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Development of an analytical strategy based on LC–MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France

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ABSTRACT

It is important to evaluate the impact of pesticides on human health because exposure to these compounds has been linked to harmful effects in many research studies. This exposure may be particularly harmful during the early stages of development (e.g. the prenatal period). The aim of the present study was to develop an analytical strategy for quantifying a number of pesticides and their metabolites in meconium (the neonate's first faeces), in order to characterize the extent of foetal exposure. The meconium sample was dried and grinded in order to homogenize the sample, prior to solid–liquid extraction and a purification by solid-phase extraction using a weak anion mixed-mode polymeric sorbent. Analyte separation and quantification was performed by liquid chromatography coupled to electrospray-triple quadrupole mass spectrometry. Five pesticide families (carbamates, organophosphates, pyrethroids, phenylureas and phenoxy herbicides) and their metabolites could be quantified in meconium with limits of quantification ranging between 0.2 ng/g and 200 ng/g. This method was applied to a set of 171 meconium samples collected in the Picardie region of northern France. The highest prevalence was observed for metabolites of organophosphates and carbamates (57.9% and 22.8%, respectively). The parent pesticides were rarely present and were only found at very low concentrations, except for the pyrethroids cyfluthrin and cypermethrin, which were found in 7.6% of meconium samples at concentrations of between 43.8 and 480 ng/g. The most frequently detected contaminant was the organophosphate metabolite dimethyl thiophosphate detected in 49.1% of the samples and quantified with a median concentration of 344 ng/g. These data evidence significant foetal exposure to organophosphate pesticides, pyrethroids and carbamates.

1. Introduction

Pesticide use has been rising steadily since the 1940s. Worldwide, one million tonnes are now dispersed each year. The intensive use of pesticides (particularly in industrialized countries) has increased farming yields and reduced the impact of many plant pests and diseases. However, pesticides now contaminate all environmental compartments (air, water and soil) and food sources. Human exposure to pesticides through the environment and food is therefore inevitable. Some of these contaminants have neurotoxic, endocrine and carcinogenic properties and have notably been linked to the occurrence of breast cancer (Lopez Carrillo et al., 1996; O'Leary et al., 2004; Cohn et al., 2007), pancreatic cancer (Garabrant et al., 1992), skin cancer, prostate cancer, brain cancer and digestive tract cancer (De Brito Sa Stoppelli and Crestana, 2005). Prenatal exposure (even at very low concentrations) can cause serious developmental disorders. Indeed, several studies have shown that maternal exposure to pesticides is correlated with low weight and small size at birth

Abbreviations: LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; LC–MS/MS, Liquid chromatography–tandem mass spectrometry; UPLC, ultra-performance liquid chromatography; MRM, multiple reaction monitoring; SPE, solid-phase extraction; DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DEP, diethyl phosphate; DETP, diethyl thiophosphonate; EU, ethyl urea; ETU, ethyl thiourea; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropanecarboxylic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MCPB, (2-methyl-4-chlorophenoxy)butyric acid; IPPU, 1-(4-isopropylphenyl) urea

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(Whyatt et al., 2004), a shorter gestation period (Eskenazi et al., 2004; Fenster et al., 2006) and abnormal neurological development (notably with impairments in learning ability (Eskenazi et al., 2007), psychomotor development (Torres-Sanchez et al., 2007), reflex development (Young et al., 2005; Chanda and Pope, 1996; Chanda et al., 1995) and balance (Muto et al., 1992). A correlation between parental exposure and childhood leukaemia and brain cancers has also been reported (Buckley et al., 1989; Shim et al., 2009). Foetal exposure is therefore a major public health problem, since the foetal immune system is very immature and the brain is growing and developing rapidly (Eskenazi et al., 1999).

Most studies of foetal pesticide exposure have been based on the analysis of urine and/or blood samples (Bradman et al., 2005; Whyatt et al., 2005; Ye et al., 2008, 2009; Barr et al., 2010; Berman et al., 2011). However, these matrices are only representative of recent exposure because xenobiotics are rapidly metabolized and eliminated within a few days. Therefore, xenobiotics are only detected in these matrices if the neonate has been exposed four or five days before birth. Furthermore, urine is not easy to collect in newborns and blood collection is invasive. Other matrices (such as breast milk or adipose tissue) have also been used to study lipophilic compounds, such as organochlorine pesticides (Lopez Carrillo et al., 1996; Jaraczewska et al., 2006; Jimenez Torres et al., 2006; Szyrwinska and Lulek, 2007; Polder et al., 2008, 2009; Nguyen Minh et al., 2010; Zhou et al., 2011; Fujii et al., 2011). However, these matrices are specific for certain classes of pesticide and cannot be used as a guide to overall pesticide exposure.

