Study of Evoked Otoacoustic Emissions and suppression effect on workers exposed to pesticides and noise

Estudo das Emissões Otoacústicas Evocadas e efeito de supressão em trabalhadores expostos a agrotóxicos e ruído

ABSTRACT

Purpose: To analyze the findings for Evoked Otoacoustic Emissions (EOEA) and suppression effect in workers with normal hearing exposed to pesticides and noise. Methods: The sample consisted of 55 participants with normal hearing, with ages ranging from 18 to 35 years, divided into two groups: one group exposed to noise and pesticides (GRA) and a control group (CG). The GRA was composed of 25 participants exposed to a representative average level of daily occupational noise exposure of 86 dBA and organophosphate-type pesticides, whereas the GC was composed of 30 participants who were not exposed to noise or pesticides. All participants underwent Transient Evoked Otoacoustic Emissions (TEOAE) and Distortion Product Otoacoustic Emissions (DPOAE) examinations and research of the suppression effect. Results: The findings revealed differences between the results of TEOAE and DPOAE exams between GRA and GC groups. GRA participants showed worse results in EOAE findings. Regarding the suppression effect, lesser effects were observed in the GRA. Conclusion: The results suggest that the study of EOAE and suppression effect can be used in early identification of hearing damage to workers simultaneously exposed to pesticides and noise.

RESUMO

Objetivo: Analisar os achados das Emissões Otoacústicas Evocadas (EOAE) e efeito de supressão em trabalhadores normo-ouvintes expostos a agrotóxicos e ruído. Métodos: A amostra foi constituída por 55 participantes normo-ouvintes, com faixa etária variando de 18 a 35 anos, distribuídos em dois grupos: grupo ruído e agrotóxicos (GRA) e grupo controle (GC). O GRA foi composto por 25 participantes expostos a um nível médio de ruído, representativo da exposição ocupacional diária, de 86 dBA e agrotóxicos do tipo organofosforado; já o GC foi composto por 30 participantes não expostos nem a ruídos e/ou agrotóxicos. Todos os participantes foram submetidos ao exame das Emissões Otoacústicas Evocadas por Estímulo Transiente (EOAET) e Produto de Distorção (EOAEPD) e pesquisa do efeito de supressão. Resultados: Os achados revelaram haver diferença entre os achados das EOAET e EOAEPD entre os grupos, GRA e GC. Os participantes do GRA apresentaram piores resultados nos achados das EOAE. Quanto ao efeito de supressão, foi observado menor efeito de supressão no GRA. Conclusão: Os resultados sugerem que o estudo das EOAE e efeito de supressão podem ser usados na identificação precoce dos agravos à audição dos trabalhadores expostos simultaneamente a agrotóxicos e ruído.

Conflict of interest: nothing to declare.
INTRODUCTION

Scientific literature on the joint effect of exposure to noise and chemical agents on the auditory system is extensive\(^{(1)}\). However, in Brazil, there are no specific recommendations to assess the hearing of workers exposed to chemical agents. Legally, only workers exposed to noise levels higher than 85 dBA (Leq/8 hours) are assessed audiometrically according to the recommendations of the NR7 Regulatory Norm\(^{(2)}\). Therefore, only a portion of workers exposed to chemical agents (whose exposure to noise is considered excessive) is included in hearing loss prevention programs.

The peripheral and central effects of metals, solvents and some asphyxiants are known\(^{(1,3,4)}\); however, the auditory effects of exposure to noise and pesticides must be analyzed further\(^{(4)}\).

It is suspected that the medial efferent olivocochlear auditory system is affected by the action of organophosphate pesticides through the inhibition of enzymes responsible for the hydrolysis of acetylcholine, a primary neurotransmitter of protein of external ciliate cells present in the neuromuscular plates of contractile cells\(^{(5)}\). Its action is very important for the synapses of external ciliate cells\(^{(5)}\). However, when there is an accumulation of acetylcholine neurotransmitters, this situation could lead to acute intoxication by cholinesterase inhibition\(^{(6)}\). Another hypothesis is that the ototoxicity consequential of intoxication by organophosphates is the result of the genesis of reactive oxygen species\(^{(7)}\).

