Determination of Malathion Urinary Metabolites by Isotope Dilution Ion Trap GC/MS

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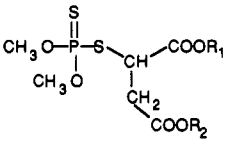
Human exposures to malathion insecticide are monitored by determination of malathion diacid (MDA) in urine using an isotope dilution GC/MS method. The procedure relies on solid-phase extraction with strong anion exchange cartridges to isolate urinary acids. Samples are adjusted to pH 8 and spiked with deuterium-labeled MDA prior to extraction, and acids eluted from the resin are methylated with diazomethane. Use of isotope dilution quantitation improves method precision (relative to conventional external standard quantitation), resulting in a 3-fold reduction in the method detection limit (MDL). Method accuracy also is improved as biases associated with extraction and derivatization are minimized. The quadrupole ion trap GC/MS detects as little as 5 pg of each metabolite in the mass scanning mode and affords an MDL of 6.5 ng of MDA/mL in human urine. The new isotope dilution method was used to monitor malathion exposures in date plantation workers.

INTRODUCTION

The analysis of urinary residues is an important tool for both epidemiologic and toxicologic studies of pesticide exposure in man. Biomonitoring also has the potential for use in enforcing occupational exposure standards. Metabolite determination is required for biodegradable compounds including the organophosphorus (OP) insecticides, which are rapidly hydrolyzed before excretion in the kidney. Biomonitoring exposures to malathion insecticide, [(dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester (Figure 1), has been accomplished by determination of dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP) (Shafik et al., 1969, 1971, 1973), or the carboxylesterase metabolites, malathion α-monoacid (α-MMA) and malathion diacid (MDA) (Figure 1) (Bradway and Shafik, 1977). The carboxylesterase-derived markers are the major metabolites and are malathion-specific, unlike the alkyl phosphates which are metabolites of a number of common OP insecticides.

Determination of urinary OP metabolites is more reliable for characterizing exposures than other approaches. Blood cholinesterase monitoring (Kay et al., 1952) is limited by low sensitivity and a sizable range in "normal" values (Shafik et al., 1971). Patch techniques, in which dermal exposure is extrapolated from residues on attached cloth pads, is poorly correlated to internal dose (Lavy et al., 1980; Franklin et al., 1981; Draper and Street, 1982; Wojeck et al., 1981; Franklin, 1984; Grover et al., 1986). In contrast, urinary metabolites correlate well with insecticide dose in both experimental animals (Shafik et al., 1973) and man (Morgan et al., 1977).

Various analytical methods for determination of malathion's urinary metabolites have been reported. Shafik and co-workers pioneered methods for determination of DMDTP, DMTP, and other alkyl phosphates in human blood and urine. These procedures are based on solvent extraction, alkylation with diazoalkane reagents, and gas chromatography using a flame photometric detector (FPD). The Shafik procedure is widely used but is reported to have variable extraction and derivatization efficiencies (Ito et al., 1979) and a major background interference due to endogenous inorganic phosphate (Moody et al., 1985). Moreover, the thiophosphate metabolites undergo variable thiolo–thiono rearrangement on derivatization with diazoalkanes (Shafik et al., 1970). Through derivatization of DMTP directly on the analytical GC column with trimethylammonium hydroxide, some of these problems have been avoided (Moody et al., 1985).



Malathion	$R_1, R_2 = C_2H_5$
Malathion Diacid	$R_1, R_2 = H$
α Malathion Monoacid	$R_1 = H, R_2 = C_2H_5$
β Malathion Monoacid	$R_1 = C_2H_5, R_2 = H$

Figure 1. Structures of malathion and its carboxylesterase-derived metabolites.

As metabolic pathways for malathion include oxidation to malaoxon [rapidly hydrolyzed to dimethyl phosphate (DMP)] and carboxylester hydrolysis, alkyl phosphate determination alone may significantly underestimate the internal malathion dose. The total malathion residue can be approximated by hydrolysis of the two main metabolites, MDA and α-MMA, to DMTP and DMDTP (Fenske, 1988; Fenske and Leffingwell, 1989), which have been determined after derivatization with pentafluorobenzyl bromide (Reid and Watts, 1981). To distinguish malathion exposure from that of other OP insecticides, however, it is essential to determine the carboxylesterase metabolites directly.

Lores and Bradway (1977) first used ion-exchange resins to isolate alkyl phosphates from urine. More recently Muan and Skare (1989) described an anion-exchange, solid-phase extraction procedure for malathion metabolites including MDA, α-MMA, DMDTP, DMTP, and demethylmalathion. After derivatization with diazomethane, these metabolites are quantified by GC with a nitrogen–phosphorus detector (NPD). Muan and Skare report detection limits of 2 μg/mL for each analyte while confirmation by quadrupole GC/MS was 100–1000-fold less sensitive.

