Biological Monitoring of Exposure to Organophosphorus Insecticides in a Group of Horticultural Greenhouse Workers

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Exposure to selected organophosphorus insecticides (OPs), malathion, diazinon and acephate, was evaluated in a group of horticultural greenhouse workers. This was achieved through measurements of the cumulative urinary excretion time courses of specific and non-specific biomarkers over a 24 h period following the onset of work exposure. For malathion, the absorbed daily doses were estimated from the 24 h cumulative urinary amounts of the specific mono- and di-carboxylic acid metabolites (the sum of MCA and DCA) through the use of a kinetic model. The observed 24 h urinary levels were also compared with a biological reference value (BRV) of 57 nmol kg⁻¹ of body weight established in a previous work on the basis of a human no-observed-effect level exposure dose. Excretion values were found to be 2.5% or less of the BRV, suggesting a negligible health risk. Both median and 95th percentile concentrations of DCA (n = 57 samples) were, however, slightly higher than the baseline values determined by the Centers for Disease Control and Prevention (CDC) in the US civilian population (MCA was not analyzed by the CDC). The cumulative urinary excretion time course of the methyl phosphoric (MP) derivatives, which are metabolites of malathion but also of several other OPs, was also determined. Though relatively low, the MP levels were from 3 to 31 times higher than would be expected on the basis of the malathion specific MCA and DCA excretions, indicating that MP excretions stem from sources other than malathion exposure. Accordingly, only the time courses of MCA and DCA excretion rate (nmol h⁻¹) were compatible with the time of work exposure. Urinary biomarkers of exposure to diazinon and acephate were also measured. Urinary concentrations were essentially below or equal to the analytical limit of detection of 1 µg l⁻¹ for 2-isopropyl-4-methyl-6-hydroxypyrimidine (n = 54) and of 0.8 µg l⁻¹ for acephate and methamidophos (n = 59): values within the baseline range of the US civilian population, like the observed phosphoric metabolite concentrations. The workers under study thus appeared to be only slightly more exposed to malathion than the general population. However, their overall exposure to OPs, as measured by non-specific phosphoric metabolites, was similar to that of the general population, whose exposure occurs mainly through the ingestion of contaminated food. These results question the relevance of measuring non-specific phosphoric metabolites when attempting to assess low-dose occupational exposure to a specific OP.

Keywords: acephate; biomonitoring; diazinon; malathion; occupational exposure; organophosphorus insecticides

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INTRODUCTION

Organophosphorus insecticides (OPs) are neurotoxic substances widely used worldwide in agriculture and horticulture. In humans, as in insects, they exert their neurotoxic action through a common mechanism, the inhibition of nervous system acetylcholinesterases, enzymes responsible for the degradation of acetylcholine at nerve junctions (Koelle, 1994; Sidell, 1994). To help evaluate health risks associated with the occupational use of OPs, it is important to have accurate information on the extent of exposure, particularly in workers frequently in contact with these insecticides.

Exposure to OPs may occur through multiple routes. In occupational environments, exposure occurs primarily by skin contact and, to a smaller extent, by inhalation (Bonaugh and Maibach, 1999; Dowling and Seiber, 2002). In the general environment, it can arise from the ingestion of contaminated food (and, in children, also ingestion of contaminated dust or soil), from the inhalation of contaminated ambient air or through skin contact during domestic uses (Simcox et al., 1995; Ripley et al., 2000; Dowling and Seiber, 2002; Fenske et al., 2002a, b).

To obtain an indication of the overall exposure by the different routes-of-entry, one can rely on the measurement of the chemical or its metabolites in biological matrices, such as blood and urine (Gil and Pla, 2001). This approach automatically accounts for the fact that: (i) the exposure scenario varies widely from one individual to another, (ii) the amounts absorbed through the lungs depend on several factors such as the respiratory rate or the wearing of masks, (iii) the amounts absorbed through the skin differ according to the exposed anatomical site and depend on individual protections (clothes, gloves, etc.) as well as the ambient temperature and humidity (Lauwerys and Hoet, 1993; Bronaugh and Maibach, 1999).

