ELSEVIER

Journal of Chromatography A, 939 (2001) 79–89

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Gas chromatographic–tandem mass spectrometric analytical method for the study of inhalation, potential dermal and actual exposure of agricultural workers to the pesticide malathion

M. Cruz Márquez, F.J. Arrebola, F.J. Egea González, M.L. Castro Cano, J.L. Martínez Vidal*

Department of Analytical Chemistry, University of Almeria, 04071 Almeria, Spain

Received 30 March 2001; received in revised form 1 October 2001; accepted 1 October 2001

Abstract

New analytical gas chromatographic–tandem mass spectrometric approaches have been developed for assessing both potential and actual exposure of agricultural workers to malathion. The metabolites α- and β-malathion monocarboxylic acids have been determined after a derivatisation process in order to obtain their hexafluoroisopropyl esters. Whole body dosimetry was used for potential dermal exposure assessment. Potential exposure by inhalation was estimated using personal air samplers and polyurethane foam plugs as sorbents. The intern dose measurements were carried out by analysing samples of urine after solid-phase extraction with C_{18}. The recoveries of the analytes of the three matrices were between 90 and 102%. Quantification limits were lower than 0.24 ng L^{-1}. The proposed methods have been applied to evaluate potential and actual exposure of applicators spraying malathion in greenhouses.

Keywords: Extraction methods; Malathion; Pesticides; Polyurethane

1. Introduction

The legislative basis for the regulation of pesticides in the European Union is Directive 91/414/EEC [1]. It states that members shall not allow a pesticide to be authorised unless it is scientifically shown that normal use has no risk of harmful effects on humans, establishing exposure data requirements. The approaches for assessing dermal exposure have been well described in several reviews [2–4], where patch and whole body dosimetry are discussed as sampling methods. It is concluded that there is a need for systematic research on sampling and analytical methods in order to choose the adequate sampling media and to establish performance parameters of analytical procedures. One advantage of the whole body dosimetry is its compatibility with biological monitoring. The analysis of the coverall worn by applicators to obtain potential dermal exposure, and the use of biological monitoring to measure the internal dose appears as the most sophisticated tool in exposure assessment.

Concerning the potential exposure, the Organisation for Economic Co-operation and Development (OECD) [5] reports a guidance for the study of occupational exposure, including a quality assurance

*Corresponding author. Tel.: +34-950-01-5429; fax: +34-950-01-5483.

E-mail address: jlmartin@ual.es (J.L. Martínez Vidal).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01347-4

and quality control procedure. In this sense, aspects which might affect the reliability of results, such as the matrix effect in the quantification of pesticides, or the influence of the retention characteristics of different clothes used for whole body dosimetry, have been studied [6,7]. For the ambient air monitoring, most of references address the active sampling using solid sorbents attached to personal pumped air samplers as the best methodology for determining pesticides in air. In this aspect it is important to consider both the generation of pesticide standard vapours for validation purposes, and the range of air concentrations in which the sorbent is suitable without saturation and/or breakthrough [8,9].

Malathion, diethyl (dimethoxythiophosphorylthio) succinate, is a very widely non-systemic insecticide and acaricide with contact, stomach, and respiratory action. It is a cholinesterase inhibitor of low mammalian toxicity. Many studies have assessed the metabolism of malathion on insects [10], rats and humans [11]. The principal route of malathion metabolism in animals is via de-esterification to the α- and β-malathion monocarboxylic acids (α- and β-MMA) followed by further metabolism to the dicarboxylic acid (MDA). This is a facile esterase-catalysed detoxification route, which is considered to be responsible for the low toxicity of vertebrate to malathion. Another metabolic pathway is the oxidative desulfuration that leads to the formation of malaoxon, an active acetylcholinesterase inhibitor. The main metabolites detected in humans are α- and β-MMA. Appreciable amounts of MDA are also found but malaoxon is a minor metabolite. Additional human metabolites identified were *O,O*-dimethyl phosphorodithioate, *O,O*-dimethyl phosphorothioate, dimethyl phosphate and monomethyl phosphate. Malathion is metabolised and then excreted predominantly in the urine (85–89%) and faeces (4–15%). Therefore, the best way to determine the actual exposure to malathion is analysing MMA and MDA in urine. The analysis of MDA presents the difficulty that it is not easily obtainable in pure state for its usage as analytical standard. Additionally, it is not very stable. Unlike MDA, MMA is stable in pure state and can be commercially obtainable.

Gas chromatography–tandem mass spectrometry (GC–MS–MS) has been successfully applied for determining other metabolites and pesticides in biological monitoring [12–15]. The combination of the techniques above mentioned allows the analysis of ultra trace levels of target compounds in complex matrices such as biological fluids (urine or blood). Additionally, the confirmation of the results carried out by MS–MS spectra, obtained in special experimental conditions reduces the possibility of false positives compared with other mass spectrometric techniques (Selected Ion Monitoring or full scan modes) used as GC detectors.