Studies show that, on their own, pesticides can affect the auditory system, both peripheral and central, causing a marked difficulty in the comprehension of speech sounds uttered in the midst of noise\(^{(7-15)}\). For this reason, it is necessary to monitor the peripheral and central auditory system of workers exposed to these substances, and to include them in hearing preservation programs\(^{(4,6,12)}\), even though it is not mandatory, as per the national labor law, to monitor these individuals epidemiologically through audiometry\(^{(2)}\) and/or complementary tests.

Scholars have shown the necessity of conducting tests that complement occupational audiologic assessments, considering that tonal audiometry has not been sufficient to assess the extent of lesions caused by otoacoustically aggressive agents, such as noise and chemical agents\(^{(3,4)}\).

As an alternative complementary test, the study of Evoked Otoacoustic Emissions (EOAE) can contribute to audiologic monitoring and the early detection of hearing loss, above all in the presence of chemical agents such as pesticides\(^{(3,4,16)}\).

Among the existing EOAE tests, the assessment of emission suppression effects is characterized by applying a contralateral masking noise against the ear analyzed. This procedure enables the assessment of the actions performed by the medial olivocochlear efferent auditory system\(^{(17-24)}\), one of the mechanisms most affected by the action of pesticides\(^{(6)}\). The contralateral noise provokes a decrease in the number of EOAE responses, thus indicating normal efferent functioning\(^{(5,17-24)}\). It is worth highlighting that the olivocochlear efferent auditory system is possibly responsible for sound localization, auditory attention, auditory sensitivity and detection of acoustic signals in the presence of noise\(^{(25-27)}\).

Considering what has been exposed so far, in the present study we aimed at analyzing findings concerning Otoacoustic Emissions Evoked by Transient Stimulus, Distortion Product, and suppression effect among workers exposed to pesticides and noise.

METHODS

The present observational and transversal study was initiated after the approval granted by the Ethics Committee and the Central Research Coordination (CCPq) of Universidade do Oeste Paulista (UNOESTE), under protocol number 647/11. The procedures were carried out after all participants signed the Informed Consent. The individuals were allowed to withdraw their participation at any moment. The Informed Consent had two copies, one for the researchers and one for the participant, elaborated on a single page, dated and signed by the parts involved, and filed for 5 years.

The sample was composed of 55 normal listeners with auditory thresholds of up to 25 dBA, according to the criteria suggested on paragraph 19 of NR7’s Appendix I\(^{(2)}\). They were allocated in two groups: Group exposed to noise and pesticides (NPG) and control group (CG).

The criteria for inclusion in the NPG were the participants’ age (between 18 and 35 years), and no alteration in the external acoustic meatus, inspected by means of an otoscope and type A tympanometry. To be included in the study, the participants in the CG could not have a history of occupational exposure to high levels of sound pressure and/or pesticides, and otologic complaints.

The participants of the NPG were referred by the Workers’ Health Referral Center (Centro de Referência em Saúde do Trabalhador [CEREST]). Upon being selected in accordance with the inclusion prerequisites, they were contacted and invited to participate in the study as volunteers, without participation onus and with permission to quit at any time. The information about their occupational exposure, individual safety equipment, and noise assessments, contained in the medical charts of each participant and in the environmental risk prevention program, were collected at CEREST.

The participants in the CG were personally invited to participate in the study. They were part of the staff of a Post-Secondary Institution. We hereby declare that the supervisor responsible for the workers of this institution was notified about the study. Upon signing the “Authorization to Contact Individuals for a Research Study,” he allowed the workers to participate.

Thus, the CG was composed of 30 individuals, 19 women and 11 men, aged between 18 and 35 years (mean of 27.43 years; standard deviation of 5.03).