The measurement of urinary biomarkers of OP exposure is of special interest, being non-invasive and highly sensitive. Experimental studies in volunteers indicate that within a few hours following exposure to OPs, whatever the route-of-entry, OP metabolites become easily measurable in urine even at absorbed doses well below those necessary to induce any sign of toxic effects (Morgan et al., 1977; Richter et al., 1992; Carrier and Brunet, 1999; Dennis and Lee, 1999; Griffin et al., 1999; Bouchard et al., 2003; Gosselin et al., 2005). Through measurements of the amounts of specific biomarkers accumulated in urine over a certain time period, it is possible to estimate the absorbed dose of a particular OP. Conversely, the measurement of OP metabolites in a spot urine sample, as is usual, does not allow for a good estimation of the dose absorbed in the body since the absorption rate of these insecticides and the excretion rate of their metabolites vary significantly with time and depend on the route-of-entry and the temporal exposure scenario (Maibach et al., 1971; Feldmann and Maibach, 1974; Carrier and Brunet, 1999; Bouchard et al., 2003; Gosselin et al., 2005).

There are few data available in the literature on the cumulative excretion time courses of OP metabolites, in particular of specific biomarkers in an occupational setting. The available data in workers pertain mostly to the urinary excretion time profiles of non-specific alkyl phosphate metabolites of OPs (Fenske and Leffingwell, 1989; Krieger and Dinoff, 2000; Cocker et al., 2002), although a few time course data are also available for mono- and di-carboxylic acid (MCA and DCA) specific metabolites of malathion (Krieger and Dinoff, 2000; Cruz Marquez et al., 2001; Tuomainen et al., 2002).

Recently, a toxicokinetic modeling approach has been developed to describe the disposition kinetics of some OPs and their metabolites following different exposure routes and temporal scenarios (Carrier and Brunet, 1999; Bouchard et al., 2003, 2005; Gosselin et al., 2005). Using these kinetic models, the absorbed dose of an OP compound can be reconstructed starting from measurements of the cumulative amounts of its metabolites over given time periods. The models also enable the establishment of cumulative urinary levels of biomarkers corresponding to a human no-observed-effect level (NOEL) for a chosen critical biological effect, in the case of OPs, the inhibition of blood cholinesterase. These threshold urinary metabolite levels can be used as biological reference values (BRVs) to assess health risks.

The objective of this study was to document the extent of exposure to OPs for a specific group of horticultural greenhouse workers who are often in contact with these chemicals, in order to assess their health risks, through measurements of the cumulative urinary excretion courses of specific and non-specific biomarkers following spraying episodes of selected OPs: malathion, diazinon and acephate.

METHODS

Studied workers

During the summer of 2003, 18 workers of a horticultural greenhouse company were recruited to participate in a biological monitoring study. In their workplace, chemical pesticides (insecticides and fungicides mainly) are applied on an almost daily basis during the evening, when most workers have left the premises. Only three or four persons apply pesticides but ~150 individuals are later in contact with the treated flowers or plants.

In the current study, exposure to three OPs, malathion, diazinon and acephate, was evaluated through
measurements of urinary biomarkers. Given the difficulty in recruiting volunteers, the high costs of metabolite analysis, and since this study aimed at assessing individual exposure, it was limited to eight participants for each substance studied: two individuals applying the pesticides, five workers manipulating treated plants (through cutting, weeding, watering or transporting plants) and one individual without direct contact with treated plants. The three studied OPs were applied on different evenings. Some individuals participated in the study of more than one insecticide. It was, however, verified that the participants had not been occupationally exposed to any OP through spraying during the 3 days preceding the onset of sampling. All participants agreed to sign an informed consent. The protocol and consent form were approved by the Comité d’éthique de la recherche de la Faculté de médecine of the Université de Montréal.

Urine sampling

Each participant provided all urine voided over roughly a 24 h period following the onset of an exposure in open greenhouses. Each voiding was collected in separate 500 ml polypropylene Nalgene® bottles. Applicators collected all their micturitions during the 24 h period following the onset of an application of either malathion, diazinon or acephate. Individuals manipulating plants collected all their micturitions over a 24 h period after starting plant handling in an area that had been sprayed the evening before with the studied OP. Individuals without direct contact with treated plants collected all their micturitions during a 24 h period following the beginning of an 8 h work shift, on the day following the application of the studied OP.

