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Investigation of gastrointestinal effects of organophosphate and carbamate pesticide residues on young children

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ABSTRACT

This prospective study was designed to investigate whether there is any association between gastrointestinal effects and pesticide residue exposure (as measured by metabolite levels in urine and faecal samples) in young children and to describe background levels of pesticide residues in samples from healthy children in the UK. Children ($N = 136$) between the ages of 1.0 and 4.2 years were recruited. Of these, 107 provided background baseline samples and 26 provided samples when suffering from gastrointestinal symptoms. Urine samples (from all populations) were positive for (non-specific) carbaryl metabolite (urine 19/78, faeces 9/99), organophosphate metabolites (urine 103/135, faeces 47/111) and pirimicarb metabolite (urine 72/175, faeces 45/135). There were no statistically significant differences between samples from children when healthy or unwell. The urinary 95th percentile values for the healthy population of young children in this study were 31 nmol/l (carbaryl metabolite), 2156 nmol/l (total organophosphate metabolites) and 139 nmol/l (pirimicarb metabolite). In this study, samples from children suffering gastrointestinal symptoms were no more associated with anti-cholinergic pesticide metabolite levels or rotaviral infection than samples from healthy children. Background levels of anti-cholinergic pesticide metabolites in healthy UK children were in agreement with previously reported levels from the US and Germany.

Introduction

It has been suggested that anticholinergic organophosphate and/or carbamate pesticide (OP/C) exposure in humans may cause gastrointestinal (GI) effects and that children, having the highest short-term intakes of OP/C residues on a body weight basis, may be at particular risk. Certainly in poisoning cases, diarrhoea has been reported as a symptom in children (Sofer et al., 1989). A review (Hughes et al., 2002) of the effects of OP pesticide exposure on young children recommended further work including a biomonitoring survey and greater elucidation of the sources of OP exposure. Non-governmental organisations such as Pesticides Action Network UK have also questioned the possible effects of OP exposure on children (Pesticide_Action_Network_UK, 2012).

The majority of young children presenting to the Accident and Emergency department of a British hospital with diarrhoea have contracted an infectious agent or are suffering from 'food poisoning'. The most common agents are viruses with rotavirus being the most commonly identified pathogen. Outbreaks are generally seasonal. Less commonly, bacterial pathogens such as *Salmonella* or *Escherichia coli* are identified.

General symptoms of organophosphate or carbamate poisoning can range from dizziness and nausea through muscle twitching and tremor to bradycardia, seizures and coma in severe poisoning cases. Symptoms usually appear within 4 h but may take longer to fully manifest themselves. Early symptoms may be vague and include headache, dizziness, abdominal pain and nausea (Mitchell et al., 1995). Because of these indistinct early symptoms, and the problems of interrogating young patients, diagnosing pesticide poisoning in children is difficult. Symptoms can mimic other problems including traumatic brain injury, diabetic ketoacidosis and respiratory tract infections (Zwiener and Ginsburg, 1988). Also, common signs such as diarrhoea and salivation may not be obvious in very young children.

Lifshitz et al. (1997) compared symptoms of 36 children (median age 2.5 years) and 24 adults (median age 22 years), all of whom had been admitted to hospital following oral ingestion of carbamates. Serum cholinesterase activity was determined in all patients and was quoted as being similar between adults and children. The predominant symptoms in young children were depression of the central nervous system (CNS) and hypotonia, with diarrhoea the most common muscarinic effect. These effects did not occur in the adult patients, where most suffered miosis and fasciculation. The

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authors conclude that young children present different symptoms of carbamate poisoning compared to adults. In a significant number of cases, these symptoms will include gastrointestinal effects such as diarrhoea.

Compared to adults, children may be more susceptible to pesticide exposure and the reasons for this are twofold. First, they have greater potential exposure to pesticides: children drink and eat more per unit body weight than adults and tend to eat proportionally more of foods that typically have pesticide residues. Their behaviour, such as hand to mouth contact and playing close to the ground, can increase exposure to pesticides and they spend more time indoors at home (house dust has been shown to contain significant numbers, and levels, of pesticides). Use of headlice shampoos can also be a source of pesticide exposure. Secondly, children may be more biologically vulnerable to the effects of pesticides because they are still growing and developing. Children therefore have immature metabolic pathways and immune systems. This may render children less capable of detoxifying harmful chemicals that are absorbed and disruption to the development of the immune system may cause permanent dysfunction. Children are overall more likely to be susceptible to harmful effects from pesticides and more likely to have a higher non-occupational exposure to pesticides. It has been argued (NRDC, 1989) that these considerations have not been taken into account when calculating acceptable risks and daily doses.