The NPG counted 25 participants, 11 women and 14 men, aged between 22 and 35 years (mean of 26.29 years; standard deviation of 3.77).

According to the medical charts consulted at CEREST, all NPG participants were agents dealing with endemics (100%) and exposed to an organophosphate pesticide commercially known as Malathion. This pesticide is used during...
anti-vectorial campaigns that occur annually between the months of December and July. They work with educational prevention initiatives during the remaining months of the year. The daily exposure time to this pesticide is 6 hours. The chemical substance is spread with the aid of motorized back atomizers, with an average weight of 10 kg, generating a mild level of noise of 86 dBA when operated, which corresponds to their daily occupational exposure.

Because they are exposed to noise during their work activities, 20 (80%) NPG participants use Hearing Protection Equipment: 15 (60%) use earplugs and earmuffs, 1 (4%) uses earplugs, and 4 (16%) use earmuffs.

In addition to Hearing Protection Equipment, all NPG participants (100%) make use of other protection equipment, such as masks, pants, capes, boots, gloves, helmets, goggles and repellents, given that they are exposed to chemical agents.

With the purpose of investigating Transient Stimulus Otoacoustic Emissions (TSOAE), Distortion Product (DPOAE) and suppression effect, we utilized a cochlear electrophysiological device produced by Bio-logic Systems Corp., model Audix Plus, and a Scout application software attached to a microcomputer.

The procedure consisted in placing a catheter in the external acoustic meatus with a signal generator, microphone, amplifier and filters (to reject any unwanted noise).

First, we investigated the TSOAE using a non-linear click stimulus, because this stimulus type is brief and it encompasses a wide gamma of frequencies (1000, 1500, 2000, 3000, and 4000 Hz) at 80 dBSPL (sound pressure level). The responses were obtained after 3.5 ms within a timeframe of 16.6 ms.

We analyzed the TSOAE relative response levels (signal/noise relation) within a latency period of 3.5–16.6 ms. We considered TSOAE as present when the reproducibility value was ≥50%, the stability of the catheter was ≥70%, and the level of relative response was ≥3 dBSPL in at least three consecutive frequencies at 1000, 1500, 2000, and 4000 Hz.

Next, we registered the DPOAE. The stimulus evocation employed to investigate this type of emission consisted of two pure tones (f1 and f2) presented simultaneously with very approximate sound frequencies (f2/f1=1.22), using sound pressure levels of L1=65 dBSPL and L2=55 dBSPL. The frequency spectrum ranged from 750 to 7969 Hz, in accordance with the classification, which suggests the presence of DPOAE when the signal/noise ratio is ≥6 dBSPL. This value was also standardized by the equipment used.

After registering the TSOAE and the DPOAE, we investigated the Suppression Effect of the TSOAE only with the participants who showed present responses for the TSOAE established in the aforementioned criteria. We applied a masking noise to the ear that was contralateral to the ear analyzed, and registered it bilaterally.

Owing to the fact that the equipment used to register the EOE in the population studied lacked a suppression system, we utilized a clinical audiometer with TDH-29 phones to apply the masking noise. We applied a broadband White Noise noise at the intensity of 60 dBSPL.

The parameter employed to register the suppression effect of the TSOAE was the application of a click-type linear stimulus at the frequency ranges of 1000, 1500, 2000, 3000, and 4000 Hz. The response was obtained after 3.5 ms within a time period of 16.6 ms.

In the analyses, we surveyed the phenomena observed by verifying if the participants in the NPG presented different suppression effect responses than in the CG, considering the overall response. To quantify the suppression, we subtracted the amplitudes of the responses with and without competitive noise.

Following the data collection, we returned the results to each participant, orally and in writing. In addition, the participants in the NPG received instructions about hearing protection measures, use of individual protection equipment, and explanations about the harms provoked by noise and pesticides to the organism.