This paper presents a methodology, based on GC analysis and tandem mass spectrometry detection, to assess the exposure of greenhouse applicators to malathion, by combining potential dermal, inhalation and internal dose measurements. The analytical methods have been validated establishing performance parameters for the analysis of malathion in protective clothing and polyurethane foam, which are used as sampling media for potential dermal assessment and air analysis respectively. It is also used for the analysis of malathion and its metabolites α- and β-MMA in urine samples. Finally the exposure levels obtained for three pesticide applicators have been determined.

2. Experimental

2.1. Chemicals and materials

Residue analysis grade *n*-hexane, diethyl ether, acetone, and methanol (Panreac, Barcelona, Spain) were used as received. Organic-free water was prepared by distillation and then by passing through Milli-Q SP column (Millipore, USA). Analytical-grade anhydrous sodium sulfate and potassium carbonate were purchased from Merck (Darmstadt,Germany). 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) and diisoproylcarbodiimide (DIC) were from Aldrich (Steinheim, Germany) and were used as received.

Pure malathion and methyl-chlorpyrifos (internal standard, I.S.) as well as MMA in ethylacetate (10 $\mu g\ mL^{-1}$) were available from Dr. Ehrenstorfer (Ausburg, Germany). Individual stock solutions of malathion and methyl-chlorpyrifos at 400 $\mu g\ mL^{-1}$ were prepared in acetone and stored in a freezer

(−30°C). The working solutions for biological monitoring determinations were obtained by appropriate dilution of the stock solution with the same solvent, and stored in a refrigerator (4°C). Matrix-matched calibration solutions of malathion were prepared for whole body analysis using acetone extracts from uncontaminated coveralls. Sigmasil A was purchased from Sigma (St. Louis, MO, USA). A Sep-Pak cartridge for solid-phase extraction packed with 500 mg C_{18} was from Waters (Milford, MA, USA).

Malathion 90 (malathion 90%, w/v, EL, Lainco, Barcelona, Spain) was used for treatment of vegetables in the greenhouse. Latex gloves, disposable coveralls (65% cotton, 35% polyester purchased from Iturri, Sevilla, Spain) and protective masks (3M Model 4251) were worn by applicators as protection equipment during treatments. Air concentration of malathion was monitored using three SKC personal samplers Model PCEX3KB, connected to polyurethane foam (PUF) plugs 10 cm length, 2 cm diameter and 0.022 g cm^{-3} density (Pikolin, Zaragoza, Spain), working at a sampling flow rate of 21 min^{-1}.

2.2. Equipment

A Saturn 2000 gas chromatograph-ion trap mass spectrometer (Varian Instruments, Sunnyvale, CA, USA) was used. The gas chromatograph was fitted with an 8200 autosampler, a split/splitless temperature programmable injector 1078 operated in splitless mode. The column used was a DB5-MS 30 m×0.25 mm I.D., 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). The ion trap mass spectrometer was operated in the electron ionisation mode (El, 70 eV) and the MS–MS option was used. The data handling system had an EI-MS–MS library specially created for the target analytes under our experimental conditions. In addition, other EI-MS libraries were also available. The carrier gas used was helium (purity N50).

A test tube shaker with a variable speed controller was purchased from IkaWorks (Wilmington, NC, USA). An overhead mixer (Agitator) to hold containers (1 L capacity with lid) was used for cold extraction of contaminated clothes. A Soxhlet was used for the extraction of PUF plugs.

2.3. Field trial design

A total of three applications of malathion were carried out by three different applicators in three flat roof greenhouses of polyethylene (200 μm thickness, 15×40×2.50 m), located in Almeria (Spain). The lateral windows remained closed during the experiment and climatological conditions were registered. The crops were green beans (cultivar Helda), tomato (cultivar Daniela) and cucumber (cultivar Almeria). They were 2 m in height with a 1-m inter-row distance, allowing the applicators to walk between each row during the applications. For spraying, they used semi-stationary high volume application equipment with one circular nozzle operating at a flow rate of 4 L min^{-1}. The volume sprayed was 375 L in each case, corresponding approximately to a dose of 1 kg ha^{-1} of malathion. For the three applications the spray liquid was prepared by dispersing 600 mL of malathion 90 EL in a tank containing 400 L of water.

Each of the treatments lasted approximately 90 min. Applicators sprayed following a similar application pattern, walking between the rows spraying one side of the crop, and returning along the same row spraying the other side of the row. The protection equipment of the three applicators consisted of a cotton T-shirt for the first applicator and a full cotton–polyester coverall for the other two. All of them wore a mask.

2.4. Sampling procedure

Gloves and coveralls were used as sampling media for the whole body methodology. After applications, they were carefully removed and dried in the shade. Applicator 1 did not wear any protective equipment so that potential dermal exposure of this applicator was not assessed. In order to assess inhalation exposure, during the applications, each operator carried a personal sampler, sampling air at 2 l min^{-1} flow-rate, and connected to a PUF plug, which was fitted downwards on the upper part of the chest, avoiding accidental contamination by dripping or contact with contaminated items. After 1 h of application, PUF was replaced in order to avoid saturation and breakthrough. Biomonitoring was performed taking between 7 and 10 urine samples

from the three volunteers up to 24 h after the application of malathion. Samples of personal protective equipment were stored in bags out of the light at 4°C until analysis; PUF plugs were stored in glass containers out of the light at −30°C until analysis; urine samples were stored in sterilised polyethylene containers, frozen immediately and kept at −30°C until analysis.