This study was funded by the UK Government Department of Environment, Food and Rural Affairs to determine whether there was any evidence for anticholinergic pesticide exposures causing gastrointestinal effects in young children. A previous pilot study (Jones, 2002) demonstrated that urine and faecal samples could be obtained from young children suffering from GI effects and that existing analytical methodologies were suitable to detect the metabolites of OP/C pesticides in these samples. The pilot study included 15 children suffering from GI problems (urine and/or faecal samples) and 12 controls (urine samples only). The control population had detectable levels of OP pesticide metabolites in urine and the levels found were comparable to previously reported studies (Aprea et al., 2000). However, the samples obtained from the symptomatic population contained lower levels of OP pesticide metabolites. We have suggested that this was due to the time delay between the onset of symptoms and the provision of samples. Some of the children were not admitted to the hospital accident and emergency department until several days after the onset of symptoms and, because of the very nature of the symptoms, had eaten virtually nothing since that time. Metabolites of OP pesticides are mostly eliminated within the first 24 h when exposure is by the oral route (Griffin et al., 1999; Garfitt et al., 2002). There was also some concern over the influence of viral infection in GI symptoms and how this might compromise the study (some 50% of cases of GI symptoms are thought to be caused by viral infection (Amar et al., 2007)). These issues were addressed in the study design reported here.

Subjects and methods

Study outline

The study was designed to recruit young children (aged 1.5–4.5 years) and for them to provide three sets of samples (one urine sample, one faecal sample per set). Two sets of samples would be provided, when healthy, six months apart to determine any seasonal variations in background levels. One set would be provided (within 24 h of onset) if the child suffered from gastrointestinal symptoms (vomiting, diarrhoea, stomach pains, etc.).

Children were recruited prospectively by the Sheffield Children's Hospital from non-related clinics (such as ENT and orthopaedics) and from local nurseries in the Sheffield area (South Yorkshire, UK). The study was reviewed and approved by the North Sheffield Research Ethics Committee. Children of both sexes within the target age range who were generally healthy with no long-term history of stomach complaints were eligible for inclusion in the study. A target population of 100 children was proposed based on a two-sided Wilcoxin Rank-sum test for the organophosphate metabolite DMTP (this was chosen as the most frequently detected metabolite, with highest mean value and large variation) using data from the pilot study. Recruitment took place over two years.

Questionnaire data

Brief questionnaires were taken with each set of samples provided. These provided details of fruit and vegetables, bread and drinks consumed in the previous 24 h and details of any recent flea treatments for pets, headlice treatments or household pesticide use.

Sample analysis

Samples (both urine and faecal) were analysed for generic metabolites of organophosphates (dialkyl phosphates) and specific metabolites of the anti-cholinergic carbamates, pirimicarb and N-methyl carbamates (carbaryl, carbofuran and methiocarb). The methods used were well established in our laboratory for urinary biomonitoring. None of the methods were validated for faecal analysis but, due to the lack of published literature, the faecal results were used only for internal comparison i.e. between healthy and unwell samples. The faecal samples were analysed as they may have represented a more symptom-relevant sample matrix than urine in this study.

Urine samples were analysed for creatinine using the alkaline picrate method on an automated biochemical analyser (Cocker et al., 2011). Faecal samples were weighed prior to being dispersed in phosphate buffer. Due to the volume of sample required for a complete set of pesticide analyses (~15 ml), it was not possible to analyse all samples for all analytes. Actual samples numbers analysed for each analyte in each matrix are given in the results section.

Adenovirus/rotavirus analysis

All faecal samples were analysed for adenovirus and rotavirus using VIKIA® Rota-Adeno test kits (bioMérieux, France). These tests provide a visual result within 10 min with high sensitivity (100% rotavirus, 98% adenovirus) and specificity (100%).

Organophosphate metabolites

Urine and faecal samples were analysed using HSL's method for dialkylphosphates (Garfitt et al., 2002). Dialkylphosphates (dimethyl phosphate (DMP), dimethylthio phosphate (DMTP), dimethyldithio phosphate (DMDTP) and their ethyl analogues (DEP, DETP, DEDTP)) are generic metabolites of many of the organophosphates currently registered for use and so give a useful indication of 'total organophosphate' exposure although individual pesticide exposures cannot be elucidated.

Aliquots of sample (2 ml) were acidified and extracted into diethyl ether:acetonitrile (50:50, v/v). The organic layer was removed and evaporated under nitrogen. The residue was then reconstituted in acetonitrile and derivatised (40 °C, 14–16 h) with pentafluorobenzyl bromide (50 μl) in the presence of anhydrous potassium carbonate. Samples were analysed by gas