-sectional study carried out between 2010 and 2012. Participants were evaluated with two behavioural procedures to investigate CAF, the random gap detection test (RGDT) and the dichotic digit test in Portuguese (DDT). *Study sample*: A total of 22 growers exposed to pesticides (study group) and 21 subjects who were not exposed to pesticides (control group) were selected. *Results*: No significant differences between groups were observed for pure-tone thresholds. A significant association between pesticide exposure and the results for RGDT and DDT was found. Significant differences between pesticide-exposed and nonexposed subjects were found for RGDT frequency average and DDT binaural average, when including age and hearing level as covariates. Age was significantly associated with RGDT frequency average, DDT left ear score, DDT binaural average and DDT right ear advantage. Hearing levels were not significantly associated with any of the test scores. The relative risk of failing the DDT and RGDT for the study group was 1.88 (95% CI: 1.10–3.20) and 1.74 (95% CI: 1.06–2.86), respectively, as compared with the control group. *Conclusions*: The results showed that tobacco growers exposed to pesticides exhibited signs of central auditory dysfunction characterised by decrements in temporal processing and binaural integration processes/abilities.

Keywords: Hearing loss, binaural integration, temporal resolution, pesticides, hearing

Introduction

The planting of tobacco occupies a prominent place in the Brazilian trade balance (Associação dos Fumicultores do Brasil [AFUBRA], 2012). The country is the largest exporter of tobacco leaves. The Southern Region, with the states of Parana, Santa Catarina and Rio Grande do Sul, produces about 95% of the country's tobacco (AFUBRA, 2012; Departamento de Estudos Sócios-Econômicos Rurais [DESER], 2013). Studies have shown that tobacco farming uses pesticides in large amounts, which can affect the overall health of rural workers and their entire family (AFUBRA 2012; BRASIL, 2012).

Brazil is considered the largest consumer of pesticides in the world and is responsible for about 86% of the use of such products in Latin America. The State of Paraná in 2011 ranked third in the country in terms of consumption, using more than 112,500 tons of pesticides in farms (BRASIL, 2012).

It is known that occupational exposure to pesticides relates to a range of harmful effects on human health such as neurological, psychological, immunological, endocrine, hematologic, skin, liver and kidney problems as well as congenital malformations (Araújo et al, 2007; BRASIL, 2012). In addition, pesticides have been associated with auditory dysfunction in both mice and humans (Johnson & Morata, 2010; Gatto et al, 2014).

Some studies suggest that organophosphate (a type of pesticide) or paraquat (a type of herbicide) can promote the formation of reactive oxygen species (ROS) within the perilymphatic space in the cochlea. The resulting formation of ROS can be toxic to the cochlea (Bielefeld et al, 2005; Jayasinghe & Pathirana, 2011). Other studies have indicated that pesticides, including organophosphates, can modify the action of the efferent auditory system by inhibiting acetylcholinesterase, causing acetylcholine build-up in the peripheral auditory system and sensory pathways (Sidell, 1994). For the central auditory system, acetylcholine build-up affects the transmission of the efferent system's action potentials from the superior olivary nucleus to the cochlea (Cáceres et al, 2010).

In guinea pigs exposed to organophosphate pesticides (Methamidophos) concentration-dependent alterations to outer

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Acronyms/Abbreviations

AFUBRA	Tobacco Growers' Association of Brazil
ANVISA	National Sanitation and Health Agency
CAF	Central Auditory Functions
DDT	dichotic digits test
NEG	non-exposure group
OHC	outer hair cells
PEG	pesticide exposure group
REA	right ear advantage
RGDT	random gap detection test
ROS	reactive oxygen species
RS	regulatory standard
SUS	unified health system

hair cells (OHCs) and other inner ear structures were observed. The effects ranged from no pathological alterations in OHCs, utricule or saccule to frank lesions such as distortion, shortening or absence of OHCs stereocilia and alterations in the utricle and saccule (Körbes et al, 2010).

In humans, some studies show that exposure to pesticides, including organophosphate and/or pyrethroid can induce changes in peripheral and central auditory function as well as in vestibular function (Teixeira et al, 2002, 2003; Manjabosco et al, 2004; Kimura et al, 2005; Hoshino et al, 2008; Dassanayake et al, 2008; Crawford et al, 2008; Guida et al, 2009, 2010; Jayasinghe & Pathirana, 2011; Camarinha et al, 2011; Bazilio et al, 2012; Alcarás et al, 2012; Gatto et al, 2014). Importantly, it has been suggested that when organophosphate pesticides are combined with noise exposure, the auditory effect of the former can be aggravated (Teixeira et al, 2003; Guida et al, 2010).

Studies, using pure-tone audiometry, show a high-frequency sensorineural hearing loss associated with organophosphate pesticide exposure (Teixeira et al, 2003; Manjabosco et al, 2004). Guida et al (2009) found that exposed farmers had a lower percentage of present otoacoustic emissions and that the magnitude of the responses was decreased as compared to a control group without exposure to noise or pesticides. Similar findings were found by Alcaras et al (2012). The authors found that transient evoked otoacoustic emission (TEOAE), distortion product otoacoustic emission (DPOAE), and TEOAE efferent suppression results were significantly worse among workers exposed to organophosphate pesticides as compared to a non-exposed control group.