A field quality control protocol was established in order to ensure the integrity of samples during sampling, transport, storage and analysis. An aliquot of the sprayed liquid was taken from the gun 15 min after the start and at the end of each application, in order to check its concentration. Six blanks of each sampling medium (coverall, cotton gloves, PUF and urine) were taken from each operator before the applications. Three blanks were labelled as field blanks and stored in the same way as samples in order to check accidental contamination or degradation of sampling media. The rest of blank samples were spiked as follows: three cotton gloves and three pieces (30×30 cm^2) of protective coverall, were spiked with 100 μl of the spray tank (around 135 μg of malathion, depending on the spray tank concentration); three PUF plugs were spiked with 5 μl of the spray tank liquid (6.8 μg of malathion); and three aliquots of uncontaminated urine, were also spiked with malathion and MMA standards at 40 μg l^{-1} concentration level. Field blanks, field spikes and samples were stored, processed and analysed in the same batch. The criteria assumed were [5]: recovery rates of field spikes should be between 70 and 120% and relative standard deviation (RSD)<20%; field blanks should not have evidence of any contamination or sample decomposition; slopes of calibration curves should not differ more than 25% of those obtained in validation studies and should fit into straight lines with $r^2>0.95$.

2.5. Analytical procedure

2.5.1. Urine extraction procedure

A 3-mL aliquot of urine was passed through the C_{18} cartridge previously conditioned with 6 mL of methanol and 4 mL of distilled water in that order. (Note: cartridge was not allowed to dry during conditioning). To carry out a clean up step, 4 mL of distilled water were passed through the cartridge. The last drops of liquid from the cartridge were withdrawn with a vacuum pump. Analytes were eluted with 10 mL diethyl ether, which was passed through anhydrous sodium sulfate. A 100-μl volume of I.S. solution (500 ng mL^{-1} in acetone) was added to the extract and then, the solvent was removed under a soft stream of nitrogen without heating it. The residue was re-dissolved in 1 mL *n*-hexane. For derivatisation, 10 μl HFIP were added with gentle mixing, then 15 μ1 of DIC were added. After shaking for 3 min, the extract was washed with 1 mL of 5% aqueous potassium carbonate solution to neutralise the excess of the derivatising agent. The organic layer was transferred to a 2-mL autosampler vial for GC–MS–MS analysis.

2.5.2. Personal protective equipment extraction procedure

The extraction procedure was similar to that described in [6], based on the sectioning of coveralls in nine pieces and further extraction with different volumes of acetone: head and neck (250 mL), left arm (250 mL), right arm (250 mL), chest (350 mL), back (350 mL), thighs/waist front (350 mL), thighs/waist back (350 mL), lower leg left (250 mL), lower leg right (250 mL), glove left (150 mL) and glove right (150 mL). The extraction was carried out in 1 L capacity bottles placed for 30 min in an overhead shaker at 30 rpm. An aliquot of this extract was transferred to a 10 mL volumetric flask containing 3.5 μg of I.S., being ready for GC–MS–MS analysis.

2.5.3. PUF plugs extraction

PUF samples were extracted following the procedure described elsewhere [9], placing PUF plugs in a Soxhlet extractor, siphoning at 20 min $cycle^{-1}$, with 100 mL of acetone for 8 h. The extract was evaporated until almost dryness, I.S. was added (0.4 μg) and the extract diluted to 4 mL, ready for GC–MS–MS analysis.

2.5.4. GC–MS–MS conditions

A 1-μl aliquot of each extract was injected into the gas chromatograph with the split closed for 1.75 min. Injector temperature was programmed from 90 (hold 0.1 min at 90°C) to 280°C at the rate of 200°C/min and held at 280°C for 20 min. The oven

Table 1
Mass spectrometer operating conditions

Ionisation mode	EI
Multiplier voltage	1700 V
A/M amplitude voltage	4.0 V
Trap temperature	200°C
Manifold temperature	45°C
Transfer-line temperature	280°C
Emission current	80 μA
Automatic gain control (AGC)	On
AGC target	2000 counts

temperature was modified from 60 (hold for 1.75 min) to 270°C at the rate of 20°C/min (20 min held). The mass spectrometer was calibrated weekly. The operating conditions are summarised in Tables 1 and 2. MS–MS was performed in a non-resonant mode for all compounds.