In order to characterize the response levels of the TSOAE, DPOAE, and the suppression effect of the groups, the data obtained were submitted to a descriptive and quantitative statistical analysis, with application of central measures of tendency, mean, and standard deviation, taking into consideration the ears (right and left) and the frequency ranges of the tests described previously. Only the participants who showed present responses in the TSOAE, DPOAE, and TSOAE suppression effect were included in the statistical analyses. All others were excluded.

In order to analyze the variability among the TSOAE, DPOAE, and suppression effect averages between the NPG and the CG, we applied Student’s t test and Fisher’s test, with a significance level of 0.05 (5%).

RESULTS

Graph 1 displays the comparison of the occurrence of absent and present responses of the TSOAE between the control group (CG) and the study group (NPG) by ear. Using Fisher’s test with a significance level of 0.05 (5%), we verified that there was no significant relation between the present and the absent responses of the CG and the NPG in relation to the right ear (p=0.5730) and the left ear (LE) (p=0.0850).

Table 1 presents the comparison of the occurrence of absent and present DPOAE responses in the right ear and the LE in the CG and the NPG in relation to the frequencies. Upon applying the chi-square test with a significance level of 0.05 (5%), we verified that there was no significant relation in the bilateral comparison of absent and present responses between the CG and the NPG.

Table 2 shows the calculation of the mean and the comparison of relative response levels (signal/noise relation) of the TSOAE for the NPG and the CG. Upon comparison of the means of the results between the groups, it was possible to observe differences in the frequencies of 1000, 1500 Hz in
both ears, and 2000 in the LE, as well as in the overall LE mean (p<0.05). In all these cases, the response level in the NPG was lower than in the CG.

Table 3 displays the calculation of the mean and the comparison of the level of relative response (signal/noise relation) of the DPOAE for the NPG and the CG. After comparing the NPG and the CG, the DPOAE response levels in regards to the signal/noise relation at the frequencies of 6000 and 7969 Hz in both ears and 3984 in the LE were lower in the NPG than in the CG, and this is statistically significant (p<0.05). In regards to lower frequencies, 750 and 984 Hz, lower response levels were observed in the CG (Table 3).

Table 4 shows the comparison of TSOAE general response results with and without competitive contralateral noise in the CG and in the NPG. The results suggest that the general response level, with and without noise, was lower in the NPG (p<0.05).

Table 5 displays the calculation of the mean, maximum and minimum TSOAE suppression values and the comparison between the groups. Through the application of Student’s t test with a significance level of 0.05 (5%), we verified differences between the groups, as the NPG presented lower TSOAE suppression values than the CG.

**DISCUSSION**

Authors have pointed out audiometric findings in workers exposed to noise and chemical agents (8,9); however, few reinforce the importance of complementary audiologic assessments, such as the investigation of EOAE and suppression effect in this population (17-24).

It is known that exposure to pesticides can provoke auditory alterations in human beings to the extent of injuring both the peripheral and the central auditory system (7-15).

In the present study, all participants presented auditory thresholds of up to 25 dBNA (NR-7), which suggests that all could potentially present EOAE (5,16,28). However, the results shown on Figure 1 and Table 1 demonstrate that the occurrence of present and absent EOAE in the groups evaluated did not present any differences (p<0.05). This finding may suggest that the occurrence of present responses in normal listeners (<25 dBNA) does not reach 100%. In similarity to our findings, the results of a study on the exposure to a chemical agent (29), considering the analysis of both ears together, show that the occurrence of TSOAE was higher in the group exposed in comparison to the control group, but without any differences. From the point of view of workers’ health, the analysis of the occurrence of the signal/noise relation should not be considered separately; it is recommended that any audiologic findings (including anamnesis) be interpreted in combination (4,16).
Table 3. Mean calculation and comparison of the response levels concerning the Otoacoustic Emissions Evoked by Distortion Product between the control group and the noise and pesticides group (n=49)