Regarding central auditory function, Dassanayake et al (2008) showed that acute poisoning by organophosphates induces a delay in long latency auditory evoked potentials. Dassanayake et al (2009) suggest that chronic exposure to OP pesticides may delay the neurophysiological processes underlying the early stages of selective attention, and the late stages of sensory information processing that include stimulus evaluation and updating of working memory. In addition, the behavioural findings suggest that pesticide exposure also impairs the accuracy of stimulus classification (Dassanayake et al, 2009). These findings corroborate the neurobehavioral findings for humans chronically exposed to OP pesticides. However, the effect of pesticides on the brainstem, assessed by auditory evoked potentials, showed no statistically significant differences, in either absolute latencies or interpeak interval latencies, between subjects with and without organophosphates pesticide exposure (França, 2013) and organophosphate or paraquat (Jayasinghe & Pathirana, 2011). Other studies have investigated central auditory functions using behavioural tasks such as the Frequency Pattern, Duration Pattern and Gaps-In-Noise tests. Results showed that rural workers exposed to organophosphates (Camarinha et al, 2011), and workers exposed to various types of pesticides—mainly herbicides such as Roundup®, and in smaller amounts insecticides and fungicides (Bazilio et al, 2012) – presented with worse results for the aforementioned temporal processing tests as compared to control group subjects. Despite these studies, research on the use of pesticides, especially those involved in tobacco growing and their effect on central auditory functions (CAF), is still insufficient. The random gap detection test (RGDT) and dichotic digit test (DDT) have been recommended for use in the detection of adverse effects of chemical agents on CAF in exposed workers (Fuente & McPherson, 2006; Johnson & Morata, 2010). However, no studies using these clinical procedures in populations of workers exposed to pesticides have been identified in the existing literature. Thus, the aim of this study was to investigate the effects of exposure to pesticides on CAF of tobacco growers using the RGD and DD tests.

Method

The study was approved by the Research Ethics Committee of Universidade Estadual do Centro Oeste (UNICENTRO). All participants signed an informed consent form. This was a cross-sectional study of workers with and without pesticide exposure and data collection took place between October 2011 and August 2012.

Participants

Sample size estimation

The sample size was calculated based on previous reports of relative risks for central auditory dysfunction using auditory tests evaluating similar aspects of central auditory function as in this study (Teixeira et al, 2002). The sample size was calculated for a medium effect size (0.25), a significance of 0.05 with an 80% power for mean comparisons between two groups, and a ratio of 6.89. This analysis was based on the formula proposed by Fleiss et al, (2003). The minimum sample size required was 28 participants (14 participants exposed to pesticides and 14 non-exposed participants).

Study group

The study group (Pesticide Exposure Group – PEG) was comprised of 22 tobacco growers who were exposed to pesticides (9 females and 13 males). The study group participants did not report exposure to noise or other ototoxic agents other than pesticides. The mean age for this group was 38.5 years (20–57 years). All workers from this group lived in the South-Central region of the State of Parana in Brazil, and 77.3% of PEG workers had only completed primary-level education and 22.7% had completed secondary-level education.

Tobacco growers were invited to participate in the study via posters placed at the main entrances of places where local farmers regularly visited, such as health facilities, food markets, the Irati Department of Agriculture and Supply and the Tobacco Growers' Association of Brazil, among others. Inclusion criteria included right-handedness, the absence of previous episodes of acute poisoning that required hospitalisation, chronic health conditions such as diabetes, hypertension, metabolic diseases and the absence of ear problems, including previous episodes of recurrent otitis media. In addition, selected participants needed to present with an otoscopic examination without ear canal obstruction, pure-tone thresholds at 500, 1000, 2000 and 4000 Hz less than or equal to 25 dB HL in both ears (Conselho Federal de Fonoaudiologia [CFFa], 2013) and bilateral Type A tympanograms. A total of nine PEG participants were excluded from the sample due to sensorineural hearing loss, and one participant due to a mixed hearing loss. For two participants, the evaluation was inconclusive, and therefore, they were excluded.

Characterisation of pesticide exposure and use of protective equipment among tobacco growers

For tobacco growers, the mean number of years working in this type of job was 17.1. The mean age at which they commenced working in the fields was 11.3 years. The mean reported number of years using some kind of protection was 2.2 years. The application of pesticides was done manually with backpack sprayers. The personal protective equipment utilised included leather boots and latex gloves used by 19 subjects (86.36%), caps used by 18 subjects (82%), masks without a filter used by 15 subjects (68.18%), and overalls used by nine subjects (41%). Table 1 shows the most commonly-used pesticides among participants.

Control group

The control group (non-exposure group – NEG) was comprised of 21 volunteers of both genders (10 female participants and 11 male participants) between 16 and 68 years of age (mean age: 42). NEG participants did not present with a history of exposure to pesticides or any other ototoxic agent such as chemicals and noise. 71.4% of NEG participants had only completed primary-level education, and 28.6% had completed both primary- and secondary-level education. The same inclusion criteria as for the study group were considered for the selection of NEG participants. No one from this group was excluded.

Material

All hearing tests were conducted in a double-walled, sound-treated room meeting ANSI S3.1-1991 standards for ambient sound pressure levels. For otoscopy, a Kole otoscope was used. For pure-tone audiometry, a Madsen Itera II clinical audiometer (GN Otometrics A/S, Denmark) was used with TDH-39 headphones, calibrated according to ANSI S.3.6-2010. For immittance audiometry an Audiotest 425 (The Binding Site Ltd, England) middle-ear analyser was used. Finally, for the evaluation of the CAF, the RGDT (Keith, 2000) and the DDT (Pereira & Schochat, 1997) were used. Each test was recorded on a compact disc (CD). A CD player connected to the audiometer mentioned above was used for CAF assessment.

Procedures

Otoscopy

Only subjects from both groups with no visible pathologic alterations of either the ear canal or the tympanic membrane were considered for inclusion in the final sample. One participant from the study group was excluded due to tympanic membrane perforation.

Immittance audiometry

Tympanometry and contralateral acoustic reflexes were obtained. Bilateral type A results (Jerger, 1970) in tympanometry and presence of contralateral reflexes at 500, 1000 and 2000 Hz were required at 70–100 dB HL in order to be included in the sample.

