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Feasibility of hair sampling to assess levels of organophosphate metabolites in rural areas of Sri Lanka

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ABSTRACT

Measuring chronic pesticide exposure is important in order to investigate the associated health effects. Traditional biological samples (blood/urine) are difficult to collect, store and transport in large epidemiological studies in settings such as rural Asia. We assessed the acceptability of collecting hair samples from a rural Sri Lankan population and found that this method of data collection was feasible. We also assessed the level of non-specific metabolites (DAPS) of organophosphate pesticides in the hair samples. The median concentration (pg/mg) of each DAP was: diethyl phosphate: 83.3 (IQI 56.0, 209.4); diethyl thiophosphate: 34.7 (IQI 13.8, 147.9); diethyl dithiophosphate: 34.5 (IQI 23.4, 55.2); and dimethyl phosphate: 3 (IQI 3, 109.7). Total diethylphosphates were recovered in $>80\%$ of samples and were positively correlated with self-reported pesticide exposure.

1. Introduction

It is estimated that nearly 2.8 million tons of pesticides are used each year globally Ecobichon (2001). Farmers in developing countries increasingly rely on these chemicals Ecobichon (2001). In low and middle income countries, like Sri Lanka, the majority of farmers use pesticides as the main pest management practice; applying pesticides at a concentration 35% higher than recommended, often without following safe practices Nagenthirarajah and Thiruchelvam (2010). In addition the improper disposal, storage and cleaning of spraying equipment is likely to increase environmental contamination and consequently increase pesticide exposure of non-farming individuals. Therefore the level of chronic (long-term) exposure of pesticides may be higher in these settings than in high income countries.

It is not clear whether individuals in developing countries experiencing this chronic exposure are at increased risk of ill health. Acute exposure to pesticides has been associated with negative health effects, such as birth defects, cancer, respiratory and neurological disease, infertility and death Jeyaratnam (1990); Michalakis et al. (2014); Kanavouras et al. (2011); Baltazar et al. (2014); Mehrpour et al. (2014); Zaganas et al. (2013).Investigations into the health effects of chronic exposure are limited because traditional biological assays like blood and urine only indicate short-term exposure and are difficult to collect, store and transport in large population studies Kavvalakis and Tsatsakis (2012); Koutroulakis et al. (2014), especially in developing countries. An alternative is to use hair samples, these provide a measure of longer term exposure (dependent on hair length). Studies in European populations have shown that it is possible to assess pesticide exposure using non-specific dialkyl phosphate metabolites (DAPs) using hair samples from the general population Tsatsakis et al. (2010); Kokkinaki et al. (2014). In Sri Lanka, hair is believed to be used in sorcery practices (*gurukam*), and hair sampling is not a routine procedure, therefore collection in this setting maybe problematic Senarthna (2014). The aim of this study was to assess the feasibility and acceptability of obtaining hair samples from people living in rural Sri Lanka, and to use these samples to assess long-term exposure to pesticides by measuring levels of non-specific metabolites (DAPs) of organophosphate pesticides to inform future epidemiological studies.

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2. Methods

2.1. Sampling

This cross-sectional feasibility study was based in the North Central Province of Sri Lanka in a rural village with a mixture of agricultural and non-agricultural households. This area has both rice and vegetable farmers, and therefore represents a village with moderate/high levels of chronic exposure. Using key informants we invited villagers to a public meeting to describe the study. Participants were recruited via home visits and we purposively sampled 50 adults ($\geq$ 18 years) in order to collect data from broad age and gender categories during the area's dry season (Yala).The amount of pesticide used in the Yala season by rice farmers (and hence acute exposure) is likely to be less than during the main rice cultivation season (Maha) Konradsen et al. (2007).

Participants were asked to give a hair sample of a pencil width diameter from the back of their head. For individuals with limited head hair we collected chest, arm or leg hair. We also collected demographic details and self-reported information about pesticide exposure.

To assess the feasibility and acceptability of hair sampling, we asked participants for feedback on their experience. Participants were given a 750 Rs (5 USDollars) shopping voucher for their participation. To ensure that the voucher did not impact on the informed consent process, participants were given an information sheet to read, but the gift voucher was not highlighted by researchers until the end of the interview. The samples were analysed by the laboratory of Toxicology and Forensic Chemistry, University of Crete Medical School, Greece.

Organophosphates (OPs) are commonly used in this area and their mode of action is by targeting the nervous system of pests, a system that is also shared by humans. Samples were analysed for DAP metabolites of OPs: dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP). These metabolites are frequently used as biomarkers for OPs exposure in humans and reflect long-term exposure to Ops Kavvalakis and Tsatsakis (2012); Margariti and Tsatsakis (2009a); Maravgakis et al. (2012); Margariti and Tsatsakis (2009b); Tsatsakis et al. (2009). The parent pesticide compounds of these DAP metabolites are summarised in (Table 1).