<table>
<thead>
<tr>
<th>Ear and frequencies (Hz)</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>10.1</td>
<td>17.0</td>
<td>5.9</td>
</tr>
<tr>
<td>NPG</td>
<td>14.1</td>
<td>18.4</td>
<td>7.3</td>
</tr>
<tr>
<td>CG</td>
<td>16.9</td>
<td>18.4</td>
<td>6.8</td>
</tr>
<tr>
<td>NPG</td>
<td>21.6</td>
<td>18.8</td>
<td>9.2</td>
</tr>
<tr>
<td>CG</td>
<td>17.9</td>
<td>18.7</td>
<td>7.9</td>
</tr>
<tr>
<td>NPG</td>
<td>19.9</td>
<td>16.5</td>
<td>7.0</td>
</tr>
<tr>
<td>CG</td>
<td>18.1</td>
<td>12.4</td>
<td>7.8</td>
</tr>
<tr>
<td>NPG</td>
<td>15.5</td>
<td>5.2</td>
<td>10.4</td>
</tr>
<tr>
<td>LE</td>
<td>10.6</td>
<td>18.4</td>
<td>6.3</td>
</tr>
<tr>
<td>RE</td>
<td>12.9</td>
<td>17.8</td>
<td>9.7</td>
</tr>
<tr>
<td>LE</td>
<td>19.6</td>
<td>17.2</td>
<td>8.6</td>
</tr>
<tr>
<td>RE</td>
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<tr>
<td>LE</td>
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<tr>
<td>LE</td>
<td>20.6</td>
<td>10.4</td>
<td>8.1</td>
</tr>
<tr>
<td>LE</td>
<td>17.5</td>
<td>6.3</td>
<td>11.1</td>
</tr>
</tbody>
</table>

*p<0.05 (Student’s t test).

Caption: CG = control group; NPG, noise and pesticides group; RE, right ear; LE, left ear; Hz, hertz.

Table 4. Mean calculation and comparison of the overall response levels concerning the Transient Stimulus Otoacoustic Emissions with and without contralateral noise between the control group and the noise and pesticides group

<table>
<thead>
<tr>
<th>Ear</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>10.9</td>
<td>17.0</td>
<td>5.0</td>
</tr>
<tr>
<td>NPG</td>
<td>11.1</td>
<td>17.8</td>
<td>4.4</td>
</tr>
<tr>
<td>CG</td>
<td>1.2</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>NPG</td>
<td>1.0</td>
<td>0.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*p<0.05 (Student’s t test).

Caption: CG = control group; NPG, noise and pesticides group; RE, right ear; LE, left ear.

Table 5. Mean calculation, maximum and minimum suppression values of the Transient Stimulus Otoacoustic Emissions and comparison between the control group and the noise and pesticides group

<table>
<thead>
<tr>
<th>Group and ear</th>
<th>n</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG–RE</td>
<td>27</td>
<td>9.6</td>
<td>2.2</td>
<td>18.1</td>
<td>4.7</td>
<td>0.0303</td>
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<tr>
<td>NPG–RE</td>
<td>22</td>
<td>6.9</td>
<td>0.4</td>
<td>14.8</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>CG–LE</td>
<td>27</td>
<td>10.1</td>
<td>1.7</td>
<td>18.9</td>
<td>4.5</td>
<td>0.0089</td>
</tr>
<tr>
<td>NPG–LE</td>
<td>19</td>
<td>6.5</td>
<td>0.6</td>
<td>16.4</td>
<td>4.3</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 (Student’s t test).

Caption: CG = control group; RE, right ear; NPG, noise and pesticides group; LE, left ear.

Upon comparison of the relative response level in relation to TSOAE (Table 2) and DPOAE (Table 3), we observed that the NPG presented worse results than the CG. This finding possibly demonstrates the cochlear effect caused by exposure to noise and pesticides among NPG participants. This finding has also been verified in recent studies[8,12].

Some authors have highlighted that the exposure to pesticides can provoke alterations in the morphology of the cochlea, which justifies the alterations in the EOAE registers[11].

In regards to the findings concerning the DPOAE (Table 3), scholars have pointed out that higher frequencies are more susceptible to alterations through exposure to noise and other ototoxicologically aggressive agents[12,17,22]. DPOAE are an important instrument to assess and monitor individuals exposed to noise and chemical agents.