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This paper describes modifications in the Muan and Skare procedure which allow detection limits in the low nanograms per milliliter (parts per billion) range for MDA, sensitivity necessary to detect low-level dietary and extraneous exposures. The new procedure relies on isotope dilution GC/MS quantitation with a quadrupole ion trap instrument. This technique is as sensitive as those based on GC with element-specific detectors, and the full-scan spectra provide simultaneous residue confirmation. The method has been used to study low-level malathion exposures in the Mediterranean fruit fly eradication program in southern California and to examine much higher exposures in agricultural workers applying the insecticide in date production as described here.

EXPERIMENTAL PROCEDURES

Chemicals. Malathion dicarboxylic acid was prepared in a three-step sequence beginning with the reaction of phosphorus pentasulfide and methanol. Intermediate *O,O*-dimethylphosphorodithioic acid was then reacted with maleic anhydride and the adduct hydrolyzed to obtain the desired product.

O,O-Dimethylphosphorodithioic Acid. Phosphorus pentasulfide (10 g, 45 mmol) was added to 11 mL of dry benzene, and the mixture was stirred at 40 °C. Absolute methanol (8.2 mL, 200 mmol) was added dropwise to the stirred slurry over 1 h. The reaction mixture was filtered, and the solvent was removed by rotary flash evaporator to obtain a colorless liquid (crude yield, 12.5 g, 79 mmol, 88%). This compound was used without further purification as an intermediate in the preparation of *O,O*-dimethyl *S*-(1,2-dicarboxyethyl)phosphorodithioate.

O,O-Dimethyl S-(1,2-Dicarboxyethyl) Phosphorodithioate (MDA). Maleic anhydride (2.1 g, 21.4 mmol) and *O,O*-dimethylphosphorodithioic acid (3.4 g, 21.5 mmol) were heated at 80 °C for 15 h. Thereafter, 1 mL of water was added to the pink liquid product, and this mixture was heated at 80 °C for an additional 1 h. The mixture was cooled, and excess water was removed by adding benzene and azeotroping under reduced pressure. The solid product isolated after removal of water was recrystallized from chloroform to obtain white crystals: mp 125–7 °C (lit. 127–9 °C; Wolfe et al., 1975); ^{1}H NMR ($CDCl_3$) δ 4.20 (ddd, J = 13.8, 9.2, and 9.2 Hz, 1 H), 3.85 (d, J = 2.0 Hz, 3 H), 3.81 (d, J = 2.0 Hz, 3 H), 3.08 (d, J = 9.2 Hz, 1 H), 3.05 (d, J = 4.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 176.7, 176.6, 54.6, 44.8, 37.9; FAB ms (rel intensity) m/z 549 (11, $[2M + H]^+$), 275 (100, MH^+), 159 (20), 143 (51), 125 (22, $[(CH_3O)_2PS]^+$); negative FAB ms (rel intensity) m/z 547 (41, $[2M - H]^-$), 273 (100, $[M - H]^-$), 157 (97, $[(CH_3O)_2P(S)_2]^-$), 141 (40).

d_7-O,O-Dimethylphosphorodithioic acid was prepared exactly according to the procedure described for *O,O*-dimethylphosphorodithioic acid except that d_4-methanol (8.2 mL) was substituted for methanol.

d_6-O,O-Dimethyl S-(1,2-dicarboxy-2-d-ethyl) phosphorodithioate (d_7-MDA) was prepared exactly according to the procedure described for *O,O*-dimethyl *S*-(1,2-dicarboxyethyl) phosphorodithioate except that d_7-*O,O*-dimethylphosphorodithioic acid was used in place of the unlabeled reactant: mp 124–6 °C; ^{1}H NMR ($CDCl_3$) δ 4.20 (multiplet, 1 H), 3.06 (multiplet, 1 H); FAB ms m/z 563 (7, $[2M + H]^+$), 282 (99, MH^+), 165 (31), 149 (100), 131 (44, $[(CD_3O)_2PS]^+$); negative FAB ms (rel intensity) m/z 561 (18, $[2M - H]^-$), 280 (90, $[M - H]^-$), 163 (100, $[(CD_3O)_2P(S)_2]^-$), 147 (43).

Analytical Standards. Samples of MDA and *O,O*-dimethyl *S*-(1-carboxy-2-carbethoxyethyl) phosphorodithioate (malathion monoacid, α isomer) (α-MMA) and *O,O*-dimethyl *S*-(1-carboxy-2-carbethoxyethyl) phosphorodithioate (malathion monoacid, β isomer) (β-MMA) were donated by American Cyanamid Co. (Princeton, NJ). The standard provided for the α- and β-monoacids was a 1:5.7 (w/w) isomer mixture, respectively.

Strong anion exchange (SAX) solid-phase extraction cartridges (500 mg, 2.8 mL, catalog no. 1210-2044) were obtained from Analytichem International (Harbor City, CA). Ethereal diazomethane was prepared by alkaline hydrolysis of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald, Aldrich Chemical Co.) in a commercially available distillation apparatus specifically designed for this purpose. The reagent was stored at −5 °C in a vial with a Teflon-lined, plastic screw-top closure. Caution: Due to the volatility and toxicity of diazomethane, a high-draft hood, gloves, and other protective clothing should be used. Concentrated diazomethane solutions may be explosive, and therefore special safety precautions (detailed in the Diazald product literature) must be followed in preparation and storage of the reagent.