Bilateral pure-tone audiometry

Hearing thresholds were obtained at 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz for air conduction. The modified Hughson & Westlake (1944) procedure described by Carhart and Jerger (1959) was used to obtain hearing thresholds.

Random gap detection test (RGDT, Keith 2000)

This test was used to assess temporal resolution. It is commercially available from Auditec, St. Louis. RGDT falls within the test category of temporal auditory processing (AAA, 2010). At 50 dB HL, stimuli comprising two tone bursts that differed in their onset times were presented binaurally. Subjects were asked to state whether they heard one or two tones at each presentation. The duration of each tone burst was 17 ms with a rise/fall time of 1 ms. Stimulus pairs were presented with silent intervals of 4.5 s. The silent interval between the two tone bursts ranged from 0 to 40 ms (0, 2, 5, 10, 15, 20, 25, 30 and 40 ms, randomly presented). In this research, subtest 1 (screening/practices) which uses a 500 Hz tone burst and subtest 2 for 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz tone bursts, were used. Thresholds for each frequency tested (500,

Table 1. Main pesticides reported to be used by tobacco growers.

Name of pesticide	*Active ingredient*	*Application*	*Toxicological classification*	*Percentage of PEG reporting the use of the specific pesticide*
Gamit	Clomazone	Herbicide	Level – IIIModerately toxic	95.4%
Primeplus	Flumetralin	Growth regulator	Level – IExtremely toxic	81.8%
Roundup	Glyphosate	Herbicide	Level – IIHighly toxic	68.1%
Boral 500SC	Fluazolate	Herbicide	Level – IVSlightly toxic	59.0%
Orthene	Acephate	Insecticide organophosphate	Level - IIHighly toxic	59.0%
Rovral	Iprodine	Fungicide	Level – IIIModerately toxic	36.3%
Confidor	Imidacloprid	Insecticide	Level – IVSlightly toxic	18.1%
Ridomil	Mancozeb	Fungicide	Level – IIIModerately toxic	13.6%
Poast	Sethoxydim	Herbicide	Level - IIHighly toxic	13.6%
Dithane Pm	Mancozeb	Fungicide Acaricide	Level – IExtremely toxic	9.0%
Manzate	Dithiocarbamate	Fungicide	Level – IIIModerately toxic	4.5%

Toxicological classification - (BRASIL. Ministério da Saúde, 1992).
PEG: Pesticide-exposed group.

1000, 2000 and 4000 Hz) were calculated by identifying from the score sheet the interval in milliseconds when the subject consistently commenced detection of two stimuli instead of one. In addition, the overall average gap detection threshold for the four tone burst frequencies was calculated. Based on previously reported normative data, gap detection thresholds above 13.5 ms were considered as below the normal range (Fuente & McPherson, 2006).

Dichotic digit test (DDT, Pereira & Schochat, 1997)

This test was used to assess binaural integration. DDT falls within the test category of dichotic listening (speech) tests (AAA, 2010). Twenty sets of two pairs of digits in Portuguese (80 numbers totally, 40 numbers presented to each ear) were presented dichotically at 50 dB SL (according to the average of pure-tone thresholds at 500, 1000 and 2000 Hz). Subjects were asked to repeat back each set of four numbers. The repetition task involved free recall. Scores in percentage of correctly repeated items per ear were obtained. Thus, a right ear score and a left ear score were obtained. A total percentage score or binaural average was also obtained by combining the scores of both ears ([RE score + LE score]/2). In addition, a right-ear advantage (REA) was calculated by subtracting left ear scores from right ear scores. Based on previously reported normative data, results below 95% were considered as below normal ranges (Pereira & Schochat, 1997).

Data analyses

Initially, Mann–Whitney *U*-tests were used to determine possible significant differences between groups for pure-tone thresholds at each frequency in each ear. A Bonferroni adjustment of the p value was used as multiple comparisons for pure-tone thresholds were performed. Significant differences for pure-tone thresholds between groups were considered at a p value of 0.006 (0.05 divided by 8, the total number of comparisons per ear). Subsequently, the percentage of abnormal results for RGDT (frequency average for 500, 1000, 2000 and 4000 Hz), and DDT (binaural average) were calculated for each group of subjects. Cut-off scores for assigning normal or abnormal results were taken from previous studies reporting normative scores for these tests (see above). Chi-Square tests were used to determine whether abnormal results for RGDT frequency average and DDT were significantly associated with the presence of pesticide exposure. Then, an analysis of covariance (ANCOVA) was performed to compare the mean values of the dependent variables (results for RGDT frequency average and DDT) between pesticide-exposed and nonexposed subjects. Age and hearing thresholds were included in the ANCOVA as covariates. For RGDT frequency average, DDT binaural average, and DDT REA, hearing level was defined as the average of the right and left ear hearing thresholds for the frequencies 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz. For DDT right ear, hearing level was defined as the average of the right ear hearing thresholds for the frequencies 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz. For DDT left ear, hearing level was defined as the average of the left ear hearing thresholds for the frequencies 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz. Differences for test results between pesticide-exposed and non-exposed subjects were obtained. In addition, the mean test scores adjusted for age and hearing level were obtained for each group of subjects. Finally, relative risks (RR) were estimated taking the higher likelihood of overestimating the effect size from odds ratio into account. For this, logistic regressions for DDT and RGDT were performed. Both response variables were dichotomised as normal or abnormal results. For DDT, the average score was compared with previously reported normative values. Those participants with scores below normative values were considered as failing this test. For RGDT, each subtest (500, 1000, 2000 and 4000 Hz) was also compared with previously reported normative values. Participants obtaining two or more RGDT subtests with scores below the norms were considered as failing this test. Probabilities for failing the DDT and RGDT were estimated for each group (exposed and non-exposed) controlling for the covariates of age and binaural average for hearing thresholds (i.e. average of the right and left ear hearing thresholds for the frequencies 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz). Taking the asymmetric distribution of RR (from zero to infinite) into consideration, the transform-the-endpoints method suggested by Cummings (2011) was used. This method provides a nonbiased estimation for the confidence intervals (Cummings, 2011).

