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Absorption and excretion of organophosphorous insecticide biomarkers of malathion in the rat: Implications for overestimation bias and exposure misclassification from environmental biomonitoring

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ABSTRACT

Malathion is an organophosphorous (OP) insecticide widely used for crop protection. Its degradates, malathiondiacid (MDA), malathion monoacid (MMA), dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP) are formed in strawberries and other produce. These same chemical biomarkers are measured in urine in human studies as quantitative measures of exposure. The excretion of malathion and its common biomarkers including MDA, MMA, DMP, DMTP and DMDTP at equal molar doses (73 μmol/kg b.w.) was studied following oral dosing of female Holtzmann rats (240–300 g). Following MDA administration, 36.3 ± 5.4% was recovered as MDA, 0.05 ± 0.02% as DMP, 5.5 ± 0.3% as DMTP, 3.8 ± 2.9% as DMDTP (mole percent), and totally 45.6 ± 7.0% of administered dose in urine after 120 h (over 94% in the first 24 h). Following DMTP administration, 8.3 ± 7.7% was recovered as DMP, 46.6 ± 16.5% as DMTP, and totally 55.0 ± 10.3% of administered dose in urine after 120 h (over 92% in the first 24 h). Similar results were obtained with other malathion biomarkers. Preformed biomarkers of malathion and other OP insecticides when ingested in produce are readily absorbed and excreted. Low level human dietary and non-occupational urine biomonitoring studies will be confounded by preformed pesticide biomarkers used to infer potential human pesticide exposure. This has profound implications for epidemiology studies where subject's biomarker excretion is used as a surrogate for OP exposures that cannot be related to a particular insecticide residue.

1. Introduction

Malathion is a broad spectrum organophosphorous insecticide (OP) used worldwide for over 50 years. This dimethylphosphorothionate is one of a large group of OP insecticides that comprise an even larger family of pesticides that have been used in global crop protection, structural pest control, medicine and public health since the 1950s. The majority of economically important OPs are structurally related pentavalent phosphorus acid esters, and in addition to sharing acetylcholinesterase inhibition as a common mechanism of action, the large majority of economically important active ingredients are either dimethyl- or diethylphosphorothionates (Chambers et al., 2010).

Following OP insecticide exposures, relatively rapid absorption, metabolism, and excretion of more hydrophilic metabolites occurs in animals and humans (Chambers and Levi, 1992). The metabolites excreted in urine can be convenient biomarkers of occupational exposure since they represent internal or absorbed dose and require fewer measurements to reliably estimate worker exposure (Ross et al., 2008). Analytical methods and techniques are available for quantitative analysis of biomarkers of exposure in blood and urine in amounts well below toxic levels signaled by high levels of cholinesterase inhibition (Sudakin and Stone, 2011). Reliable and responsible use of urine biomarkers as indicators of OP exposure requires an appreciation of their fate and disposition in animals, people, and the target environment (e.g., plants to which they are applied crop protection).

The dialkyl phosphates (DAPs) including dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP) and diethyldithiophosphate (DEDTP) were first used as biomarkers of occupational OP exposure (Shafik et al., 1973). Their measurement in urine was usually coupled with knowledge of OP use, and assessment of cholinesterase status of exposed workers (Duggan et al., 2003). The metabolites are not anticholinesterases at concentrations found on food plants or in and on food products.

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The DAPs may be capable of inhibiting cholinesterase at millimolar concentrations (Imaizumi et al., 1993), exceeding exposures experienced by even the most highly exposed workers. Short persistence and rapid excretion minimize body burden.

Malathion metabolism is broadly similar in plants and animals (Roberts and Hutson, 1999) and rats and humans (Bradway and Shafik, 1977; Krieger and Dinoff, 2000; Bouchard et al., 2003; Buratti and Testai, 2005). Malathion is relatively safe among the OPs (EPA, 2000) for mammals including humans as a result of efficient detoxication to malathion monocarboxylic acid (MCA) catalyzed by carboxyesterasewith lesser cytochrome P-450 catalyzed desulfuration activity catalyzing the formation of malaoxon, the metabolite responsible for acetylcholinesterase inhibition and acute toxicity (Buratti and Testai, 2005). The specific and generic malathion biomarkers of concern here have been studied in strawberries and other produce. The dynamic relationship between the biomarkers is illustrated in Fig. 1 presenting biomarker data from six successive harvests (Chen et al., 2012).DAPs such as DMP, DMDTP and DMTP, which are metabolic products of dimethylphosphorothionate insecticides, have long been used as general urinary biomarkers for this class of OP insecticides (Bouchard et al., 2003; Bradway and Shafik, 1977; CDC, 2010; Cocker et al., 2002; Coye et al., 1986; Fenske et al., 2000; Forsberg et al., 2011; Griffith and Duncan, 1985; Heudorf and Angerer, 2001).