Urine handling and analysis

The participants kept urine samples in coolers, with ice packs, or in freezers until our research group collected them. Samples were brought to the University at the end of the 24 h collection period and stored at −20°C. Within a few days of the collection period, they were sent on ice for analysis to the Laboratoire de toxicologie humaine of the Institut national de santé publique du Québec, which is accredited by the Canadian Association for Environmental Analytical Laboratories. Once at this laboratory, they were maintained at −20°C until analysis.

For the assessment of exposure to malathion, urinary levels of specific metabolites, the MCAs (the sum of α- and β-MCA) and DCAs, were determined. Non-specific methyl phosphoric (MP) derivatives of malathion, dimethyl dithiophosphate (DMDTP), dimethyl thiophosphate (DMTP) and dimethyl phosphate (DMP), were also determined. For the assessment of exposure to diazinon, the levels of the specific metabolite 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) were measured, as were the levels of the non-specific ethyl phosphoric derivatives, diethyl thiophosphate (DETP) and diethyl phosphate (DEP). For the assessment of exposure to acephate, acephate itself as well the non-specific methamidophos and DMTP metabolites were measured.

Analysis of MCA and DCA metabolites

For the analysis of MCA and DCA, urinary samples were first acidified, extracted on C18 micro-columns and derivatized with diazomethane. Analysis was then performed using a gas chromatograph (Agilent 5890, formerly Hewlett Packard)-mass spectrometer (Agilent 5899B) system with an electron capture negative ionization source operating in single ion monitoring mode (GC-MS-ECNI-SIM). The GC system was equipped with a fused silica capillary column HP-5MS (60 m × 0.25 mm, 0.25 μm). The analytical limit of detection (LOD) for both MCA and DCA was 0.2 μg l⁻¹. Average recoveries of urine samples spiked with 8 μg l⁻¹ of MCA and DCA authentic reference standards were 97 and 92%, respectively (n = 3 samples). Inter-day coefficients of variation for replicate analysis of the same urine sample spiked with 10 μg l⁻¹ of MCA and DCA reference standards were 8.1 and 2.7%, respectively (n = 8 days).

Analysis of alkyl phosphates and IMPY

For the analysis of alkyl phosphates (DMDTP, DMTP, DMP, DEP, DETP) and IMPY, urinary samples were first spiked with isotopically labeled alkyl phosphate analogues and derivatized using pentafluorobenzyl bromide at 70°C for 2 h. The derivatives were then extracted with hexane and methylene chloride. Analysis was performed using a gas chromatograph (Agilent 6890)-mass spectrometer (Agilent 5973N) system with an electron impact ionization source operating in single ion monitoring mode (GC-MS-ECNI-SIM). The GC system was equipped with an Agilent capillary column HP-50 (30 m × 0.25 mm, 0.25 μm). The LOD of IMPY, DMDTP, DMTP, DEP, DETP was 1 μg l⁻¹ and that of DMP was 2 μg l⁻¹. Average recoveries of urine samples spiked with 5–15 μg l⁻¹ of authentic reference standards were 104, 102, 85, 99, 91 and 96% for IMPY, DMDTP, DMTP, DMP, DEP, DETP, respectively (n = 5 samples). Inter-day coefficients of variation for replicate analysis of the same urine sample spiked with 40 μg l⁻¹ IMPY, 102 μg l⁻¹ DMDTP, 199 μg l⁻¹ DMTP, 156 μg l⁻¹ DMP, 44.3 μg l⁻¹ DEP and 33.4 μg l⁻¹ DETP were 3.9, 5.6, 3.0, 6.8, 3.8 and 7.2%, respectively (n = 20 days).