Results

Pure-tone thresholds

Figure 1 displays the distribution of hearing thresholds (250–8000 Hz) for both groups for the right and left ears. No significant differences between groups for pure-tone thresholds were observed at any frequency tested ($p > 0.006$).

Temporal resolution and binaural integration

Figure 2 displays the score distribution for both groups for the RGDT for all frequencies tested (500–4000 Hz) as well as for the average result. Figure 3 displays the score distributions for the DDT for both groups for the right and left ears, as well as for the binaural average and REA. Mean values for both tests were lower among pesticide-exposed subjects than non-exposed subjects.

The percentage of abnormal results for each group for the RGDT frequency average and for the combined right and left ear scores (binaural average) for DDT was calculated. Among pesticide-exposed subjects, 17 (77.3%) presented abnormal results for the RGDT, and 19 (86.4%) presented abnormal results for DDT. Among non-exposed subjects, eight (38.1%) presented abnormal results for the RGDT and eight (38.1%) presented abnormal results for DDT. A Chi-square test was carried out in order to explore the possible association between the categorical variables of pesticide exposure and abnormal results for each test. Yates' correction for continuity was used. The assumption of 2 concerning minimum expected cell frequency was not violated, as all expected cell sizes were greater than 5. The two Pearson Chi-square values after continuity correction were 6.7 ($p < 0.01$) and 10.7 ($p < 0.01$) for RGDT and DDT, respectively. Therefore, a significant association between pesticide exposure and abnormal results for RGDT and DDT was found.

Differences between groups for temporal resolution and binaural integration adjusted for age and hearing thresholds

Table 2 shows the adjusted mean test scores (RGDT frequency average and DDT) for age and hearing levels for both groups. Significant differences between pesticide-exposed and non-exposed

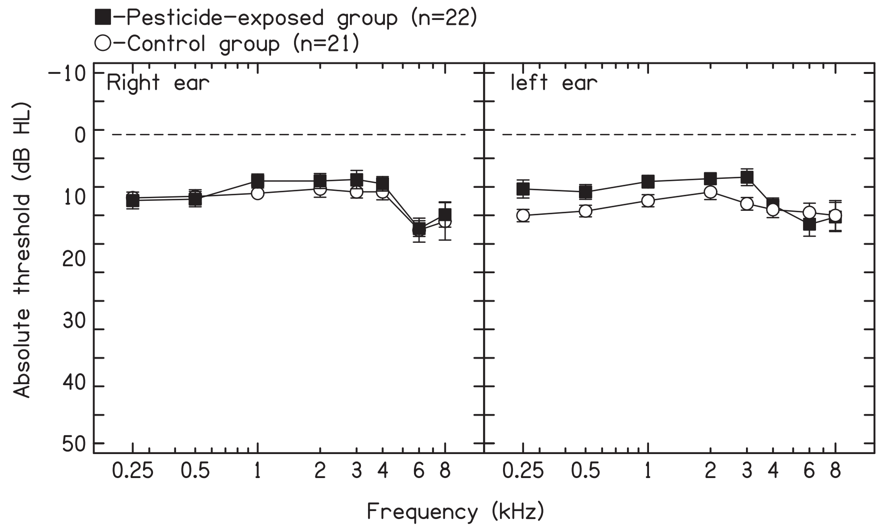

Figure 1. Mean right and left ear pure-tone thresholds (250–8000 Hz) and standard errors for both groups of subjects.