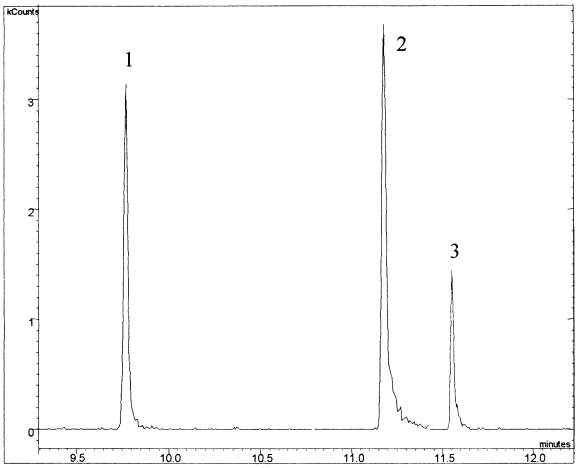

Fig. 1. Gas chromatogram of a clean urine fortified with 10 μg L^{-1} of each analyte. (1) MMA, (2) I.S., and (3) malathion.

3. Results and discussion

3.1. Instrumental variables

A derivatisation process is necessary due to the low volatility and high polarity of MMA. Several derivatising agents (fresh diazomethane, Sigmasil A and 1,1,1,3,3,3-hexafluoroisopropanol) were investigated and the chromatographic properties of various derivatives were studied. The methyl esters obtained with diazomethane [16] presented a questionable stability and low sensitivity. In addition, the derivatising agent is a carcinogenic reactive and may exploit. The trimethylsilyl esters were obtained by a reaction of dried eluent with with Sigmasil A [14]. Although, trimethylsilyl derivatives were much more sensitive than methyl derivatives, the excess of the derivatizing agent and its by-products produced several interfering peaks. HFIP derivatives, which are produced by a rapid coupling of the eluent with HFIP in the presence of DIC reaction, are highly sensitive and do not produce interfering substances.

α- and β-MMA HFIP derivatives co-eluted in the selected gas chromatographic conditions at the retention time of 9.79 min. They were not separated, being quantified as the sum of both isomers. The retention times for I.S. and malathion were 11.20 and 11.57 min respectively. A gas chromatogram obtained in the selected conditions is shown in Fig. 1.

In order to optimise sensitivity for mass detection, the automatic gain control (AGC) was switched on by filling the trap with target ions. For that, the AGC target was fixed at 2000 counts because higher values caused electrostatic interactions among ions in the ion trap chamber. For analysis, a parent ion was chosen for each analyte taking into consideration its m/z and its relative abundance (both as high as

Table 2
MS–MS conditions[a]

Compound	Activation time (min)	Range (m/z)	Parent ion (m/z)	Mass defect (mu/100 u)	Excitation amplitude (V)	Excitation storage level (m/z)
MMA	9.0–10.8	60–305	295	+74	37.5	80
Methyl-chlorpyrifos (I.S.)	10.8–11.4	125–295	286	0	66.0	80
Malathion	11.4–18.0	90–185	173	0	70.0	89

[a] Excitation time = 40 μs; isolation window = 2 u; non-resonant waveform.

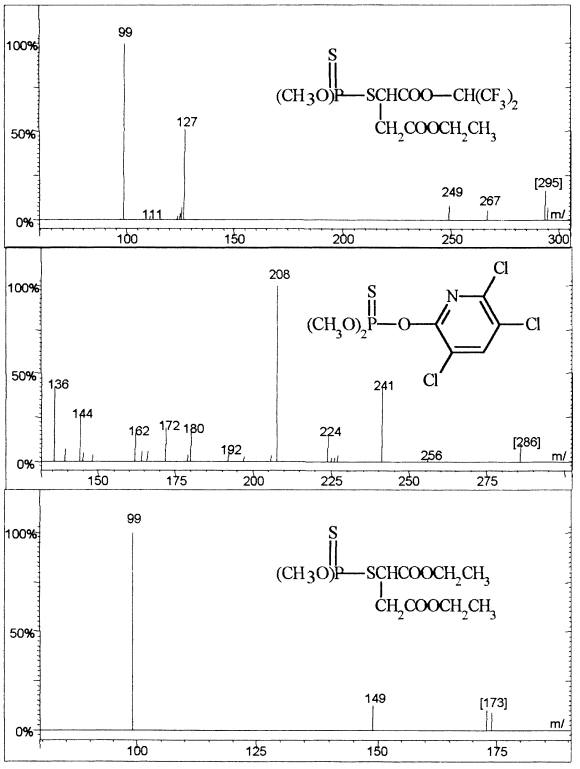

Fig. 2. MS–MS and structural formulae. (1) MMA, (2) methylchlorpyrifos (I.S.), and (3) malathion.

possible), so as to improve sensitivity. A non-resonant waveform (second ionisation) was selected for all the compounds. The excitation storage level and excitation amplitude were selected with the objective of generating spectra with the parent ion as their molecular peaks (between 10 and 20% of relative abundance). The MS–MS spectra obtained in the selected experimental conditions are shown in Fig. 2. The base peak was selected for all quantifications.

