Effect of the co-exposure to organophosphate pesticides and noise on the auditory function of farm workers

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INTRODUCTION

On a daily basis, farmers use mobile and fixed machinery generating noise levels from 85 to 120 dBA (EU-OSHA 2008; Health and Safety Executive 2009). Therefore, it is not surprising that noise-induced hearing loss is common among farmers (Plakke & Dare 1992; Hwang et al. 2001; McBride et al. 2003). However, noise is rarely the sole contaminant in most work settings (Campo et al. 2009). Studies have demonstrated that concomitant exposure to noise and a variety of chemicals (solvents, asphyxiants, heavy metals, pesticides) increases the risk and/or severity of acquiring hearing loss (Campo et al. 2009; Johnson & Morata 2010; Vyskocil et al. 2011). Furthermore, organic solvents can by themselves produce otoneurotoxic effects in both humans and experimental animals (Vyskocil et al. 2009). Farmers use a variety of pesticides, among other methods, to manage pests. Organophosphate insecticides (OPs) are widely used worldwide in agriculture and horticulture because of their efficacy and rapid degradation (Maroni et al. 2000). Within this category of pesticides, frequently encountered active ingredients are malathion, chlorpyrifos, terbufos, diazinon and parathion-methyl (U.S. EPA 2004). OPs are known neurotoxic substances (Hawkers et al. 1989). They exert their neurotoxic action through the inhibition of nervous system acetylcholinesterases (AChE), enzymes responsible for the degradation of acetylcholine (ACh) at nerve junctions (Koelle 1994; Sidell 1994).

In the auditory system, the olivocochlear bundle (OCB) is mostly cholinergic and projects from the superior olivary complex (SOC, medial-MSOC, lateral-LSOC) in the brainstem to the hair cells (outer-OHC & inner-IHC) in the cochlea (Figure 1). ACh release from the olivocochlear terminals leads to a hyperpolarization of the outer hair cells (Cooper & Guinan 2006). The hyperpolarized outer hair cells elongate which in turn reduces the cochlear sensitivity (Guinan 1996; Lustig 2006). According to animal and human studies, this mechanism seems to improve signal detection in noise (Cooper & Guinan 2006). Under OPs intoxication, ACh accumulates in the synaptic space due to the inhibition of AChE, altering the transmission of action potentials from the OCB to the outer hair cells (Morata & Keith 2007). Outer hair cells are also more vulnerable to noise exposure and, in the case of a combined exposure with OPs, an interaction of effect could be expected.

Only a few human and animal studies have looked at the effect of OPs on the auditory function (Beckett et al. 2000; Teixeira et al. 2002, 2003; Beckett et al. 2004; Hoshino et al. 2008; Mac Crawford et al. 2008). Beckett et al. (2000) surveyed 185 farmers on pesticide exposure. According to these authors, 48.6 % of the farmers showed a high frequency hearing loss (measure by pure-tone audiometry) that might be related to the use of pesticides in the year prior to the study. However, noise exposure was not documented, which could explain the prevalence of hearing loss in this cohort. In a follow-up study conducted with a small subgroup of the same farm-