Analysis of acephate and methamidophos

Urinary levels of acephate and methamidophos were determined by a high performance liquid
chromatography-mass spectrometry method (HPLC-MS-MS) after clean-up using a sorbent-immobilized liquid extraction cartridge (Chem Elut, Varian). Analysis of acephate and methamidophos was performed using a HPLC system (Waters Alliance 2690) equipped with a C8 Waters Symmetry column (2.1 mm × 50 mm, 5 μm), a tandem mass spectrometer system (Micromass Quattro LC) with an electrospray source operating in MRM mode. The LOD of acephate and methamidophos was 0.8 μg l⁻¹. Average recoveries of urine samples spiked with 10 μg l⁻¹ of acephate and methamidophos authentic reference standards were 31 and 30%, respectively (n = 3 samples). Urinary values were corrected to compensate for the incomplete recovery. Inter-day coefficients of variation for replicate analysis of the same urine sample spiked with 50 μg l⁻¹ of acephate and methamidophos reference standards were 1.7 and 2.4%, respectively (n = 3 days).

**Questionnaires**

Participants completed a self-administered questionnaire, which was filled at the time of urine collection. Personal data [gender, body weight (b.w.), address] was documented as well as information related to the urine sampling day of each of the studied insecticide (work shift date and hours, duration of application of the studied insecticides or time spent in the treated area, personal protection, contact with pesticides during the 3 days preceding urine sampling, at work or outside work).

**Data analysis**

Both the concentrations (nmol l⁻¹, μmol mol⁻¹ creatinine) and amounts (nmol kg⁻¹ b.w.) of biomarkers of exposure to each studied OP were determined in every urine sample provided by the participants. Samples with undetectable levels were assigned a value of half the LOD. The cumulative urinary excretion time courses of the measured biomarkers in workers (nmol kg⁻¹ b.w.) were then determined over a ≈24 h period following the onset of exposure episodes in greenhouses.

Health risks of exposure to malathion for each worker were assessed by comparing total amounts of biomarkers observed over the 24 h urine collection period to available BRVs below which the risks of adverse health effects can be considered negligible (Bouchard et al., 2003). The BRVs for malathion were derived using a toxicokinetic model specific to this insecticide (Bouchard et al., 2003), which allows reconstruction of the absorbed dose of malathion starting from measurements of the cumulative urinary amounts of malathion metabolites over given time periods (the most accurate estimate being obtained with the sum of the specific MCA and DCA metabolites). Since a NOEL dose for the inhibition of blood cholinesterase can be inferred from published data (Moeller and Rider, 1962), the cumulative biomarker levels corresponding to a human NOEL can thus be established since the model links dose absorption scenarios to the time course of cumulative levels of biomarkers. The relationship between the excretion rate of carboxylic acids and that of MP derivatives in each urine sample was also tested by linear regression.

In addition, urinary concentrations of the various OP metabolites measured in the current study were compared with baseline values established in the civilian US population by the Centers for Disease Control and Prevention (CDC, 2005), as part of the National Health and Nutrition Examination Survey (NHANES) 1999–2002.

**RESULTS**

**Malathion exposure**

Table 1 describes questionnaire data. Fig. 1 depicts the cumulative urinary excretion time courses of the specific MCA and DCA metabolites of malathion for each of the greenhouse workers. The time profiles of MCA and DCA urinary excretion generally evolved in the same manner but the 24 h cumulative MCA molar amounts were on average 2.2 (SD = 0.59) times higher than DCA values.

Table 2 shows the total amounts of the sum of MCA and DCA excreted in the urine of workers over the entire collection period (~24 h). These excretions amounted at most to 2.5% of the BRV of 57 nmol kg⁻¹ b.w. per day established by Bouchard et al. (2003) on the basis of a non-significant inhibition of plasma and erythrocyte cholinesterase activities.