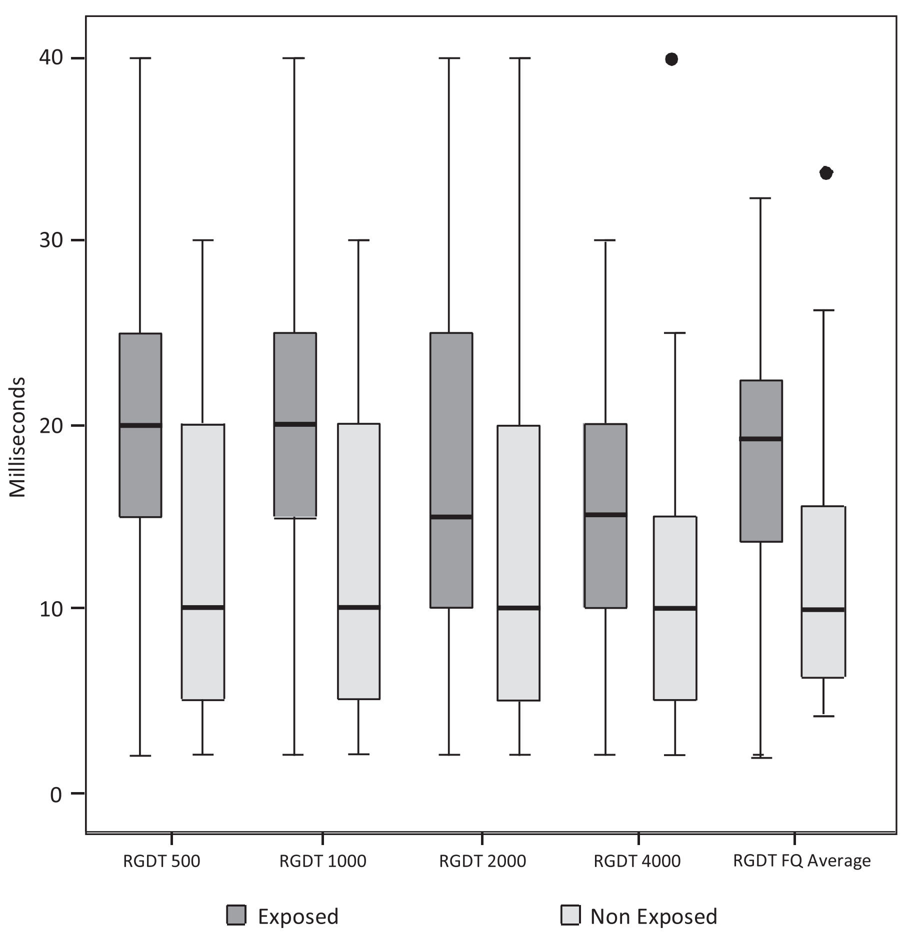

Figure 2. Boxplots of the scores of pesticide-exposed subjects ($n = 22$) and nonexposed control subjects ($n = 21$) for the random gap detection subtests. RGD500: Subtest 500 Hz; RGD1000: Subtest 1000 Hz; RGD2000: Subtest 2000 Hz; RGD4000: Subtest 4000 Hz; RGD FQ AV: mean value for the previous four subtests.

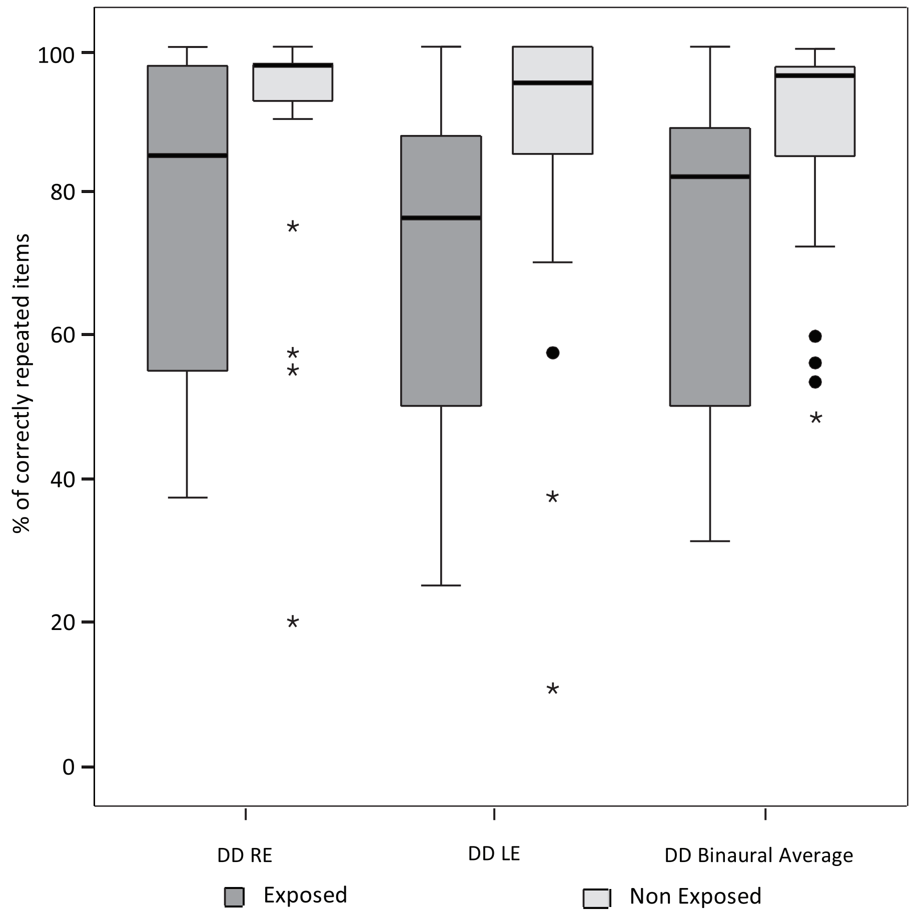

Figure 3. Boxplots of the scores of pesticide-exposed subjects ($n = 22$) and control nonexposed subjects ($n = 21$) for dichotic digit test. DD RE: dichotic digit scores for the right ear. DD LE: dichotic digit scores for the left ear. DD total: dichotic digit total score (right and left ear scores combined).

Table 2. Mean test results for RGDT and DDT, adjusted for age and hearing level.

	Pesticide exposed			*Non-exposed*		
Test	*Mean*	*SE*	*95% CI*	*Mean*	*SE*	*95% CI*
RGDT Frequency average, ms[a]	18.1	1.5	15.0–21.38	12.5	1.6	9.40–15.95
DDT right ear, %	76.6	4.6	67.36–86.02	88.0	4.7	78.43–97.56
DDT left ear, %	70.8	4.2	62.12–79.52	82.2	4.2	73.55–90.95
DDT binaural average, %[a]	73.5	3.6	66.14–81.01	85.8	3.7	78.20–93.45
DDT REA, %	3.8	4.4	−5.21–12.94	6.9	4.6	−2.37–16.23

CI: confidence interval; DDT: dichotic digits test; ms = milliseconds; % = percentage of correctly repeated items; REA: right ear advantage; RGDT = random gap detection test;SE: standard error.

subjects were found for RGDT frequency average ($F = 5.7$, $p < 0.05$) and DDT binaural average ($F = 5.0$, $p < 0.05$) when including age and hearing level as covariates. The adjusted R squared for the RGDT model was 0.26 ($F = 5.81$ $p < 0.01$, partial Eta squared = 0.309 and an observed power = 0.931). The adjusted R squared for the DDT binaural average model was 0.35 ($F = 8.43$, $p < 0.0001$, partial Eta squared = 0.394 and an observed power = 0.988). Age was significantly associated with RGDT frequency average ($F = 4.2$, $p < 0.05$), DDT left ear ($F = 16.7$, $p < 0.001$), DDT binaural average ($F = 6.8$, $p < 0.05$) and DDT REA ($F = 10.7$, $p < 0.01$). Hearing level was not significantly associated with any of the test scores.