ers, Beckett et al. (2004) were not able to replicate their findings. Teixeira et al. (2002) examined central auditory processing in a group of 98 Brazilian workers exposed to noise and OPs as compared to a non-exposed control group (n=54). Both groups were stratified according to noise exposure level (interview). Pitch-pattern and Duration-pattern tests were administered. Results revealed that 56 % of the OP-exposed subjects showed abnormal performance on these tests compared to 7 % in the control group (χ^2 = 32.77; p<0.001). In addition, workers with a longer duration of OP exposure (>6 years) showed a larger proportion of abnormal performance (χ^2 = 8.46; p<0.004). The results were similar across noise exposed subgroups. In 2003, the same authors reported audiometric data for the same workers. Results showed a greater proportion of hearing loss (> 25 dBHL) in the workers exposed to OPs for more than 6 years (71.4 vs 57.1 %); the severity of the hearing loss and the impaired frequency range were also more important for the workers exposed to both noise and OPs as compared to workers exposed only to noise. However, none of these differences were statistically significant. More recently, Hoshino et al. (2008) examined 18 Brazilian workers exposed to OPs and found a prevalence rate of 39 % of high frequency hearing loss and abnormal peripheral vestibular function in 89 % of the workers. This study did not include a control group. As in all cited studies, exposure data for OPs were obtained using a questionnaire and no specific measures were taken. Mac Crawford et al. (2008) looked at self-reported hearing loss and pesticide exposure (including OPs) in a cohort of 14,229 American white male. Compared with controls, the odds ratio for hearing loss with OPs exposure was 1.17 [95IC: 1.03-1.31] after controlling for age, smoking, noise, solvents, and metals. All the studies reviewed here have serious limitations: lack of exposure data for OPs and/or noise, use of audiometric measures that are not sensitive to OP mode of action on the hearing system, absence of control group. Therefore, no clear evidence of an ototoxic effect for OPs can be drawn.

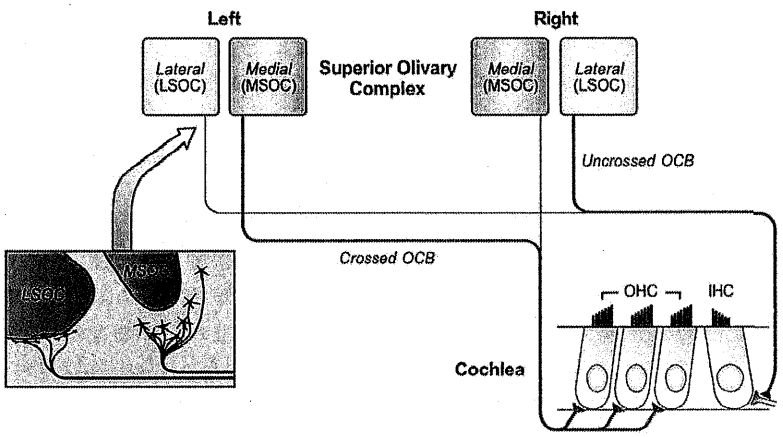

Figure 1: The mammalian olivocochlear bundle (Tan 2009)

The present study examines the effect of noise exposure and contamination by OPs on the auditory function of farm workers using standard audiometric measures and tests sensitive to dysfunctions of the efferent auditory system.

METHODS

Experimental subjects were farm workers exposed for at least 2 years to malathion, chlorpyrifos, diazinon, azinphos-methyl, phosmet or phosalone. Subjects suffering from hypertension, renal or hepatic problems, presenting alcohol or drug abuse, using ototoxic medications, exposed to solvents or heavy metals were excluded. The study group consisted of 5 workers exposed to OPs and noise, 8 noise-exposed workers, and 12 non-exposed workers for a total of 25 subjects. Subjects' characteristics are summarized in Table 1.

Table 1: Sampling description for experimental and control groups

Groups	Group 1 OPs + Noise	Group 2 Noise only	Group 3 Controls	Total
Sample size	5	8	12	25
Women	1	4	6	11
Men	4	4	6	14
Mean age (± sd)	46.4 ± 11.4	53.3 ± 12.4	45.0 ± 15.9	47.9 ± 13.4
Weight (kg)	77.8 ± 14.3	76.5 ± 16.0	72.9 ± 17.1	
Height (m)	1.74 ± 0.06	1.68 ± 0.12	1.72 ± 0.1	
Use of tobacco	20 %	25 %	16.7 %	