Gas Chromatography/Mass Spectrometry. MDA was quantitated by using a Finnigan ITS40 (San Jose, CA) quadrupole ion trap instrument coupled to a Varian 3400 (Walnut Creek, CA) chromatograph. The GC was fitted with a Model 1075 split/splitless inlet and a 30 m × 0.25 mm (0.25-μm film) J&W DB-5 (Rancho Cordova, CA) column for routine use. The GC operating conditions were as follows: inlet temperature, 180 °C; splitless injection mode, 1.0 min purge off time; split vent flow, 60 mL/min; oven temperature program, 60 °C for 3 min, 60–275 °C at 7 °C/min, 275 °C for 10 min; He carrier gas head pressure, 10.5 psi. Under these conditions typical retention times (t_R) were 24.1 min for d_7-MDA, 24.2 min for MDA, 25.1 min for β-MMA, and 25.2 min for α-MMA. When identical operating conditions and a 30 m × 0.32 mm (0.25-μm film) J&W DB-1 column were used, the retention times were 20.17, 20.23, and 21.1 min for d_7-MDA, MDA, and β-MMA, respectively.

The mass spectrometer scanned from 50 to 400 amu each second with a minimum peak threshold setting and automated ionization control. Both transfer line and manifold temperature were 275 °C, and the multiplier voltage was 1450 V. The instrument was tuned once at the beginning of the study by using the automated tuning program with perfluorotributylamine (PFTBA) on the m/z 69, 131, 219, 264, 414, 502, and 614 ions.

Urine Extraction. The solid-phase extraction procedure developed by Muan and Skare (1989) was modified for trace analysis of malathion residues by isotope dilution GC/MS analysis. A 25-mL urine sample is spiked with 50 μL of 100 ng/μL d_7-MDA in methanol. The sample is adjusted to pH 8.0 by dropwise addition of 0.5 N sodium hydroxide with pH confirmation by test paper. A SAX column is washed with 2 mL of methanol followed by 2 mL of deionized water. Urine (5-mL aliquot) is passed through the column by applying slight pressure. The eluent and a 2-mL distilled water wash are discarded. The metabolites are recovered by elution with 1 mL of 2 N methanolic HCl. The eluate is reduced to ~0.5 mL under a stream of nitrogen, combined with 1 mL of acetone, and agitated with a vortex mixer. The sample is methylated by adding ethereal diazomethane with intermittent vortex agitation until the yellow color of diazomethane persists. After standing 10 min at room temperature, the sample was reduced to 0.5 mL in a nitrogen evaporator and the sample extracted with 4 mL of cyclohexane–acetone (3:1 v/v) by agitating in a vortex mixer for 2 min. The organic layer is reduced just to dryness and redissolved in 1.00 mL of cyclohexane–acetone for a 2.0-μL manual injection volume.

Isotope Dilution Calibration. Quantitation was accomplished by use of summed areas for three quantitation ions (Q ions) for each analyte: d_7-MDA (m/z 99, 131, 164); MDA (m/z 93, 125, 158); α-MMA and β-MMA (m/z 93, 125, 159). Reconstructed ion current chromatograms for each of the seven Q ions were plotted individually to confirm both the coelution of diagnostic fragment ions and relative abundances. The concentration of each metabolite was determined from the ratio of the metabolite area to that of the internal standard (IS), $\text{area}_{\text{metabolite ions}}$:$\text{area}_{\text{IS ions}}$.

To evaluate the mass spectrometer linearity, the deuterated IS and malathion metabolites were combined in ratios between 0.020:1 and 50:1 ([metabolite]:[IS]). The compounds (25 mg) were derivatized with ethereal diazomethane and a 1000 μg/mL stock solution was prepared for each. The stock solutions were diluted in cyclohexane–acetone (4:1 v/v) prior to GC/MS analysis. For daily, continuing calibration a single midrange standard was chromatographed to monitor retention times and detector response factors.

Calculations. Equation 1 uses MDA as an example of data manipulation for determination of metabolite concentrations in urine using the labeled IS

$$AR_{sample}AR_{std}^{-1}[IS]_{std}[MDA]_{std}^{-1}[IS]_{sample} = [MDA]_{sample} \quad (1)$$

where AR_{sample} is the sum of MDA Q ions_{sample}/sum of IS Q ions_{sample} and AR_{std} is the sum of MDA Q ions_{std}/sum of IS Q

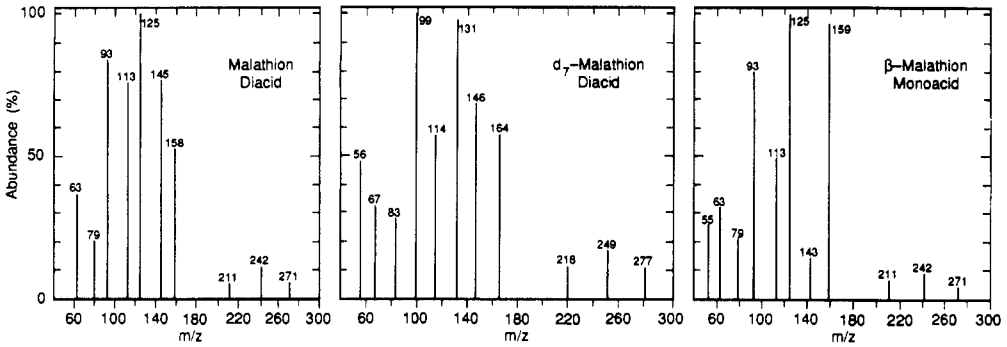

Figure 2. Electron impact mass spectra of methylated MDA, d_7-MDA, and β-MMA.