The urinary excretion time courses of the non-specific MP derivatives of malathion (the sum of DMDTP, DMTP and DMP) were also examined (Fig. 1). Total amounts of the sum of the MP metabolites excreted over the 24 h collection period were 2.2–14.9 times higher than carboxylic acids values (the sum of MCA and DCA). This is different from what would be expected from the metabolism of malathion, where the ratio of total urinary amounts of MP derivatives (in moles) to that of total carboxylic acids is on average *circa* 0.5 (Jellinek, Schwartz and Connolly, Inc., 2000; Bouchard et al., 2003). If this latter ratio were applied, the expected cumulative urinary excretion of MP derivatives over 24 h should be below the values observed for MCA and DCA, which is clearly not the case in this study (Table 2). There was, accordingly, no significant correlation between the observed urinary excretion rate of MP derivatives and that of carboxylic acids (the sum of MCA and DCA) ($R^2 = 0.0045$) (Fig. 2). Only the time courses of MCA and DCA...
excretion rate (nmol h\(^{-1}\)) were compatible with the time of work exposure (data not shown). It was, however, calculated that the total amounts of MP derivatives excreted over the 0–24 h collection period in the current study were far below the BRV of 62 nmol kg\(^{-1}\) b.w. per day proposed by Bouchard et al. (2003) as an indicator of health risks on the basis of blood cholinesterase activity (i.e. from 0.8 to 8% depending on the worker).

Table 3 shows the distribution of MCA, DCA, DMDTP, DMTP and DMP urinary concentrations (\(\mu g \text{ l}^{-1}\)) in the 57 urine samples analyzed compared with the baseline range values determined by the CDC (2005). Median and 95th percentile values of DCA and DMP values were slightly higher than the baseline values observed in the civilian US population while DMDTP and DMTP values were similar to the baseline ranges (MCA was not measured by the CDC).

**Diazinon exposure**

Urinary excretion values of the specific metabolite of diazinon, IMPY, in all of the 54 urine samples were below or equal to the analytical LOD of 1 \(\mu g \text{ l}^{-1}\). The cumulative urinary excretion time course of the non-specific ethyl phosphoric derivatives of diazinon (the sum of DETP and DEP) was also determined for each worker (Fig. 3). Cumulative urinary amounts over the 24 h collection period ranged from 0.06 to 1.62 nmol kg\(^{-1}\) b.w. for DEP and from 0.04 to 0.12 nmol kg\(^{-1}\) b.w. for DETP. Table 3 shows that the distribution of DETP and DEP urinary concentrations (\(\mu g \text{ l}^{-1}\)) in the 54 urine samples analyzed was similar to known non-occupational baseline ranges.

**Acephate exposure**

In all of the 59 urine samples provided by the participants, concentrations of acephate were below the LOD (0.8 \(\mu g \text{ l}^{-1}\)) except for one individual, who showed detectable levels of acephate in four of the nine samples he provided (concentrations between 1.4 and 3.5 \(\mu g \text{ l}^{-1}\)). Concentrations of methamidophos in all the 59 urine samples provided by the participants were also below the LOD (0.8 \(\mu g \text{ l}^{-1}\)). DMTP, a non-specific metabolite of acephate, was further measured in the urine of workers following the onset of an exposure to acephate in greenhouses. Total urinary amounts of DMTP during the 24 h collection period were on average (range) 1.19 (0.06–3.47) nmol kg\(^{-1}\) b.w., which is similar to the values of 1.07 (0.10–2.71) nmol kg\(^{-1}\) b.w. observed upon exposure to malathion in this study.

**Discussion**

The present study was undertaken to estimate the extent of exposure to malathion, diazinon and acephate in a group of horticultural greenhouse workers and to assess the associated health risks. This was achieved by determining the cumulative urinary excretion time course of biomarkers of exposure to these OPs in workers, particularly specific biomarkers for which there is little published data (Krieger and Dinoff, 2000; Cruz Marquez et al., 2001; Tuomainen et al., 2002).

On the basis of malathion specific metabolite measurements, it appeared that the workers under study were slightly more exposed to malathion than the

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**Table 1. Questionnaire data for the individuals exposed to malathion in greenhouses**