Relative risks

After adjusting for age and binaural average for hearing thresholds, the relative risk for pesticide-exposed participants compared with non-exposed participants was 1.88 (95% confidence interval [CI] = 1.10–3.20) and 1.74 (95% CI= 1.06–2.86) for failing the DDT and RGDT, respectively.

Discussion

Pure-tone thresholds

No significant differences for pure-tone thresholds between groups were observed. This is mainly because all participants from both groups presented with normal hearing thresholds, as this was one of the requirements for subjects to be included in the sample. Some authors have reported an association between organophosphate pesticide exposure and increased (i.e. lower) hearing thresholds (Teixeira et al, 2003; Manjabosco et al, 2004; Hoshino et al, 2008; Guida et al, 2010; Camarinha et al, 2011).

Beckett et al (2000) showed a significant association between pesticide exposure and presence of hearing loss in workers with a history of spraying crops. However, information about the type and quantity of the pesticides used by the workers was not reported by Beckett et al (2000). Teixeira et al (2003) in a group of 98 workers exposed to organophosphates and pyrethroids found the presence of hearing loss in 63.8% of workers exposed only to insecticides and in 66.7% of workers exposed to both insecticides and noise. Teixeira et al (2003) concluded that the use of organophosphate and pyrethroid insecticides independent of noise may have been the cause of the hearing losses observed in workers. In addition, Manjabosco et al (2004) found that 60% of workers exposed to organophosphate and pyrethroid pesticides had a hearing loss (92% of them presented with a sensorineural hearing loss). Guida et al (2010) in a study conducted on 43 workers exposed to organophosphates, found the presence of hearing loss in 55% of workers exposed to pesticides compared to 42.5% of workers exposed solely to noise. Similar results have been found by Camarinha et al (2011) who reported that 64% of workers exposed to organophosphate pesticides presented with hearing loss.

Central auditory functions

As for RGDT results (Figure 2 and Table 2), the mean value was worse in the exposed group than in the non-exposed group. In addition, a significant association between pesticide exposure and abnormal results for RGDT was found. Finally, the relative risk of failing this test was 1.74 for the group of participants exposed to pesticides as compared with non-exposed control participants. These findings suggest that pesticide exposure may be associated with decrements in temporal resolution abilities. This result is in agreement with Camarinha et al (2011) who evaluated temporal resolution and temporal ordering abilities in rural workers exposed to pesticides. They found that this population presented with worse results in the frequency pattern, duration pattern and gaps-in-noise (GIN) tests than non-exposed control subjects. Bazilio et al (2012) found that organophosphate pesticide-exposed workers presented with abnormal results (based on previously reported norms) for duration pattern and GIN tests. Also, Teixeira et al (2002) studied the effects of occupational exposure to organophosphates and pyrethroid insecticides on the central auditory function. Central auditory system functions were assessed through frequency pattern and duration pattern tests (both tests evaluate temporal processing). Fifty-six per cent of exposed workers presented with central auditory dysfunction. The relative risk was 7.58 for the group exposed to insecticides (95% CI 2.9–19.8) when compared to the non-exposed group. In addition, the group exposed to insecticides and noise had a relative risk for central auditory dysfunction of 6.5 (95% CI: 2.2–20.0) when compared to the non-exposed group, and 9.8 (95% CI: 1.4–64.5) when compared to the group exposed only to noise. No other studies investigating temporal processing in pesticide-exposed workers have been identified in the existing literature. However, studies conducted in populations of workers exposed to organic solvents have found that these chemicals may adversely affect temporal resolution abilities (Fuente et al, 2011, 2013).

Regarding the DDT, abnormal results were found in 86.4% of pesticide-exposed workers as opposed to 38.1% of non-exposed workers. Pesticide exposure was significantly associated with abnormal results for this test. Indeed, the relative risk for failing this test was 1.88 for the group of participants exposed to pesticides as compared with non-exposed control participants. Fuente and McPherson (2007) investigated a group of subjects exposed and non-exposed to solvents. Solvent-exposed subjects presented with significantly worse results than non-exposed subjects for a number of central auditory function tests, including the DDT.

Recommendations arising from the results of this study

The results from this study showed that workers exposed to pesticides presenting with hearing thresholds similar to non-exposed workers may still show signs of auditory dysfunction. Specifically, pesticide-exposed workers presented with a significantly worse performance for temporal resolution and binaural integration abilities than non-exposed workers. This finding re-emphasises the previously suggested recommendation that pure-tone audiometry may be insufficient when evaluating the auditory system in workers exposed to chemicals, including pesticides (Morata & Little, 2002; Fuente & McPherson, 2006; SESA, 2013). Therefore, and similar to suggestions arising from other authors (Morata & Little, 2002; Johnson & Morata, 2010; Campo et al, 2013), results from this study suggest that workers exposed to pesticide should be evaluated with a comprehensive audiological test battery. This test battery should comprise procedures exploring different aspects/functions relating to audition. Procedures, such as RGD and DD tests, should be used when evaluating hearing in pesticide-exposed workers. This is because in this research, results for these procedures have been shown to be poor among pesticide-exposed workers. In addition, both tests are easy to administer, they do not require sophisticated equipment, and they can both be completed in approximately 10 minutes. Also, the RGDT does not use verbal material as stimuli and the DDT uses numbers as stimuli, a low-level commonly accessible material. Therefore, both tests, especially the RGDT, are free from language problems that may interfere with test results.