Meconium (the neonate's first faeces) is a more appropriate matrix for measuring foetal exposure to xenobiotics during a wide window of pregnancy. Indeed, xenobiotics accumulate in meconium from the third month of gestation until birth. Hence, all exposure over the last six months of a term pregnancy will be detected in this matrix. Furthermore, postnatal meconium collection is easy and non-invasive. Studies having compared infant hair, cord blood and meconium have found that the latter matrix is the most sensitivity (Ostrea et al., 2008, 2009). Meconium has already been previously used to detect exposure to illicit drugs (such as cocaine, cannabinoids, opiates, amphetamines and methadone (Callahan et al., 1992; Ostrea et al., 1992, 1993, 1998; Mirochnick et al., 1995; Moore et al., 1998; Pichini et al., 2003, 2005)), nicotine metabolites (Ostrea et al., 1994; Baranowski et al., 1998; Gray et al., 2008, 2010; Xia et al., 2011), alcohol metabolites (Bearer et al., 1999; Klein et al., 1999; Pichini et al., 2008; Morini et al., 2010; Hutson et al., 2011), heavy metals (Ostrea et al., 2002) and medications (Ostrea et al., 1998; Alano et al., 2001; Madej, 2010).

In contrast, there are few data on the detection and quantification of pesticides in meconium (Whyatt and Barr, 2001; Hong et al., 2002; Ostrea et al., 2002, 2008, 2009; Bielawski et al., 2005; Zhao et al., 2007; Tsatsakis et al., 2009). The earliest studies targeted organochlorine pesticides, which are very persistent compounds. In 2002, Hong et al. detected *p,p*'-dichlorodiphenyldichloroethylene (DDE, a metabolite of dichlorodiphenyltrichloroethane (DDT)) in 5% of 60 samples collected in Germany 25 years after the use of organochlorine pesticides had been prohibited. In 2002, Ostrea et al. quantified four organochlorine and four organophosphate pesticides in a cohort of 200 meconium samples from rural population in the Philippines. The most frequently quantified compounds were lindane (73.5%) and malathion (53%), whereas chlorpyrifos was only found in 11% of the samples. More recently, a study of 90 meconium samples from China found that DDE and hexachlorobenzene (HCB) were present in all meconium samples (Zhao et al., 2007). Two other studies have investigated the levels of five classes of pesticides (carbamates, organochlorines, chloroacetanilide, organophosphates and pyrethroids) in a Philippine population (Bielawski et al., 2005; Ostrea et al., 2008); the high contamination rates were seen for propoxur and cypermethrin (32.53% and 6.02% for the first study and 23.8% and 1.9% for the second, respectively). Lastly, two studies focused on six dialkylphosphates (metabolites of organophosphate pesticides) in meconium samples from New York City (Whyatt and Barr, 2001) and from rural areas of Crete (Tsatsakis et al., 2009). Diethyl thiophosphonate (DETP) and diethyl phosphate (DEP) were found in, respectively, 100% and 95% of the 20 meconium samples from New York (Whyatt and Barr, 2001), whereas dimethyl phosphate (DMP) was found in 92.1% of the Greek meconium samples.

This body of literature data demonstrates that foetuses are exposed to high levels of pesticides. However, there are no data on foetal exposure in France. Hence, the aim of the present study was to develop and validate a multi-residue method for the quantification of 18 pesticides and their metabolites in samples by liquid chromatography–tandem mass spectrometry (LC–MS/MS), and generate the first exposure data for the foetus in France. To the best of our knowledge, this study is the first to have investigated as many parent pesticides and metabolites at the same time and to have used liquid chromatography to measure pesticides in meconium. All previous studies having been performed with gas chromatography (GC) coupled to mass spectrometry. Liquid chromatography enables the direct analyses of a wider range of compounds than gas chromatography, since the latter only targets volatile and apolar compounds and usually requires a derivatization step. The meconium samples were all collected in the Picardie region of northern France, in which pesticide use is extensive.

We therefore targeted the pesticides most used in the Picardie region. Each compound's contamination of air and water compartments (based on their physicochemical properties) was estimated using the Sph'Air and SIRIS software packages developed at INERIS (Verneuil-en-Halatte, France). The pesticide's toxicity was also considered and so only compounds with known or suspected effects on reproduction or embryonic development were selected. Hence, organophosphates, pyrethroids, carbamates, phenylurea and phenoxy herbicides were selected as analytes. A number of metabolites were also targeted, including the five metabolites characteristic of all organophosphate pesticides and the carbamate metabolites EU and ETU (given that the two non-specific metabolites of mancozeb which is the most-used pesticide in Picardie). Mancozeb is metabolized very rapidly (with a half-life of 0.7 h in rat and 95% metabolization within four days (data of *Institut National de Recherche et de Sécurité*) and is therefore difficult to measure in vivo. In all, nine pesticides and nine metabolites (corresponding to five different classes of pesticides) were selected as analytes (Table 1).

Table 1
Target compounds: parent pesticides and their metabolites.

Class	Parent pesticides	Pesticide metabolites
Organophosphates	Malathion	DMP (dimethyl phosphate) DMTP (dimethyl thiophosphate) DMDTP (dimethyl dithiophosphate)
	Diazinon Chlorpyrifos	DEP (diethyl phosphate) DETP (diethyl thiophosphonate)
Pyrethroids	Cypermethrin Cyfluthrin	DCCA (3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropanecarboxylic acid)
	Deltamethrin	
Carbamates	Propoxur	
	Mancozeb (*not measured*)	EU (ethyl urea: 2-imidazolidone)
	Mancozeb (*not measured*)	ETU (ethyl thiourea: 2-imidazolidinethione)
Phenylurea	Isoproturon	Desmethylisoproturon (1-(4-isopropylphenyl)-3-methylurea)
Phenoxy herbicides	MCPA (2-methyl-4-chlorophenoxyacetic acid)	