<table>
<thead>
<tr>
<th>Workers</th>
<th>Gender</th>
<th>Body weight (kg)</th>
<th>Task performed in greenhouses</th>
<th>Duration of malathion exposure in the greenhouses(^{b,c})</th>
<th>Protective equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>75.0</td>
<td>Application of malathion with a hand sprayer</td>
<td>95 min</td>
<td>Coverall, mask with filter, safety glasses, gloves and rubber boots</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>63.6</td>
<td>Application of malathion with a hand sprayer</td>
<td>95 min</td>
<td>Coverall, mask with filter, glasses, gloves and rubber boots</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>56.8</td>
<td>Verification of plant watering</td>
<td>15 min</td>
<td>Pants, long-sleeved shirt, boots</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>48.2</td>
<td>Plants watering and cleaning</td>
<td>8 h 45 min</td>
<td>Short pants, short-sleeved shirt, apron</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>43.2</td>
<td>Plants transportation</td>
<td>9 h</td>
<td>Short pants, short-sleeved shirt</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>54.6</td>
<td>Watering, cutting and moving of plants</td>
<td>5 h 30 min</td>
<td>Short pants, short-sleeved shirt, work gloves</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>50.0</td>
<td>Cutting of plants</td>
<td>9 h</td>
<td>Short pants, short-sleeved shirt, work gloves</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>77.3</td>
<td>Work in greenhouses but without direct contact with treated plants</td>
<td>9 h</td>
<td>Short pants, short-sleeved shirt</td>
</tr>
</tbody>
</table>

\(^a\)F = female and M = male.

\(^{b}\)For applicators, exposure duration corresponds to spraying period; for the individuals manipulating treated plants, it corresponds to the time spent in the area treated with malathion; for the individual without direct contact with treated plants, it corresponds to the whole work shift.

\(^{c}\)All the participants reported not to have been in contact with malathion during the 3 days preceding urine sampling, at work or outside work.
general population. However, total 24 h urinary amounts of malathion specific metabolites (MCA and DCA) were well below the BRVs proposed by Bouchard et al. (2003); on that basis, the workers would incur a negligible risk of adverse health effects. Imprecisions in this estimation are, nevertheless, possible and are related in part to the urine collection period and the BRVs. For one, the choice of a 24 h
Fig. 1. Cumulative urinary excretion time courses of malathion mono- (○) and di- (■) carboxylic acids (pmol kg\(^{-1}\) b.w.) together with methyl phosphoric (MP) derivatives (△) (the sum of DMDTP, DMTP and DMP) in workers following the onset of a malathion exposure in greenhouses: two applicators, five individuals manipulating treated plants and one individual without direct contact with treated plants.
The period was retained as a compromise between practical reasons and scientific considerations. Over such a period, errors may arise if some of the workers did not follow the protocol meticulously; all workers however have asserted full compliance. Another source of uncertainty stems from different inter-subject absorption rates. To ensure a margin of safety, the BRVs (Bouchard et al., 2003) were derived considering the most conservative absorption scenario. It was shown there that reference values based on a workday dermal exposure scenario, with slow absorption and thus slow urinary output, are safer than those based on the faster urinary outputs of rapidly absorbed malathion from respiratory or oral exposures.

The workers of the current study appear to be on average 50 times less exposed than the greenhouse workers of a recent Spanish report (Cruz Ma´rquez et al., 2001) where the mean amount of MCA was calculated to be roughly 15 nmol kg\(^{-1}\) b.w. in the 24 h urine samples of three applicators following malathion spraying as compared with 0.3 nmol kg\(^{-1}\) b.w. in our study. It was, however, reported in the Spanish study that one of the applicators did not wear a coverall during the application. The workers of the present study also appear to be on average 8 times less exposed than botanical garden workers of a previous study (Bouchard et al., 2003), where the mean amount of carboxylic acid metabolites of malathion

![Table 2. Total amounts of malathion metabolites in 24 h urine collections of workers following an exposure to malathion in greenhouses, estimated absorbed dose and comparison with a biological reference value below which health risks appear negligible](image-url)

<table>
<thead>
<tr>
<th>Workers</th>
<th>Observed amounts of carboxylic acids(^a) in 24-h urine collections (nmol kg(^{-1}) b.w.)</th>
<th>Amounts of methyl phosphoric derivatives(^b) in 24-h urine collections (nmol kg(^{-1}) b.w.)</th>
<th>Reconstructed absorbed malathion dose (nmol kg(^{-1}) b.w.)(^c)</th>
<th>% of the biological reference value(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.21</td>
<td>3.17</td>
<td>0.10</td>
<td>0.32</td>
</tr>
<tr>
<td>2</td>
<td>1.12</td>
<td>3.60</td>
<td>0.54</td>
<td>1.66</td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>4.98</td>
<td>0.17</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>1.05</td>
<td>1.37</td>
<td>0.50</td>
<td>1.55</td>
</tr>
<tr>
<td>5</td>
<td>0.24</td>
<td>1.98</td>
<td>0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td>0.44</td>
<td>0.99</td>
<td>0.21</td>
<td>0.65</td>
</tr>
<tr>
<td>7</td>
<td>0.06</td>
<td>0.51</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>8</td>
<td>0.15</td>
<td>0.85</td>
<td>0.07</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(^a\)The sum of the mono- and di-carboxylic acids.