Risk assessment has traditionally relied on cholinesterase inhibition as a primary biomarker of excessive exposure to OP insecticides including malathion (EPA, 2006). Urinary DAP measurements were introduced later for occupational exposure assessment (Shafik et al., 1973) and have been widely employed in occupational exposeure assessment despite of the fact that a relationship with cholinesterase inhibition has not been demonstrated (Sudakin and Stone, 2011). For example, milligram per kilogram absorbed dosages of malathion were not associated with significant RBC cholinesterase inhibition (Krieger and Dinoff, 2000). Notwithstanding, more recent efforts to perform aggregate risk assessments related to dietary (food and water) exposures, inhalation and non-dietary exposures such as residential pesticide use have included measurement of urinary DAPs as a surrogate for OP exposure (Duggan et al., 2003). Population surveys and epidemiologic studies have utilized urinary DAP metabolites and attributed those analytes to OP insecticide exposure (Aprea et al., 2000; Curl et al., 2002; Lowenherz et al., 1997; Lu et al., 2001; Mills and Zahm, 2001; Whyatt and Barr, 2001). Similarly, the Centers for Disease Control (CDC) human biomonitoring data (Barr et al., 2004, 2011) are useful to establish trends in biomarker levels in the general US populationfollowing EPA's progressive restriction of OP agricultural use and effective elimination of residential use. More than one dozen epidemiology studies published between 2003 and 2012 have utilized background DAP concentrations in urine as proxy for exposure to OPs resulting in misclassification of dose (Krieger et al., 2012).

Both the evaluation of OP exposure levels in the general population and the regulatory efforts to reduce such levels rely, in large part, on the assumption that urinary measurements of biomarkers reflect exposure to OP's as opposed to preformed biomarkers. However, few studies have measured concurrently both pesticides and their degradation products (DPs) that are potential biomarkers of exposure in environmental media. The possible impact of preformed biomarkers on exposure assessment was discussed earlier (Duggan et al., 2003; Krieger et al., 2003; Zhang et al., 2008). Morgan et al. (2005) observed the specific chlorpyrifos biomarker TCPy (3,5,6-trichloro-2-pyridinol) in the diets of children made a substantially greater contribution to TCPy excretion than the parent insecticide. Later observational studies in a cohort of Ohio children resulted in the conclusion that TCPy and 2-isopropyl-6-methyl-4-pyrimidinol, the specific biomarker of diazinon may have been directly absorbed from the diet and environmental media and excreted (Morgan et al., 2011). The results of these studies demonstrate the need to define the relationship between parent pesticides and their putative biomarkers which may in fact be environmental DPs to avoid overestimating and/or misclassifying pesticide exposure during risk characterization.

Some animal data on DAP bioavailability do exist. The bioavailability and disposition of chlorpyrifos and its major urinary metabolites TCPy, DETP and DEP were compared following their oral administration in the rat (Timchalk et al., 2007; Busby-Hjerpe et al., 2010). A pharmacokinetic study in rats (Forsberg et al., 2011) suggests that DMP has high oral bioavailability and that about 30% of the administered dose is excreted in the urine of rats.

The current study was designed to measure the absorption and excretion of malathion and its biomarkers in rats, following oral administration. The results of these studies are expected to inform discussion of the use of malathion biomarkers as surrogates for exposure to the parent insecticide and to demonstrate the disposition malathion and several of its biomarkers in the rat, and presumably humans, as an aid in the reconstruction of OP insecticide exposure.

2. Material and methods

2.1. Chemicals and solvents

Malathion monocarboxylic acid (MMA, CAS No. 35884-76-5, 89.4% purity), malathiondicarboxylic acid (MDA, CAS No. 1190-28-9, 99.6% purity), and the methyl dialkyl phosphate biomarkers including O,O-dimethylthiophosphatedicyclohexylammonium salt (DMTP-Q, CAS No. 13941-61-2, 97.9% purity) and O,O-dimethyldi-

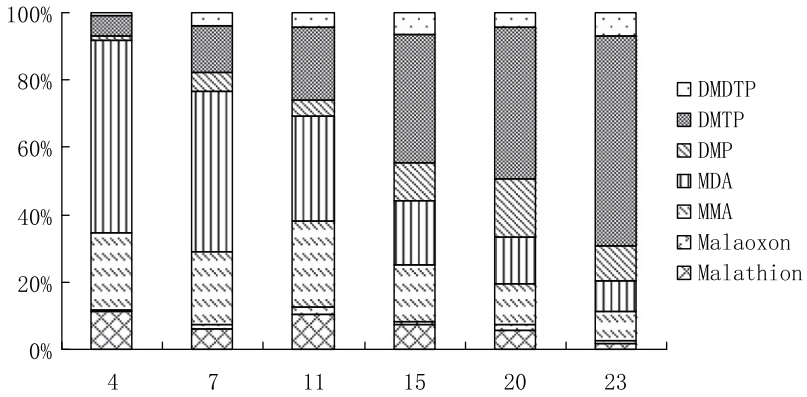

Fig. 1. Malathion, malaoxon and biomarker levels in strawberries following a single field application of malathion on day zero. Malathion and its metabolites were analyzed in 1 pound samples ($n = 8$) of fresh picked strawberries. Total nmoles/g strawberries at each day following malathion at 1 pound/A were as follows: 4d (351), 7d (151), 11d (162), 15d (230), 20d (138), 23d (185).