$ions_{std}$.

For comparative purposes quantitation was carried out by using external standards according to

$$area_{sample}[MDA]_{std}area_{std}^{-1}EV \times SV^{-1} = [MDA]_{sample} \quad (2)$$

where EV is the final extract volume (mL) and SV is the urine sample volume (mL).

RESULTS AND DISCUSSION

Mass Spectrometry of Malathion Metabolites. The electron impact spectrum of dimethyl MDA shows characteristic dimethyl dithiophosphate moiety ions: m/z 93 [98%, $[(CH_3O)_2P]^+$], 125 [100%, $[(CH_3O)_2PS]^+$], and 158 [44%, $[(CH_3O)_2P(S)SH]^+$]. Additional ions of the dicarboxyethyl side chain of MDA include m/z 113 [76%, $[CH_3OC(O)CH{=}CHCO]^+$) and 145 (61%, $[CH_3OC(O)CHCH_2COOCH_3]^+$ or $[CH_3OC(O)CH_2C(S)CO]^+$ or $[CH_3OC(O)CH{=}C(SH)CO]^+$]. In the methylated α- and β-MMA analogues the dimethyl dithiophosphate fragment ions remain abundant; i.e., m/z 93 and 125 are both greater than 95% and the m/z 158 ion is 40–42% of the base peak. The intensity of the m/z 113 acylium ion is somewhat lower, ca. 50–60%, and a second acylium ion is present of mass m/z 127 [30–37%, $[CH_3CH_2OC(O)CH{=}CHCO]^+$]. The m/z 145 ion of dimethyl MDA is replaced by an abundant m/z 159 ion [85–89%, $[CH_3CH_2OC(O)CHCH_2COOCH_3]^+$ (β) or $[CH_3CH_2OC(O)CH_2CHC(O)OCH_3]^+$ (α)]. None of the malathion metabolites exhibited major ions (>10%) of mass greater than m/z 159, although minor $[M - CH_3O]^+$ and $[M - HCOOCH_3]^+$ ions were present. EI mass spectra are shown in Figure 2.

Methane chemical ionization (CI) was investigated but not used routinely in the analysis of these compounds because of reduced sensitivity relative to EI. In methane CI the dimethyl dithiophosphate moiety ions were much lower in intensity and ions from the dicarboxyethyl side chain became prominent. $[M + C_2H_5]^+$, but not MH^+, ions were also present in the methane CI spectra.

After a period of extensive use of the instrument, progressive changes were observed in the quadrupole ion trap EI spectra. The spectra of the diacid dimethyl esters began to show increased abundances of some fragment ions including $[M - CH_3OH]^+$, $[M - CH_3O]^+$, and $[M - COOCH_3]^+$ as well as minor MH^+ signals. Two additional fragment ions, $[M - C_2H_5O]^+$ and $[M - C_2H_5OH]^+$, were present in the methylated β-MMA spectrum. Changes in the EI spectra may be associated with progressive fouling of the ion trap, resulting in chemical—and possibly surface-induced—dissociation ionization. The enhanced fragment ions, which became as abundant as 60–70% of the major fragment ions described above, did not affect the calibration curves, isotope dilution response factors, quantitative performance, or sensitivity of the instrument.

Preparation of Labeled Internal Standards. Various options were considered in labeling the MDA IS. The synthetic route to MDA involves reaction of P_2S_5 with methanol to give DMDTP, which is reacted with maleic anhydride—hydrolysis of the DMDTP–anhydride adduct gives the desired product. $^{13}C_2$-Labeled MDA could be prepared by reaction of P_2S_5 with $^{13}CH_3OH$; however, this approach was not used because the 2 amu mass difference provides inadequate separation of the isotope clusters. Alternatively, treatment of MDA with deuterium-labeled diazomethane (in deuterated solvent) was considered a means to obtain di-d_3-methyl MDA with a 6 amu mass difference. The carboxylate methyl ester labeled IS, however, would only be useful for quantitation (e.g., as a syringe spike) and would not correct for errors related to extraction or derivatization.

Deuterium labeling of the phosphate methyl groups of MDA was selected and was accomplished by reacting P_2S_5 with CD_3OD. $(CD_3O)_2P(S)SD$ adds to the activated double bond of maleic anhydride to give d_7-MDA (on hydrolysis). One deuterium atom is incorporated in the dicarboxyethyl side chain and six deuteriums are attached to the phosphate methyl groups. Alternative labeling using CD_3OH would yield d_6-MDA with no deuterium in the dicarboxyethyl side chain.