\(^b\)The sum of dimethyl dithiophosphate (DMDTP), dimethyl thiophosphate (DMTP) and dimethyl phosphate (DMP).

\(^c\)Reconstructed from carboxylic acid amounts in 24 h urine collections, considering that the absorbed dose of malathion following a work exposure in greenhouses was essentially completely eliminated from the body 24 h post-exposure and that carboxylic acids represent on average \(\approx 67.5\%\) of total metabolites excreted overall in urine following malathion exposure in volunteers (Jellinek et al., 2000; Bouchard et al., 2003) (i.e. amounts of carboxylic acids in 24 h urine collections divided by 0.675).

\(^d\)Biological reference value proposed in Bouchard et al. (2003) study based on the sum of the malathion mono- and di-carboxylic acids: 57 nmol kg\(^{-1}\) b.w. in 24 h urine collections.

\(^e\)Central estimate based on carboxylic acid values observed in this study together with the reported methyl phosphoric derivative/carboxylic acid ratio of 0.48 following exposure to malathion in volunteers (Jellinek et al., 2000; Bouchard et al., 2003).

![Fig. 2. Correlation between the excretion rates of MP derivatives (the sum of DMDTP, DMTP and DMP) and that of carboxylic acids (the sum of mono- and di-carboxylic acids) (nmol h\(^{-1}\)) in each urine sample of workers exposed to malathion in greenhouses](image-url)

\[ y = 0.386x + 0.8537 \]

\[ R^2 = 0.0045 \]
(the sum of MCA and DCA) in 24 h urine samples was 3.5 nmol kg\(^{-1}\) b.w. with minimum and maximum values of 1.0 and 13.5 nmol kg\(^{-1}\) b.w., respectively. Corresponding values in the current study are on average 0.45 nmol kg\(^{-1}\) b.w., with minimum and maximum values of 0.06 and 1.12 nmol kg\(^{-1}\) b.w., respectively.

In a study, Tuomainen et al. (2002) determined the 24 h urinary excretion time course of MCA in three greenhouse workers (applicators) following a malathion spraying. The total amounts of MCA excreted during the 24 h period following applications were calculated to be 10.9, 0.12 and 0.51 nmol kg\(^{-1}\) b.w., assuming a b.w. of 70 kg. These values represent, respectively, 0.25, 0.003 and 0.012 of the BRV for MCA proposed in Bouchard et al. (2003). In the latter two cases, the values are in the same range as those observed in the present study. In the current study, the protection measures thus appear to be sufficient to limit exposure (personal protective equipment for applicators, at least a 12 h delay between application of the pesticide and manipulation of treated plants and possibly a limited direct contact with treated plants).

With regard to the non-specific methyl phosphoric OP derivatives (DMTP, DMP and IMPY), when comparing urinary concentrations observed in our study with baseline data available in the literature for these metabolites in the general population (Barr et al., 2004; Saieva et al., 2004; CDC, 2005), it would appear that the workers under study were not seriously exposed to OPs. Based on the comparison of cumulative excretions of the specific and non-specific metabolites of malathion, it also seems that MP metabolites in the urine of workers result primarily from exposures other than malathion spraying in greenhouses. The urinary MP levels in workers were from 3 to 31 times higher than would be expected on the basis of (i) the observed excretions of malathion specific carboxylic metabolites and (ii) the documented fraction of an absorbed malathion dose that generates carboxylic acid metabolites (on average 67%) as compared with MP derivatives (on average 33%) in human volunteers exposed orally to 0.5, 1.5, 10 and 15 mg kg\(^{-1}\) of malathion under controlled conditions (Jellinek, Schwartz and Connolly, Inc., 2000; Bouchard et al., 2003). Given that the levels of non-specific MP metabolites measured in the urine of workers are in the same range as those observed in the general population (Table 3) and that some authors have shown that dietary intake of pesticides is the primary source of OP exposure in the general population (Curl et al., 2003; Fenske et al., 2005; Lu et al., 2005), this suggests that these levels arise in part from the intake of food contaminated with MP generating OPs such as phosmet, azinphos-methyl, methidathion and dimethoate. Exposure to plants contaminated with residual OPs in the greenhouses could also contribute to these levels (Samuel et al., 2002).