Data on medical and work history, noise and pesticides exposure in career, and life habits were collected through standardized questionnaires. Auditory functions were assessed in a sound-proof booth using audiometric thresholds for conventional (0.25 to 8 kHz) and very high frequencies (9 to 16 kHz), distorsion product otoacoustic emissions (DPOAEs) [parameters: f_1/f_2 de 1,22; L_1=65 dB SPL; L_1-L_2=10 dB SPL; 7 frequencies between 1 and 8 kHz; n=3 averages], contralateral suppression of transiently evoked otoacoustic emissions (TEOAEs) [parameters: click stimulus 60 dBSPL insitu, contralateral noise level 60 dBSPL, n=500 averages], acoustic reflexes thresholds and masking level difference (MLD) [parameters: S_0N_0, $S_\pi N_0$ and S_0N_π]. Normative criteria were applied to MLD data (Lynn et al. 1981) and to contralateral suppression (Berlin et al. 1993). Data were collected shortly after OP exposure (<24 h) and at least after 14 h without any noise exposure. Urine was collected in two 12-h periods, before and after exposure to OP; specific and nonspecific biomarkers of exposure to various OPs were measured (not reported in this paper). Numerical variables were studied by analysis of covariance for repeated measures variables (rANCOVAs $p<0.05$) with two within subject factors (ear, frequency), one between subject factor (group) and a covariable (age). Categorical variables were compared with chi-square (corrected $p<0.025$ for non-normal distribution). For statistical analysis, all means were adjusted for age to account for this confounding variable.

RESULTS

Table 2 shows the characteristics of OPs exposure for Group 1. The workers have been exposed to four different OPs, and none have been exposed to chlorpyrifos. The mean duration of exposure was of 132 ± 100 min and consisted of treated plants manipulation in all cases except one where one worker sprayed OPs during data collection.

Table 2: Summary of OPs exposure characteristics for Group 1 (n=5)

Characteristics of OPs exposure		Number of subjects (Proportion)
Specific OP		
Azinphos-methyl (guthion)		1 (20 %)
Chlorpyrifos		0
Malathion		1 (20 %)
Phosalone (zolone)		2 (40 %)
Phosmet (imidan)		3 (60 %)
Duration of exposure (min)	Mean	132 ± 100 [range: 30-240]
Period of exposure before collection of data		
Last 24 h		5 (100 %)
The day before		4 (80 %)
Two days before		3 (60 %)
Three days before		5 (100 %)
Tasks involving OPs		
Manipulation of treated plants		4 (80 %)
OPs spraying		1 (20 %)

Figure 2 shows the age-adjusted audiometric threshold mean for the 3 groups. The analysis revealed that workers exposed to OPs and noise showed worst hearing thresholds between 4 to 9 kHz, but only 8 kHz reached statistical significance [$F_{(2,21)}$ = 5.137, p = 0.015]. Noise-exposed subjects were showing a smaller hearing loss restricted to 3 to 6 kHz. Audiometric thresholds obtained from noise-exposed and control subjects were not statistically different.