Limitations of the study

An important limitation in the present study is the lack of environmental pesticide concentrations and/or biological marker values for pesticide exposure. This limits dose/response relationship analyses and therefore, with this study, it was not possible to determine safe levels of pesticide exposure to hearing health. In addition, workers reported being exposed to a mixture of pesticides which varied over time. Therefore, it was not possible to determine the adverse auditory effects of isolated chemical components. The deleterious auditory effects observed are rather associated with a mixture of pesticides. It was not possible to conclude that all pesticides used by workers investigated in this study are hazardous for the auditory system. Neither additive effects nor synergistic effects could be explored. Finally, this study has the limitations of all cross-sectional studies. It was not possible to conclude a causal relationship but rather indicate an association between pesticide exposure and auditory dysfunction. Workers exposed to pesticides may have some other inherent variables that may make them more susceptible to auditory dysfunction than the control group of workers investigated. Future studies should consider a longitudinal design in order to better characterise the association between pesticide exposure and auditory dysfunction.

Conclusions

The results of the present study showed that rural tobacco growers exposed to pesticides presented with significantly worse results for two clinical procedures to evaluate CAF, the RGDT and DDT, as compared to non-exposed subjects. These results were found despite there being no differences for hearing thresholds between pesticide-exposed and non-exposed workers. Therefore, it is concluded that pesticide exposure may be associated with declines in temporal resolution and binaural integration abilities. Taking these results into consideration, it is suggested that both the RGDT and DDT should be utilised to monitor CAF among pesticide-exposed workers. Further research should be conducted to determine the sensitivity of both procedures to detect CAF dysfunction associated with pesticide exposure using larger sample sizes than in the present study.

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References

Alcaras P.A.S. 2012. *Estudo exploratório das emissões otoacústicas e efeito supressão em trabalhadores expostos ao ruído e agrotóxicos.* Dissertação (Mestrado) – Universidade Tuiuti do Paraná. Programa de Distúrbios da Comunicação. Curitiba, 2012.

American Academy of Audiology - AAA (2010). Diagnosis, treatment and management of children and adults with central auditory processing disorder [Clinical Practice Guidelines]. Retrieved www.audiology.org/resources/documentlibrary/Documents/CAPD Guidelines 8-2010.pdf.

American National Standards Institute. (1991). *Maximum Permissible Ambient Noise Levels for Audiometric Test Rooms. (ANSI S3.1-1991)*. New York: ANSI.

American National Standards Institute. (2010). *Specification for audiometers (ANSI S3.6-2010)*. New York, NY: ANSI.

Araújo A.J., Lima J., Moreira J., Jacob S., Soares M., et al. 2007. *Exposição múltipla a agrotóxicos e efeitos à saúde: estudo transversal em amostra de 102 trabalhadores rurais*, Nova Friburgo, RJ. *Ciênc saúde coletiva*, 12, 115–130.

Associação dos Fumicultores do Brasil (AFUBRA). *História verde é vida.* 2012. Retrieved September 15, 2012: <http://www.afubra.com.br/index.php/conteudo/show/id/66>.

Bazilio M.M.M., Frota S., Chrisman J.R., Meyer A., Asmus C.I.F., et al. 2012. Temporal auditory processing in rural workers exposed to pesticide. *J Soc Bras Fonoaudiol*, São Paulo, 24, 174–180.

Beckett W.S., Chamberlain D., Hallman E., May J., Hwang S., et al. 2000. Hearing conservation for farmers: Source apportionment of occupational and environmental factors contributing to hearing loss. *J Occup Environ Med*, 42, 806–813.

Bielefeld E.C., Hu B.H., Harris K.C. & Henderson D. 2005. Damage and threshold shift resulting from cochlear exposure to paraquat-generated superoxide. *Hear Res*, 207, 35–42.

Brasil. Secretaria Nacional de Vigilância Sanitária, do Ministério da Saúde. Portaria n° 3 de 16 de janeiro de 1992. Anexo III. Brasília, 1992. Retrieved November 15, 2015: <http://www.brasilsus.com.br/legislacoes/anvisa/16889-3.html>.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Saúde Ambiental e Saúde do Trabalhador. Coordenação Geral de Vigilância em Saúde Ambiental. 2012. *Modelo de Vigilância em Saúde de Populações Expostas a Agrotóxicos.* Brasília.

Cáceres T., Megharaj M., Venkateswarlu K., Sethunathan N. & Naidu R. 2010. Fenamiphos and related organophosphorus pesticides: Environmental fate and toxicology. *Rev Environ Contam Toxicol*, 205, 117–162.

Camarinha C.R., Frota S., Pacheco-Ferreira H. & Lima M.A.T. 2011. Avaliação do processamento auditivo temporal em trabalhadores rurais expostos a agrotóxicos organofosforados. *J Soc Bras Fonoaudiol*, 23, 102–106.

Campo P., Morata T. & Hong O. 2013. Chemical exposure and hearing loss. *Dis Mon*, 59, 119–138.

Carhart R. & Jerger J. 1959. Preferred method for clinical determination of pure-tone thresholds. *J Speech Hear Disord*, 24, 330–345.

Conselho Federal de Fonoaudiologia (CFFa). 2013. Manual do Conselho Federal de Audiologia. Retrieved November 15, 2015: http://www.fonoaudiologia.org.br/publicacoes/Manual%20de%20Audiologia.pdf>.