Diazinon and acephate exposures following their application in greenhouses appeared very low on the basis of the observed urinary concentrations of their specific biomarkers: IMPY and acephate. This was also confirmed through the measurements of the non-specific ethyl phosphoric and methamidophos metabolites. IMPY, DEP and DETP concentrations were similar to the CDC’s non-occupational baseline ranges (CDC, 2005). As for acephate and methamidophos, concentrations were almost all below the LOD of 0.8 and 0.2 µg l\(^{-1}\), respectively, the latter being in the same value range as those reported by other authors, i.e. in the low µg l\(^{-1}\) range (Yokley et al., 2000; Olsson et al., 2003, 2004; CDC, 2005). Acephate and methamidophos were not determined in the NHANES 1999–2002 survey. Olsson et al. (2003) reported baseline values ranging from below the LOD of 0.8 to 9.9 µg l\(^{-1}\) for acephate and from below the LOD of 0.8 to 2.3 µg l\(^{-1}\) for methamidophos in 140 urine samples of the US population. In our study, the only individual with detectable acephate

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Urinary concentration observed in the greenhouse workers (µg l(^{-1}))</th>
<th>CDC’s baseline concentration in the civilian US population aged 6–54 years (µg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>1.3</td>
<td>ND(^b)</td>
</tr>
<tr>
<td>DCA</td>
<td>0.85</td>
<td>&lt;LOD of 0.29</td>
</tr>
<tr>
<td>DMTP</td>
<td>&lt;LOD of 1.0</td>
<td>&lt;LOD of 0.1</td>
</tr>
<tr>
<td>DMTP</td>
<td>3.5</td>
<td>0.45</td>
</tr>
<tr>
<td>DMP</td>
<td>4.0</td>
<td>&lt;LOD of 0.5</td>
</tr>
<tr>
<td>IMPY</td>
<td>&lt;LOD of 1.0</td>
<td>&lt;LOD of 0.7</td>
</tr>
<tr>
<td>DETP</td>
<td>&lt;LOD of 1.0</td>
<td>0.57</td>
</tr>
<tr>
<td>DEP</td>
<td>3.0</td>
<td>&lt;LOD of 0.2</td>
</tr>
</tbody>
</table>

\(a\) Results of the National Health and Nutrition Examination Survey (NHANES) 2001–2002 except for DCA, which was only measured in the NHANES 1999–2000 survey.

\(b\) ND = Not determined.

Table 3. Comparison of the urinary concentrations of malathion and diazinon metabolites in the greenhouse workers under study with the baseline values established by the Centers for Disease Control and Prevention (CDC, 2005) in the civilian US population.
concentrations in some of his urine samples showed excretion values within the baseline range reported by Olsson et al. (2003).

This study emphasized the use of the cumulative urinary excretion of specific and non-specific OP metabolites to assess occupational exposure. Only the values observed for the specific malathion metabolites (MCA and DCA) can be relied upon to reflect the low-dose occupational exposure following malathion spraying in greenhouses. The non-specific MP metabolites were not reliable to assess specific low-dose exposures to malathion given that they stem from diverse sources. As for diazinon and acephate, exposure appeared negligible on the basis of the observed levels of their specific biomarkers: IMPY and acephate. Adequate preventive measures to limit exposure to OPs, thus, appear to be applied in this workplace. Though the results cannot be generalized to all greenhouse workers given the varying measures of protection applied in this type of setting, they may be deemed representative of exposure when adequate precautions are implemented.

REFERENCES