GG–MS–MS analysis of a working standard solution consisting of the target compounds, was performed and a library was created with the MS–MS spectra obtained in the selected experimental conditions. This acted as resource/reference library. Retention time windows (RTWs) defined as a retention time averages ± 3 standard deviations (SD) of retention times were 9.6–9.8 and 11.5–11.7 min for MMA and malathion, respectively. A target analyte was searched by its RTW and confirmed by comparison with the MS–MS spectra in the library. A positive confirmation required a minimum spectral fit of >700 and the signal-to-noise ratio (S/N) >3 (for quantification ion). For quantification S/N was set at >10.

3.2. Potential dermal exposure

3.2.1. Calibration curves and limits of detection and quantification

Due to the fact that expected concentration of malathion present in the coveralls and gloves is greater than the expected one in the biomonitoring analysis, a linear range was obtained between 500 and 1500 μg L^{-1}. This was made by preparing calibration curves with pure standards and using blank extracts of uncontaminated coveralls (30×30 cm^2 area) and cotton gloves for filling up to the volume and containing the internal standard (340 μg l^{-1}). The statistical data obtained are summarised in Table 3. The limits of detection (LOD) and quantification (LOQ) were calculated analysing ten clean coveralls and following the IUPAC recommendations [17]. The values obtained (Table 3) are in the ng l^{-1} level, a good indication of the sensitivity of MS–MS.

3.2.2. Recovery and precision of the extraction method

For recovery experiments, ten pieces of coverall and ten cotton gloves were spiked at concentration levels of 720 and 1440 μg L^{-1}. After the extraction

Table 3
Limits of detection (LOD) and quantification (LOQ) and calibration data

Compound	LOD (ng L^{-1})	LOQ (ng L^{-1})	*a*	*b*	r^2
MMA	0.01	0.03	3.09	−0.08	0.9988
malathion	0.07	0.24	0.67	−0.11	0.9831

a, Intercept; *b*, slope; r^2 correlative coefficient.

and analysis, recovery rates ranged between 90.2 and 103.2% and the precision (expressed as relative standard deviation) was lower than 9.1% in all cases (Table 4). The intermediate precision was calculated by analysing three pieces of coverall and gloves, which were spiked and analysed every 2 weeks for 4 months. Relative standard deviation of these measures was $<15\%$.

The whole body method shows a matrix effect in the quantification of the analyte due to the composition of the garment and to the presence of products which are different from the active ingredient in the commercial formulation of malathion. This effect is shown when recovery rates of malathion from garments are determined in the method validation. Recovery rates obtained when spiked samples are quantified with a calibration curve prepared in solvent, are lower than the ones obtained when a matrix-matching calibration is used (83.4 versus 90.2%, respectively). Thus matrix-matching 2 calibration was chosen for the quantification of malathion in personal protective equipment samples.

Finally, stability of samples was studied by spiking 36 pieces (30×30 cm^2) of each garment with 180 μg of malathion. Spiked samples were stored in darkness at 4°C for 4 months. A set of three samples of each sampling medium were extracted and analysed the first day, and each week for 1 month. The percentage recovery besides storage time calculated by comparing the amount recovered to the amount recovered on the first day after spiking, was found to be $>91\%$ in all cases during the period studied.

Table 4
Recoveries and relative standard deviations

Sample	Fortification level	Recovery, % (RSD, %)	
		MMA	Malathion
Coverall	720 μg L^{-1}	–	90.2 (8.5)
	1440 μg L^{-1}	–	91.7 (7.6)
Air	0.2 μg^{-3} m	–	93.2 (5.7)
	10 μg^{-3} m	–	94.1 (6.0)
Cotton gloves	720 μg L^{-1}	–	103.2 (6.9)
	1440 μg L^{-1}	–	98.4 (9.1)
Urine	40 μg L^{-1}	107.2 (6.6)	114.3 (15.2)
	200 μg L^{-1}	102.1 (6.1)	109.4 (12.3)

$n=10$.

3.3. Inhalation exposure

3.3.1. Calibration curves and limits of detection and quantification

Calibration curves were prepared by diluting pure standard solution using extract of uncontaminated PUF for filling up to the volume and containing 100 μg L^{-1} of I.S. The same linear range and similar LOD and LOQ were obtained for the air analysis method using the same procedure as in the dermal exposure method [17].

3.3.2. Recovery and precision of the extraction method

The trapping efficiency of granular sorbents such as Porapak R, Chromosorb 102, Supelpak, Amberlites XAD-2 and XAD-4 was evaluated in order to sample malathion in greenhouse air, using a system for generating pesticide standard atmospheres as described in previous papers [8,9]. Different solvents were also checked for the extraction of the pesticide. The influence of variables such as sampling volume, sampling time, sampling flow-rate (1 or 2 L min^{-1}), air relative humidity (dry–100% saturation), breakthrough (until 1 m^3), and concentration of saturation (until 0.4 mg of malathion), were studied in order to validate the air sampling method. Standard atmospheres were generated by using a chromatographic oven with a hollow glass column 0.5 cm diameter. The optimised temperatures were 150°C for the chromatographic oven and 200°C for the injector and detector. Twenty (20) min of passing air at a flow rate of 2 L min^{-1} were enough to achieve the vaporisation of the analyte, obtaining recovery rates from PUF plugs of 93.2% (5.7% RSD). Any influences of the air humidity on recovery rates obtained using dry or saturated air (93.2 and 94.1%, respectively) was not observed. The experiments were carried out connecting two PUF plugs in series and sampling standard atmospheres, for 30 min, at 10 mg/m^3 concentration level, and for 8 h, at 0.2 μg/m^3. They showed a high sorption capacity of the sorbents since the analyte was not detected in the second PUF plug in such conditions and similar recovery rates as the above were obtained in the first PUF plug.