Crawford J.M., Hoppin J.A., Alavanja M.C., Blair A., Sandler D.P., et al. 2008. Hearing loss among licensed pesticide applicators in the agricultural health study. *J Occup Environ Med*, 50, 817–826.

Cummings P. 2011. Estimating adjusted risk ratios for matched and unmatched data: an update. *Stata J*, 11, 290–298.

Dassanayake T., Weerasinghe V., Dangahadeniya U., Kularatne K., Dawson A., et al. 2008. Long-term event-related potential changes following organophosphorus insecticide poisoning. *Clin Neurophysiol*, 119, 144–150.

Dassanayake T., Gawarammana I.B., Weerasinghe V., Dissanayake P.S., Pragaash S., Dawson A., et al. 2009. Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. *Clin Neurophysiol*, 120, 1693–1698.

Departamento de Estudos Sócios-Econômicos Rurais (DESER). 2013. *Fumo e mercado mundial: manipulação por parte da fumageiras.* Curitiba. Retrieved April 26, 2013: <http://www.deser.org.br/documentos/imagem/FEEMMPPDF.pdf>.

Fleiss J.L., Levin B. & Paik M.P. 2003. *Statistical Methods for Rates and Proportions* (3rd edition). Hoboken, NJ: Wiley.

França D.M.V.R. 2013. *Efeitos do uso dos agrotóxicos no sistema auditivo central dos fumicultores da Região Centro-sul do Paraná*. Curitiba. Tese (Doutorado) – Universidade Tuiuti do Paraná. Programa de Pós-Graduação em Distúrbios da Comunicação.

Fuente A. & McPherson B. 2006. Auditory processing tests for Spanish-speaking adults: an initial study. *Int J Audiol*, 45, 645–659.

Fuente A. & McPherson B. 2007. Central auditory processing effects induced by solvent exposure. *Int J Occup Med Environ Health*, 20, 271–279.

Fuente A., McPherson B. & Hickson L. 2011. Central Auditory dysfunction associated with exposure to a mixture of solvents. *Int J Audiol*, 50, 857–865.

Fuente A., McPherson B. & Hickson L. 2013. Auditory dysfunction associated with solvent exposure. *BMC Public Health*, 13, 39.

Gatto M., Fioretti M., Fabrizi G., Gherardi M., Strafella E., et al. 2014. Effects of potential neurotoxic pesticides on hearing loss: a review. *Neurotoxicology*, 42, 24–32.

Guida H.L., Morini R.G. & Cardoso A.V. 2010. Audiological evaluation in workers exposed to noise and pesticide. *Braz J Otorhinolaryngol*, 76, 423–427.

Guida H.L., Morini R.G. & Cardoso A.C.V. 2009. Audiologic and otoacoustic emission evaluation in individuals exposed to noise and plaguecides. *Int Arch Otorhinolaryngol*, 13, 264–269.

Hoshino A.C., Pacheco-Ferreira H., Taguchi C.K., Tomita S. & Miranda M.F. 2008. Estudo da ototoxicidade em trabalhadores expostos a organofosforados. *Rev Bras Otorrinolaringol*, 74, 912–918.

Hughson W. & Westlake H.D. 1944. Manual for program outline for rehabilitation of aural casualties both military and civilian. *Trans Am Acad Ophthalmol Otolaryngol*, 48, 1–15.

Jayasinghe S.S. & Pathirana K.D. 2011. Effects of deliberate ingestion of organophosphate or paraquat on brain stem auditory-evoked potentials. *J Med Toxicol*, 7, 277–280.

Jerger J. 1970. Clinical experience with impedance audiometry. *Arch Otolaryngol*, 92, 311–324.

Johnson A.C. & Morata T.M. 2010. *Occupational Exposure to Chemicals and Hearing Impairment*. *Arbet OCH Hälsa.* Gothenburg, Sweden.

Keith R.W. 2000. *Random Gap Detection Test*. St Louis, MO: Auditec St Louis Ltd.

Kimura K., Yokoyama K., Sato H., Nordin R.B., Naing l., et al. 2005. Effects of pesticides on the peripheral and central nervous system in tobacco farmers in Malaysia: Studies on peripheral nerve conduction, brain-evoked potentials and computerized posturography. *Ind Health*, 43, 285–294.

Körbes D., Silveira A., Hyppolito M.A. & Munaro G. 2010. Organophosphate-related ototoxicity: Description of the vestibulocochlear system ultrastructural aspects of guinea pigs. *Braz J Otorhinolaryngol*, 76, 238–244.

Manjabosco C.W., Morata T.C. & Marques J.M. 2004. Perfil audiométrico de trabalhadores agrícolas. *Arq Otorrinolaryngol*, 8, 285–295.

Morata T.C. & Little M.B. 2002. Suggested guidelines for studying the combined effects os occupational exposure to noise and chemicals on hearing. *Noise Health*, 4, 73–87.

Paraná. Secretaria de Estado da Saúde do Paraná - SESA. *Protocolo de avaliação das intoxicações crônicas por agrotóxicos*. Superintendência de Vigilância em Saúde, Curitiba, 2013.

Pereira L.D. & Schochat E. 1997. *Processamento Auditivo Central: Manual de Avaliação*. São Paulo: Lovise.

Sidell F.R. 1994. Clinical effects of organophosphorus cholinesterase inhibitors. *J Appl Toxicol*, 14, 111–113.

Teixeira C.F., Augusto L.G. & Morata T.C. 2002. Occupational exposure to insecticides and their effects on the auditory system. *Noise Health*, 4, 31–99.

Teixeira C.F., Augusto L.G. & Morata T. 2003. Saúde auditiva de trabalhadores expostos a ruídos e inseticidas. *Rev Saúde Pública*, 37, 417–423.