In the lower frequencies of 750 and 984 Hz, we observed a less marked relation between signal and noise in the CG when compared to the NPG (Table 3). This finding can be justified by the fact that, in this frequency range, this response suffered the influence of environmental noise and noises produced by the patient. Considering this, the DPOAE responses would have been more precise in frequencies higher than 2000 Hz[28].

In regards to the DPOAE suppression effect, another study has evidenced a larger reduction in the general response levels of emissions with suppression effect by means of TSOAE in comparison to DPOAE[17]. This influenced our choice for the protocol used in the present study to test inhibition in the mechanical activity of ciliate cells through TSOAE[17,18,27–30].

Still concerning the investigation protocol, the level of contralateral stimulus applied here (60 dBSPL) is recommended and accepted in clinical practice[5,28]. However, authors have reported that the signal/noise relation average can increase with higher stimulus levels (of up to 69 dBSPL)[17,27,28]. In other studies[17,24], scholars have considered the presence of TSOAE when the general response was higher than 6 dBSPL, with response reproducibility and stability above 70%. As previously mentioned, there is no consensus about an investigation protocol to assess the suppression effect of EOAE, which points to the need for advancements in research on this topic.

After comparing both groups, we found differences in the suppression effect averages in the general TSOAE response with and without noise (p<0.05) (Table 4). The results show that the NPG presented lower suppression effect when compared to the CG, which may suggest that the functioning of the superior olivary complex was lesser than in the CG. This finding can represent the risk posed by exposure to noise and pesticides to the auditory system.

Concerning suppression effect values (Table 5), we observed differences between the groups (p<0.05). The NPG presented lower suppression values, which suggests that the functioning of the superior olivary complex was lesser than in the CG. This finding can represent the risk posed by exposure to noise and pesticides to the auditory system.

Differences in suppression effect values are expressed in dB in the majority of studies. However, there is a broad individual variability to be considered[8,18]. These data have not been standardized, but authors have indicated that the suppression value must be higher than 1 dBSPL[5,25,28].

The results obtained in this study are conducive to reflections to be developed in future studies with the use of objective measures that will aid in the early identification of auditory effects consequential of exposure to noise and pesticides.

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Therefore, testing EOAE and the suppression effect can be considered an excellent complementary resource in the early identification of alterations in the auditory system of workers exposed to pesticides, and it can be used as an important instrument for epidemiological surveillance.

Advancements in research are fundamental for the knowledge of auditory effects consequential of exposure to noise and chemical agents, as well as possible changes in legislation, since the preventive strategies to protect the hearing of workers exposed to noise currently in place are possibly not the same as those for workers exposed to chemical agents. In these instances, the risk is assessed separately and the interaction between the agents is not taken into consideration in auditory monitoring.

Notwithstanding the evidence of risk exposed in this study and in recent publications, little attention is paid by health professionals and individuals responsible for health policies to the risks posed by chemical agents.

With the purpose of supporting the results presented here, we suggest the conduction of future studies on EOAE that compare populations exposed to pesticides, noise, and to noise and pesticides simultaneously, as well as other groups exposed to other chemical agents.

**CONCLUSION**

Based on the results, we conclude that there was no difference in the occurrence of present and absent responses between the groups. There was a reduction in the signal/noise relation of the OAE evoked by transient stimulus and by distortion product in the NPG in comparison to the CG.

Concerning the TSOAE suppression effect, the NPG presented a lower general response level and lower suppression value, which suggests that chronic exposure to noise and pesticides may have impaired the functioning of the medial olivocochlear efferent auditory system.

*PASA contributed with the elaboration of the project and timetable, literature research, data collection and analysis, paper writing and submission, and wrote the dissertation on which this article is based; ABML contributed with study and schedule elaboration, data analysis, paper writing, and supervision of the dissertation that generated this paper; JMM contributed with data analysis and the final version of this article.

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