Structures of native and labeled malathion diacid were confirmed by melting points, high-field ^1H and ^{13}C NMR, and FAB mass spectrometry. Protonated molecule ions (MH^+) were detected as intense signals (90–100% rel intensity) in positive ion mode, while the corresponding deprotonated molecule ions ($[M - H]^-$) were detected in negative ion FAB. Other FAB mass spectral features included dimer ions (e.g., $[2M + H]^+$ and $[2M - H]^-$) and abundant dimethyl phosphorodithioate anion fragments, $[(CH_3O)_2P(S)_2]^-$ and $[(CD_3O)_2P(S)_2]^-$, from dissociative e^- capture ionization.

d_7-MDA and MDA were resolved almost to baseline by the 30-m DB-5 capillary column with differences in retention time of 0.1 min (Figure 3). Unexpectedly, the higher molecular weight IS eluted prior to the native compound. The GC mass spectrum of methylated d_7-MDA (Figure 2) shows the dimethyl dithiophosphate fragment ions displaced 6 mass units, i.e., m/z 99, 131, and 164. The fragment ions of the dicarbomethoxyethyl side chain, m/z 113 and 145, are both 1 amu higher due to incorporation of deuterium. Spectra obtained with the ion trap mass spectrometer were highly reproducible over a 500-fold range of analyte concentrations. For example, between 0.2 and 100 ng the ratio of MDA Q ions m/z 93:125:158 was 4.0 ± 0.17:4.1 ± 0.18:1.8 ± 0.22, respectively. The relative intensity of individual Q ions also was repeatable from day to day, even in complex biological samples.

Detector Calibration and Linearity. The ion trap MS responded linearly to each of the malathion metabolites (Table I) with a linear dynamic range of at least 3 decades for either total ion current or the sum of Q ions.

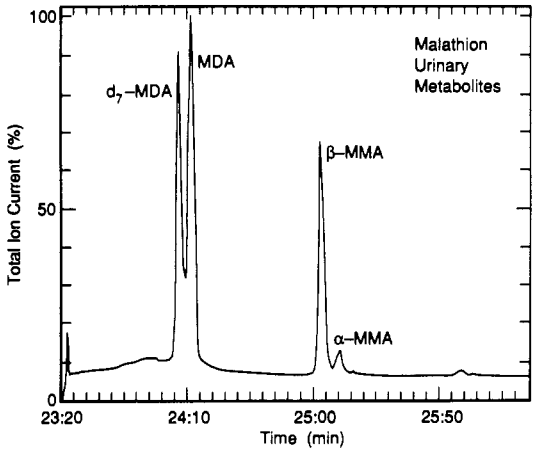

Figure 3. Total ion current chromatogram of malathion urinary metabolites and d_7-MDA internal standard.

Table I. Multipoint Detector Calibration for Malathion Metabolites and Deuterated Internal Standard[a]

metabolite concn, ng/μL	peak area × 10^{-3} (sum of Q ions)		
	MDA	β-MMA	d_7-MDA
0.020	0.55	0.21	22
0.050	1.8	0.63	30
0.10	4.3	2.1	27
0.25	13	7.1	34
0.50	33	24	53
1.0	71	52	52
10	560	550	40
50	3000	3300	58

[a] Each sample contained 1.00 ng/μL of the internal standard, and injection volumes were 2.0 μL.

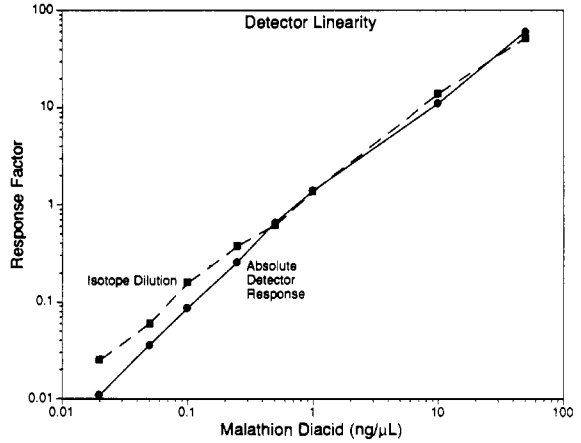

Figure 4. MS detector response curves for MDA. Absolute detector response (sum of Q ions/50) (●) and detector response normalized to the internal standard (sum of MDA Q ions/sum of IS Q ions) (■) are plotted.

Use of the IS did not improve the detector linearity (Figure 4) but markedly improved day-to-day repeatability of response factors (see below). For example, linear correlation coefficients for either MDA or β-MMA calibration curves were 0.999—when the areas were normalized to the deuterated IS, correlation coefficients were about the same, 0.997 and 0.998 for MDA and β-MMA, respectively.