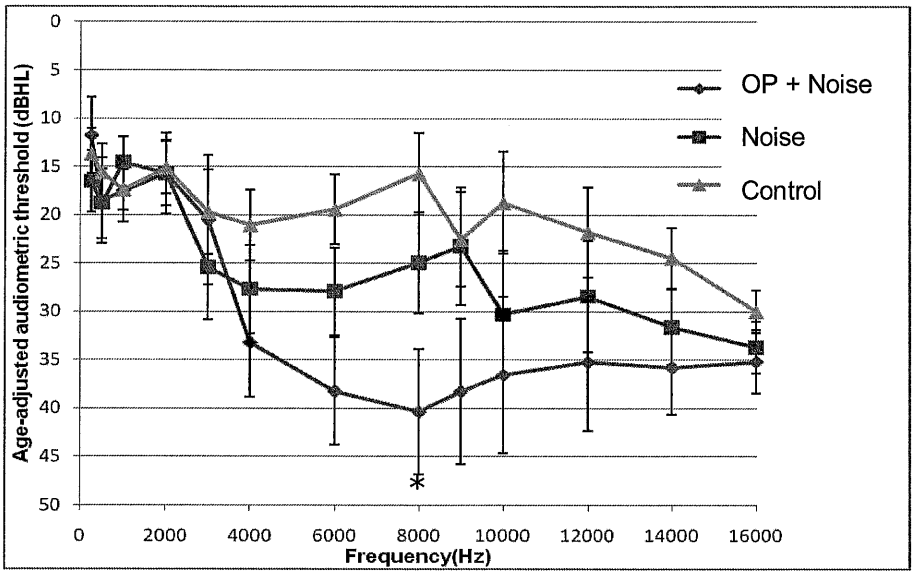

Figure 2: Group mean age-adjusted audiometric threshold

Figure 3 shows the age-adjusted DPOAE signal-to-noise ratio (SNR) mean for the 3 groups. The analysis revealed that workers exposed to OPs and noise showed smaller SNR for frequencies between 4 and 8 kHz. However, the observed differences did not reach statistical significance at any frequency [$F_{(2,21)}$ = 2.327, p = 0.121] because of weak statistical power (24 %) due to small sample size. Noise exposed and control group showed no difference.

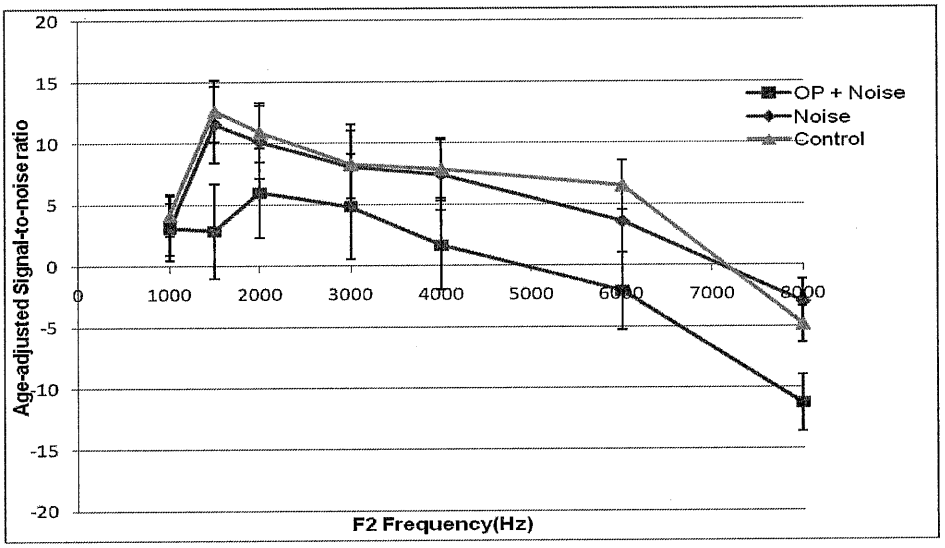

Figure 3: Group mean age-adjusted DPOAE signal-to-noise ratio

Table 3 shows the results obtained with measures sensitive to efferent system dysfunction (MLD, acoustic reflexes and contralateral suppression of TEOAEs). None of these measures showed any significant difference between OPs+noise and noise-only groups. The only significant differences were identified between controls and both experimental groups for MLD-$S_{\pi}N_0$ [χ^2_{2df} = 7.456, p = 0.024*] and MLD-S_0N_{π} [χ^2_{2df} = 9.747, p = 0.008**]. For contralateral suppression of TEAOEs, the proportion of abnormal responses was higher in the OP and noise group as compared to noise and control groups. The larger differences were noted for 1.4 and 2 kHz but failed to reach statistical significance [χ^2_{4df} = 2.625, p = 0.622].