Finally, the stability of samples was also estab-

lished for a month period, storing spiked PUF plugs at ambient temperature, 4 and −18°C, and checking recovery rates weekly (three replicates each). The samples were stored in darkness because recovery data resulted in the same range as explained above. It was observed that light affects PUF plugs, resulting in a decrease of recovery rates.

3.4. Actual exposure (biomonitoring)

3.4.1. Calibration curves and limits of detection and quantification

Calibration of the instrument was carried out using a clean urine sample fortified with each analyte in the range 1–500 μg L^{-1}. It is an adequate calibration range to the expected analyte concentrations in urine samples. The I.S. was used at 100 μg L^{-1}. Calibration graphs resulted similar to those obtained in the potential dermal exposure method (Table 3).

LOD and LOQ values were calculated by analysing ten control urine samples without malathion contamination (Table 3). The best results were obtained for MMA-hexafluoroisopropyl derivatives. This is due basically to the high sensitivity derived from hexafluoropropyl derivatives. The LOD and LOQ are low enough for monitoring exposure of pest control operators to malathion.

Worse results were found when the samples were analysed and confirmed by full scan mode (Fig. 3). This is due to the presence of interfering substances, which co-elute with the target analytes, and that are hardly removed by a background subtraction. The average spectral fit obtained when analysing ten clean urine samples spiked with 200 ng mL^{-1} of each compound, were 531 (MMA) and 397 (malathion), using full scan mode, and 882 (MMA) and 820 (malathion) using MS–MS mode. When MS–MS is used, if a co-eluted interfering has the same identification ion as the analyte, it can be avoided using special experimental conditions for the collision-induced dissociation and quantifying with a specific ion from the analyte.

3.4.2. Recovery and precision of the extraction method

On the basis of previous experiences, a SPE C_{18} cartridge was used for sample preparation, since it was found that eluents from the SPE cartridges were

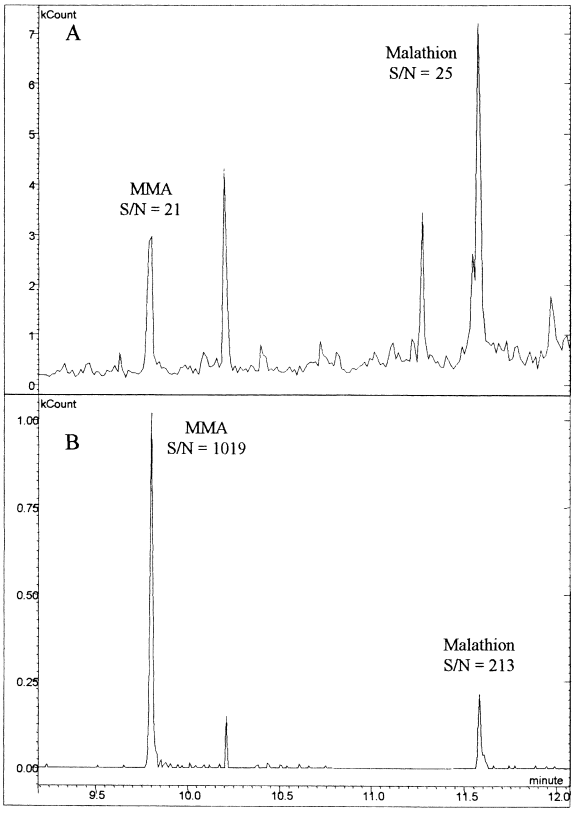

Fig. 3. Gas chromatogram of a urine containing 5 μg L^{-1} of each analyte. (A) Full scan mode, and (B) MS–MS mode.

better suited for direct GC analysis and that they seldom required clean-up prior to GC analysis. On the other hand, eluents from liquid–liquid extraction must involve a clean-up step to avoid reduction in column efficiency and contamination of injector and ion trap. After testing several elution solvents (acetone, *n*-hexane, diethyl ether, methanol, ethyl acetate, and dichloromethane), the maximum elution was found with 10 mL of diethyl ether.

To check the dependence of extraction efficiency on the initial concentration of analytes, ten urine samples were spiked with two different spiking levels (40 and 200 μg L^{-1}). The average recoveries obtained were between 102.1 and 114.3% in all the cases. The repeatability expressed as RSD was smaller than 15.2%. The results are summarised in Table 4.