Over an extended time period the mass spectrometer response to MDA and the labeled IS was tracked as part of normal continuing instrument calibration. A midrange standard containing 1.0 ng each of MDA and d_7-MDA/μL was injected manually each day of operation for 1 month. The response of MDA ranged from 8000 to 51 000 counts (sum of Q ions) with a mean of 32 000 counts and a 38% relative standard deviation (RSD). This day-to-day drift in GC/MS response is typical of both quadrupole and

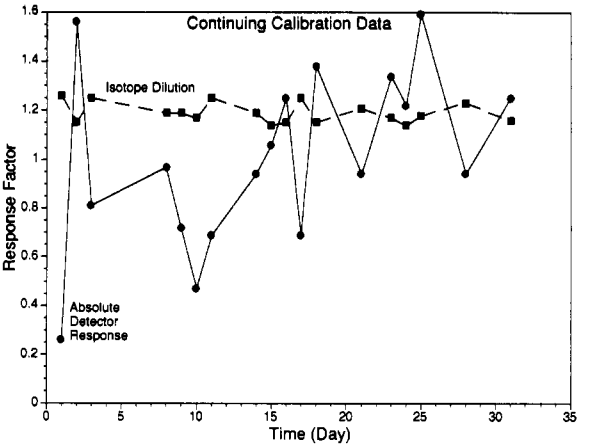

Figure 5. Continuing MS detector calibration data for MDA. Isotope dilution response factors (d_7-MDA:MDA) (■) and absolute response factors (●) are plotted.

quadrupole ion trap instruments and is attributable to numerous variables affecting chromatography, analyte ionization, and ion detection. All of the data from the ion trap mass spectrometer were accumulated after a single tuning with PFTBA, while conventional quadrupole instruments are typically tuned daily or weekly. Some of the variability was due to sampling error associated with manual splitless injection of 2.0-μL volumes; i.e., two consecutive standard analyses had a relative percent difference (RPD) of 7%. Drift in the detector response factor for d_7-MDA is shown in Figure 5.

Use of the IS significantly improved the repeatability of response factors. The d_7-MDA:MDA response factor ratio ranged from 1.14 to 1.26 with a mean of 1.19 and an RSD of only 3.5%. The high stability of isotope dilution detector response factors relative to absolute response factors is seen in Figure 5.

Recoveries of Malathion Metabolites and Method Detection Limits. To estimate method sensitivity, a method detection limit (MDL) study was carried out. A pooled urine sample was spiked with 25 ng/mL MDA and α-MMA and 140 ng/mL β-MMA. The disproportionately high level of β-MMA resulted as only a mixed standard of α- and β-MMA was available. The entire analysis of malathion metabolites was replicated seven times to determine measurement precision and thus MDLs.

The pooled urine sample studied contained trace malathion residues before spiking with less than 2000 area counts for both the MDA and β-MMA metabolites corresponding to less than 10 ng/mL each (Table II). Recoveries were approximately quantitative for MDA, but for either of the monoacid residues recoveries were in the range 55–60% (Table III). Because of the low and variable recoveries of the monoacid metabolites and the inavailability of a labeled analogue for use as an IS, they were not routinely quantitated.

The MDL is the smallest quantity of analyte that can be reliably detected in a given matrix. MDLs are proportional to measurement variability or imprecision and represent that amount of analyte which is significantly different from zero based on statistical probability. The dispersion of results reflects accumulated random errors associated with volume determinations, resin extraction and elution efficiency, diazomethane derivatization, and manual sample injection (Laitinen and Harris, 1975). Isotope dilution quantitation not only improves the repeatability of MS response factors but also improves measurement precision, resulting in reduced method detection limits. In this study normalization of analyte responses to d_7-MDA reduced random experimental errors

Table II. Comparison of Internal and External Standard Calculations for Recovery of Malathion Metabolites from Human Urine

metabolite	metabolites, ng/mL: unspiked sample	fortified urine 1	2	3	4	5	6	7
MDA[a]	7.3	36	33	36	33	31	31	34
MDA (-bkgd)[a,b]		29	26	29	26	24	24	27
MDA[c]	7.0	37	26	41	41	36	41	27
MDA (-bkgd)[b,c]		30	19	34	34	29	34	20
α-MMA[a]	ND	13	15	13	13	14	16	16
α-MMA[c]	ND	13	13	14	16	17	20	12
β-MMA[a]	5.5	88	82	80	88	78	84	78
β-MMA (-bkgd)[a,b]		83	77	75	83	73	79	73
β-MMA[c]	5.2	90	64	90	107	92	108	60
β-MMA (-bkgd)[b,c]		85	59	85	102	87	103	55

[a] Calculations based on isotope dilution quantitation with d_7-MDA IS. [b] bkgd indicates that the existing or background residue has been subtracted. [c] Quantitation based on external standard calibration.

Table III. Comparison of Internal and External Standards Method: Method Detection Limits and Recoveries for Malathion Metabolites in Human Urine

compd	IS	spike level ng/mL	found, ng/mL (± SD)	recovery, %	MDL
MDA	+	25	26.4 ± 2.1	106	6.5
MDA	-	25	28.6 ± 6.5	114	20
α-MMA	+	25	14.3 ± 1.4	57	4.4
α-MMA	-	25	15.0 ± 2.8	60	8.8
β-MMA	+	140	77.6 ± 4.3	55	14
β-MMA	-	140	82.3 ± 19	58	59

resulting in a 2–4-fold reduction in method detection limits (Table III). The detection limit for MDA was lowered from 20 to 6.5 ng/mL.