Table 3: Summary of results for MLD, Acoustic reflexes and Contralateral suppression of TEOAE

MLD - Abnormal response (%)					
Group		**SoNo-SπNo**		**SoNo-SoNπ**	
OP + Noise (n=5)		40		40	
Noise (n=8)		50		63	
Controls (n=12)		0*		0**	
Acoustic reflexes threshold - Mean ± SD (dB SPL)					
Frequency	**Group**	**Ipsilateral**		**Contralateral**	
		Left Ear	**Right Ear**	**Left Ear**	**Right Ear**
500 Hz	**OP + Noise**	98.0 ± 8.4	95.0 ± 11.2	97.0 ± 13.0	92.0 ± 11.5
	Noise	99.4 ± 9.0	98.8 ± 6.9	102.6 ± 6.5	101.9 ± 7.5
	Controls	91.7 ± 6.5	92.1 ± 6.2	96.7 ± 9.8	96.3 ± 10.0
1000 Hz	**OP + Noise**	94.0 ± 6.5	92.0 ± 9.1	90.0 ± 11.7	92.0 ± 10.4
	Noise	93.1 ± 7.0	94.4 ± 7.3	95.6 ± 7.8	95.6 ± 9.4
	Controls	89.6 ± 5.0	90.4 ± 6.2	89.6 ± 6.2	88.3 ± 6.5
2000 Hz	**OP + Noise**	96.0 ± 10.8	97.0 ± 9.1	90.0 ± 12.7	93.0 ± 11.0
	Noise	95.0 ± 9.3	94.4 ± 8.2	97.5 ± 8.0	94.4 ± 10.2
	Controls	92.1 ± 5.4	91.7 ± 6.9	90.0 ± 5.2	89.6 ± 5.8

Table 3 cont.: Summary of results for MLD, contralateral suppression of TEOAE

Contralateral suppression TEOAE - Abnormal response (%)			
Frequency	**Group**	**Left Ear**	**Right Ear**
1 kHz	**OP + Noise**	80	60
	Noise	75	75
	Controls	50	50
1.4 kHz	**OP + Noise**	80	80
	Noise	38	50
	Controls	50	33
2 kHz	**OP + Noise**	60	80
	Noise	25	38
	Controls	50	33
2.4 kHz	**OP + Noise**	80	80
	Noise	63	75
	Controls	75	76
4 kHz	**OP + Noise**	80	80
	Noise	63	63
	Controls	75	83

CONCLUSIONS

Our results suggest that a combined OP and noise exposure might adversely affect high frequency hearing in farm workers. Audiograms obtained from workers exposed to noise only showed a more restricted hearing loss in the 3-6 kHz range as predicted by epidemiological database (ISO-1999 1990). Workers exposed to both contaminants showed a significantly more pronounced hearing loss at 8 kHz and worst hearing threshold for a larger frequency range between 4 and 9 kHz. Similar results were reported by Teixeira et al. (2003) for workers exposed to OPs for more than 6 years. DPOAEs signal-to-noise ratio (SNR) were reduced in the same frequency range between 4 and 8 kHz in the OPs and noise group. Animal studies by Job et al. (2007) and Carpentier et al. (2010) demonstrated a reversible DPOAE amplitude decrease after soman administration, clearly showing the sensitivity of this test to OP intoxication. However, the fact that no difference in DPOAE SNR was detected between noise-exposed and control groups is certainly not in agreement with previous studies that have clearly demonstrated that DPOAEs are sensitive to noise-induced hearing loss (Arnold et al. 1999) and to noise-induced outer hair cell dysfunction (Robinette & Glattke 2007). In this study we used a questionnaire to assess subjects noise exposure for their whole career. The absence of specific level of noise exposure, using field measurement data, has probably led to imprecise stratification as low and high exposure are pooled together. This limitation might explain why no DPOAE difference was identified.

Results of three tests sensitive to efferent system dysfunction failed to identify significant differences between OPs and noise group as compared to noise exposed and control groups. A tendency for a larger proportion of abnormal results in the contralateral suppression of TEOAEs was observed in the OPs and noise group for 1.4 and 2 kHz. Bernardi (2000) reported similar results for workers exposed to toluene and noise when compared to workers exposed to noise only and to control subjects. However, the difference observed in our study did not reach statistical significance, which might be attributable to the weak statistical power due to our small sample size. These preliminary results need further confirmation using a larger sample size

but, at this time, a possible effect of OPs on both cochlear function and olivocochlear efferent system cannot be ruled out.

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