3.4.3. Applications of the method

Potential dermal exposure to malathion was determined for the applicators that wore personal protective equipment, (operators number 2 and 3) and expressed as mL of spray tank deposited on the garment per hour of application. The total volume of spray liquid deposited on the suit of the operators and its distribution on the body was very similar for both applicators (72.3 and 73.0 mL h^{-1}, respectively) (Table 5).

Considering the distribution on the body, approximately 75% of total potential exposure was found on the lower body (thighs and lower legs) being both lower legs the most contaminated sections (approximately 19 mL h^{-1} each leg). The highest amount of pesticide on these regions is due to the operators who directed the spray-gun cutting the flow downwards and pointing to the legs when they passed from a row to other row. On the upper body (head, torso, back and arms) the amount found in both applicators was approximately 25% of the total, being left arm (4.8–5.1 mL h^{-1}, applicators 1 and 2, respectively) and right arm (4.3 and 3.9 mL h^{-1}, respectively) the most exposed areas.

The concentration of malathion in the breathing area during the application was 69.4 and 85.9 $\mu g/m^3$ for applicators 2 and 3, respectively.

Concerning biomonitoring results, the proposed method has been applied to the analysis of the urine of the three applicators. A number of samples ranging between 7 and 10 were collected from each worker in the 24 h after the applications. Malathion and MMA were not detected in the samples taken as blanks before the applications. Figs. 4 and 5 show the results obtained in the post-application samples. It can be seen that malathion was not detected but its metabolite MMA was present in most samples. In all cases, the highest concentration found in urine took place 5–8 h after spraying malathion (except applicator 3, 20 h after application). The total amount of excreted MMA was calculated considering the concentration found in each sample and the volume collected of them. The total amount excreted ranged between 133.75 and 671.42 μg. The highest amount determined corresponded to the urine of the applicator who did not wear a cotton coverall during the experiment. This fact reveals the important use of protection equipment (coverall) to spray malathion.

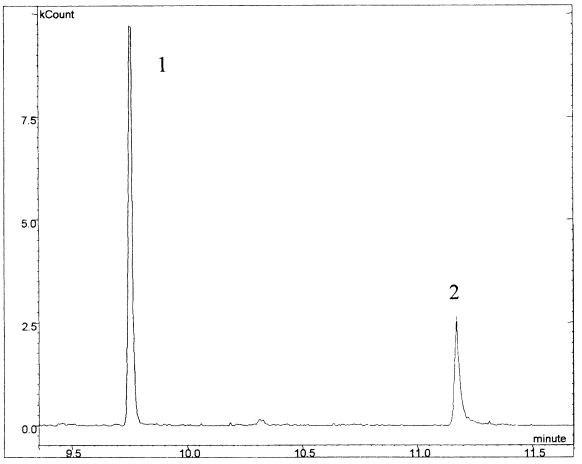

Fig. 4. Gas chromatogram of a urine sample containing MMA. (1) MMA; (2) I.S.

Table 5
Potential dermal exposure levels

Section	Description	mL h^{-1}	
		Applicator 2	Applicator 3
1	Head	0.523	0.545
2	Left arm	4.781	5.122
3	Right arm	4.276	3.873
4	Chest	3.834	3.946
5	Back	1.214	1.325
6	Upper front leg	11.140	10.834
7	Upper rear leg	2.642	2.863
8	Lower left leg	18.873	19.232
9	Lower right leg	19.041	18.958
10	Left glove	3.462	3.634
11	Right glove	2.483	2.665
Total	–	72.269	72.997

4. Conclusions

The method proposed for determination of malathion and MMA is a suitable method for the assay of malathion in human urine. The method is simple, reproducible and highly sensitive. The derivatisation process necessary, before gas chromatographic de-