Ruggedness of the Isotope Dilution Procedure. While MDL studies demonstrate the precision attainable with a method under highly controlled experimental conditions, they do not provide information on the repeatability of a method in practical use. Such information can be obtained from routine quality assurance data. In a 2-month interval during which 80 epidemiology study samples were analyzed, a sizable quality control data base was accumulated. Nine distilled water blanks were analyzed—seven contained no detectable MDA and two were positive with 1.5 and 2.9 ng of MDA/mL. Matrix spike and matrix spike duplicate (n = 16 total) samples fortified with 25 ng of MDA/mL revealed average repeatabilities of 15% RPD. The overall recovery of MDA averaged 120% when the isotope dilution GC/MS method was used.

Malathion Metabolite Levels in Date Plantation Workers. Malathion is used extensively for insect control in date production. The insecticide is applied as a dust usually with sulfur to wrapped date clusters; workers are exposed during both application and harvest. The practical utility of the isotope dilution GC/MS method was evaluated by monitoring applicators and harvesters working in date production. Urine samples also were analyzed by an independent reference laboratory using a modification of the Bradway and Shafik method. The Bradway and Shafik procedure relies on solvent extraction, derivatization, adsorption column cleanup, and GC/FPD—the reference laboratory method utilized solid-phase extraction with phenyl reversed-phase cartridges, derivatization, and quantitation by GC/FPD on an instrument equipped with an 0.53-mm capillary column.

Urine samples from agricultural workers harvesting dates contained 4.3–22 μg/mL (parts per million) of malathion diacid 1 h after the work shift (Table IV). Malathion is known to be rapidly metabolized and cleared from the body, and in this study urinary MDA appeared to have dropped by more than 2 orders of magnitude within 72 h of terminating exposure. In a related study of 11 exposed date plantation workers the half-life for urinary MDA averaged 9 h (M. Miller, 1991, personal communication). Malathion residues also were detected in urine of pesticide applicators (Table IV), although the exposures and internal doses cannot be compared with this limited data set because of differences in sampling time. Creatinine levels were relatively high in some of the urines, particularly sample AP6, where the highest malathion residue was measured. High urinary creatinine indicates concentrated urine and low urine output.

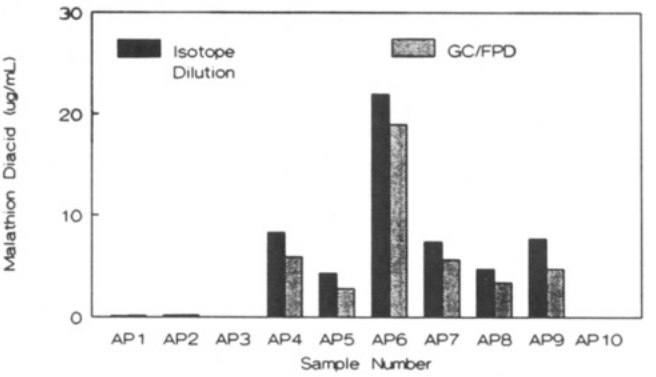

Figure 6. Urinary malathion diacid residues in date plantation workers. Results by isotope dilution GC/MS (solid bars) are compared to GC/FPD results (cross-hatched bars).

Table IV. Malathion Diacid Levels in Urine Samples from Date Plantation Workers[a]

sample	malathion diacid, ng/mL: isotope dilution GC/MS	GC/FPD	urine vol, mL	creatinine, mg/dL	RPD, %
AP1	120	130	186	183	5.0
AP2	190	180	104	193	3.8
AP3	12	ND[b]	97	284	
AP4	8300	5900	57	124	34
AP5	4300	2800	107	251	43
AP6	22000	19000	22	515	18
AP7	7400	5600	43	226	27
AP8	4700	3400	150	108	30
AP9	7700	4700	39	227	49
AP10	<MDL[c]	5	144	90	

[a] AP1 and AP2 were malathion applicators who had not been exposed for 30 h; AP3 was a date harvester who had not been exposed for 72 h; AP4–AP9 were 1 h postshift urines from harvesters; AP10 was from a control subject not known to be exposed to malathion. The isotope dilution GC/MS analyses were corrected for the typical laboratory recovery of 120%. [b] Not detected with an approximate GC/FPD detection limit of 5 ng/mL. [c] Below the 6.5 ng/mL MDL.

This limited data set cannot be used to infer typical malathion exposure levels for date plantation workers. A subsequent set of 23 urine samples from these workers had MDA levels between 0.092 and 6.9 μg/mL. A more thorough study of exposure levels and the relationship between worker practices and exposure is in progress.