Table 1
Studied dialkyl phosphate metabolites and the parent organophosphate pesticides.

Parent pesticide compounds	Dialkyl phosphate metabolites			
	DMP	DEP	DETP	DEDTP
Azinphos-Methyl	x			
Chlorethoxyphos		x	x	
Chlorpyrifos		x	x	
Chlorpyrifos-Methyl	x			
Coumaphos		x	x	
Diazinon		x	x	
Dichlorvos	x			
Dicrotophos	x			
Dimethoate	x			
Disulfoton		x	x	x
Ethion		x	x	x
Ethylparathion			x	
Fenitrothion	x			
Fenthion	x			
Isazofos-Methyl	x			
Malathion	x			
Methidathion	x			
Methylparathion	x			
Mevinphos	x			
Naled	x			
Oxydemeton-Methyl	x			
Parathion		x	x	
Phorate		x	x	x
Phosalone		x	x	x
Phosmet	x			
Primiphos-Methyl	x			
Sulfotepp		x	x	
Temephos	x			
Terbufos		x	x	x
Tetrachlorvinphos	x			
Tribufos		x	x	
Trichlorfon	x			

Dimethyl phosphate (DMP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP).

2.2. Laboratory analysis

2.2.1. Materials

The DAPs were purchased from Acros Organics (Geel, Belgium, New Jersey, USA) (dimethylphosphate 98%), from Chem Service (West Chester, USA) (diethylphosphate 98.9%) and from Sigma-Aldrich (USA) (diethylthiophosphate 98%, diethyldithiophosphate 95% and acetonitrile-LCMS grade). Toluene and potassium carbonate (K_2CO_3) were obtained from Merck (Darmstadt, Germany). Dibutyl phosphate (DBP used as internal standard) was obtained from Roth (Karlsruhe, Germany).

2.2.2. Standard and spiked solutions

The standard solutions of mixed DAPs were prepared in methanol (0–1000 ng/ml) and kept at 4 °C. Human hair samples with levels of DAPs lower than the limit of quantification (LOQ) values were pooled and used for the preparation of the spiked samples (0, 50, 100, 250, 500 and 1000 pg/mg).

2.2.3. Extraction of dialkylphosphate metabolites

The analytical procedure for the extraction of DAPs from the hair samples has been published previously Tsatsakis et al. (2010, 2012). Briefly, the decontamination step was done by washing hair samples twice with water and methanol. The dried hair samples were cut (in mm) and an amount of 100 mg was transferred in a test-tube where 2 ml of methanol and 100 ng of DBP (IS) were added. The extraction of the metabolites was performed by incubation of the samples in an ultrasonic bath for 4 h at 50 $\pm$ 5 °C. After that, liquid-solid extraction was performed for 30 min. The mixture was centrifuged at 4000 rpm for 5 min and the supernatant was filtered, 15 mg of K_2CO_3 and 50 mg $Na_2S_2O_5$ were added and evaporated to dryness. In the dry residue, 1 ml of acetonitrile, 15 mg of K_2CO_3 and 0.1 ml solution of pentafluorobenzylbromide (PFBBr) in acetonitrile (1:3 v/v) were added and incubated at 80 °C for 30 min in a water bath. When the derivatization procedure was completed and the evaporation was done the residue was re-dissolved in 50 µl of toluene.

2.2.4. Gas chromatography and Mass spectrometry conditions

Analysis of the samples was performed by a GC MS QP-2010 Shimadzu system, while the separation of the analytes was done by a Supelco Analytical SLBtm-5 ms (Bellefonte PA, USA) column of 30 m length, 0.25 mm i.d, 0.25 µm film thickness. The flow rate of helium was 1 ml/min while 2 µl of the solution was injected in the splitless mode. The temperature program started from 70 °C for 1 min, raised with a rate of 5 °C/min to 210 °C and then to 350 °C with a rate of 35 °C/min. The injector, interface and ion source temperatures were set at 270 °C, 300 °C and 230 °C, respectively. The retention time of each metabolite was 15.7, 17.9, 21.4, 23.0 and 25.04 min for DMP, DEP, DETP, DEDTP and DBP, respectively. The determination and quantification of the analytes was achieved in selected ion monitoring (SIM) using m/z 110, **306** for DMP, **258**, 334 for DEP, **350**, 274 for DETP, **366**, 185 for DEDTP and **335** for DBP (in bold the ions were used for the quantification).