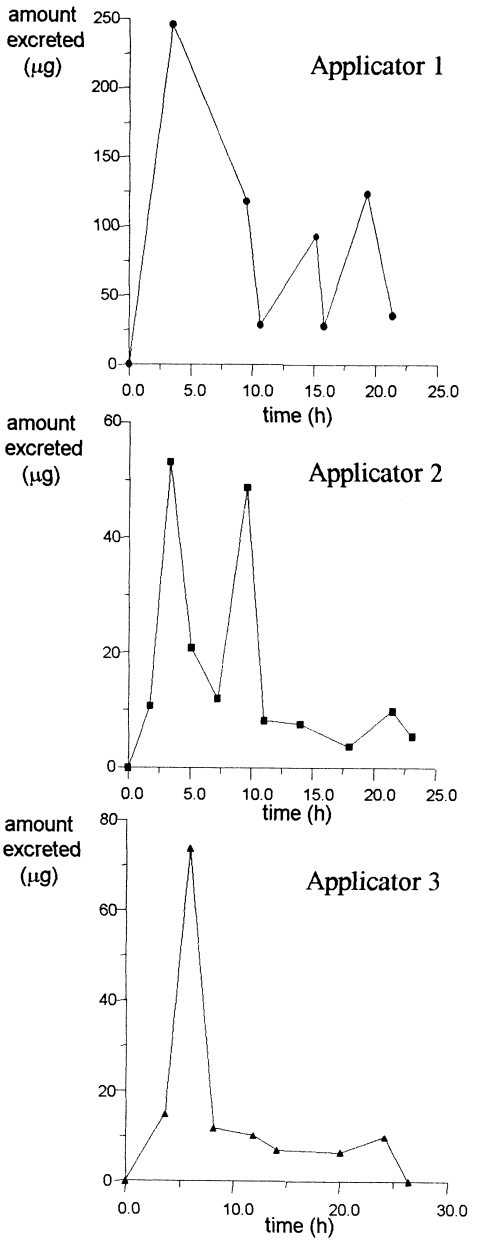

Fig. 5. Amount of MMA excreted in the urine after application of malathion.

termination, is fast, sensitive and clean and offers better results than other derivatisation reactions (methylation and trimethylsilylation). The use of MS–MS increases sensitivity and selectivity and permits the application of the method to the analysis of real samples with a low risk of obtaining a false positive. The methodology has been applied to the analysis of urine samples from three experiments carried out with volunteers who sprayed malathion in Spanish greenhouses. The analysis of the urine collected in the 24 h after application revealed the presence of the metabolite MMA in most of them. The maximum concentration of MMA in urine was reached 5–8 h after spraying pesticide.

In the same way the analytical methods developed by using whole body dosimetry and PUF plugs connected to personal samplers, were applied for assessing potential dermal exposure and inhalation exposure to malathion of two applicators. The validation of methods have been studied incorporating field and analytical quality control procedures. This is made to ensure the stability of samples during transport, storage and processing and the method performance for routine analysis. Potential dermal exposure of the applicators resulted in about 73 mL^{-1} h (including hands exposure) corresponding to 75% of the exposure to lower legs. The concentration of malathion in the air of the breathing area during the applications was 69.4 and 85.9 μg^{-3} m^3. Results showed compliance with the quality control criteria, such as, recovery rates, precision, absence of contamination and no evidence of sample decomposition during transport, storage and analysis of field blanks.

Acknowledgements

This research was supported by the European Union Project SMT4-CT96-2048 (DG-12-RSMT) and by the Comisión Interministerial de Ciencia y Tecnologia (CICYT) Project No. AMB97-1194-CE.

References

[1] Commission of the European Communities, Council Directive 91/414/EEC, Off. J. Eur. Communities No. L230, Luxembourg, 19 August 1991.
[2] G. Chester, in: P.B. Curry (Ed.), Methods of Pesticide Exposure Assessment, Plenum Press, New York, 1995, p. 179.
[3] R.A. Fenske, Ann. Occup. Hyg. 37 (1993) 687.

[4] J.J. Van Hemmen, D.H. Brouwer, Sci. Total Environ. 168 (1995) 131.
[5] OECD, Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application, Series on Testing and Assessment No. 9, OECD/GD, 1997.
[6] M.L. Castro Cano, J.L. Martinez Vidal, F. Egea González, M. Martinez Galera, M. Cruz Márquez, Anal. Chim. Acta 423 (2000) 127.
[7] J.L. Martinez Vidal, C.R. Glass, F.J. Egea González, J.J. Mathers, M.L. Castro Cano, Proceedings of 9th International Congress on Pesticide Chemistry, Royal Society of Chemistry and the IUPAC, London, 1998.
[8] J.L. Martinez Vidal, F.J. Egea González, C.R. Glass, M. Martinez Galera, M.L. Castro Cano, J. Chromatogr. A 765 (1997) 99.
[9] F.J. Egea Gonzalez, J.L. Martinez Vidal, M.L. Castro Cano, M. Martinez Galera, J. Chromatogr. A 829 (1998) 251.
[10] K. Doichuanngam, R.A. Thornhill, Comp. Biochem. Physiol. 101C (1992) 583.
[11] PSD, Pesticide Safety Directorate, Evaluation No. 135, UK MAFF, 1995.
[12] J.L. Martinez Vidal, F.J. Arrebola, A. Fernández-Gutiérrez, M.A. Rams, J. Chromatogr. B 719 (1998) 71.
[13] F.J. Arrebola, J.L. Martinez Vidal, A. Fernández-Gutiérrez, Toxicol. Lett. 107 (1999) 15.
[14] F.J. Arrebola, J.L. Martinez Vidal, A. Fernández-Gutiérrez, M.H. Akhtar, Anal. Chim. Acta 401 (1999) 45.
[15] F.J. Arrebola, J.L. Martinez Vidal, A. Fernández-Gutiérrez, J. Chromatogr. Sci. 39 (2001) 177.
[16] J. Glastrup, J. Chromatogr. A 827 (1998) 133.
[17] A. González Casado, L. Cuadros Rodriguez, E. Alonso Hernández, J.L. Vilchez, J. Chromatogr. A 726 (1996) 133.