Isotope dilution GC/MS data for date plantation workers were in good agreement with the GC/FPD method for samples with 0.1–0.2 μg of MDA/mL (Table IV). In this range RPDs of 3.8 and 5% were observed. For samples containing high MDA levels, e.g., 2–25 μg/mL, intermethod differences were between 18 and 49% RPD. MDA concentrations determined by GC/FPD were 16–39% lower than those found with the isotope dilution GC/MS procedure after correction for recovery. While these data do not represent unacceptable differences for trace-level determinations in complex samples, especially when two different laboratories and methods are compared, the

findings suggest that there are systematic differences between the methods in the parts per million range.

As with any GC/MS procedure the isotope dilution determination of malathion diacid requires the coelution of each diagnostic Q ion for qualitative confirmation. Two epidemiology study samples estimated to contain 18–19 ng of MDA/mL by GC/FPD exhibited only two of the three Q ions on GC/MS analysis. On reanalysis, however, all three Q ions were detected. While m/z 93 is less specific than the other Q ions, the simultaneous coelution of any two Q ions in capillary GC chromatograms is very strong evidence that the analyte is present. Therefore, at concentrations near the MDL the least abundant quantitation ion, m/z 158, occasionally goes undetected, and in these cases the GC/MS run should be repeated.

CONCLUSION

In practical use the isotope dilution GC/MS determination of malathion diacid gave comparable results to the conventional GC/FPD method. There was some evidence of a systematic difference for urine samples containing high parts per million levels of malathion metabolites with results on average 1.4 times higher by the isotope dilution technique. The cause of this systematic difference is under study but probably relates to reduced recovery in the modified Bradway and Shafik method (Ito et al., 1979).

Use of the isotope dilution procedure did not improve the mass spectrometer linearity, but isotope dilution response factors were far more stable in day-to-day operation. In the period of study absolute response factors varied with a 38% RSD; isotope dilution response factors showed only a 3.5% RSD. The practical advantage is that continuing calibration requirements are far more attainable.

A further advantage of the use of isotope-labeled internal standards in malathion diacid determination was a significant improvement in measurement precision. When the labeled IS is introduced before any sample manipulation, the accumulated random errors associated with each method step are accounted for. In the determination of malathion diacid, the practical advantage of this improved precision is a 3-fold reduction in the MDL from 20 to 6.5 ng of MDA/mL.

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LITERATURE CITED

Bradway, D. E.; Shafik, M. T. Malathion Exposure Studies. Determination of Mono- and Dicarboxylic Acids and Alkyl Phosphates in Urine. *J. Agric. Food Chem.* **1977**, *25*, 1342–1344.

Draper, W. M.; Street, J. C. Applicator Exposure to 2,4-D, Dicamba, and a Dicamba Isomer. *J. Environ. Sci. Health* **1982**, *B17*, 321–339.

Fenske, R. A. Correlation of Fluorescent Tracer Measurements of Dermal Exposure and Urinary Metabolite Excretion during Occupational Exposure to Malathion. *Am. Ind. Hyg. Assoc. J.* **1988**, *49*, 438–444.

Fenske, R. A.; Leffingwell, J. T. Method for the Determination of Dialkyl Phosphate Metabolites in Urine for Studies of Human Exposure to Malathion. *J. Agric. Food Chem.* **1989**, *37*, 995–998.

Franklin, C. A. Estimation of Dermal Exposure to Pesticides and its Use in Risk Assessment. *Can. J. Physiol. Pharmacol.* **1984**, *62*, 1037–1039.

Franklin, C. A.; Fenske, R. A.; Greenhalgh, R.; Mathieu, H. V.; Denley, J. T.; Leffingwell, J. T.; Spear, R. C. Correlation of Urinary Pesticide Metabolite Excretion with Estimated Dermal Contact in the Course of Occupational Exposure to Guthion. *J. Toxicol. Environ. Health* **1981**, *7*, 715–731.

Grover, R.; Franklin, C. A.; Muir, N. I.; Cessna, A. J.; Riedel, D. Dermal Exposure and Urinary Metabolite Excretion in Farmers Repeatedly Exposed to 2,4-D Amine. *Toxicol. Lett.* **1986**, *33*, 73–83.

Ito, G.; Kilgore, W. W.; Seabury, J. J. Effect of Freezer Storage on Alkyl Phosphate Metabolites in Urine. *Bull. Environ. Contam. Toxicol.* **1979**, *22*, 530–535.

Kay, K.; Monkman, L.; Windlah, J. P.; Doherty, T.; Pare, J.; Ragigot, C. Parathion Exposure and Cholinesterase Response of Quebec Apple Growers. *AMA Arch. Ind. Hyg. Occup. Med.* **1952**, *6*, 252–262.

Laitinen, H. A.; Harris, W. E. *Chemical Analysis*, 2nd ed.; McGraw-Hill: New York, 1975.

Lavy, T. L.; Shepard, J. S.; Mattice, J. D. Exposure Measurements of Applicators Spraying (2,4,5-Trichlorophenoxy)acetic Acid in the Forest. *J. Agric. Food Chem.* **1980**, *28*, 626–630.

Lores, E. M.; Bradway, D. E. Extraction and Recovery of Organophosphorus Metabolites from Urine Using an Anion Exchange Resin. *J. Agric. Food Chem.* **1977**, *25*, 75–79.

Moody, R. P.; Franklin, C. A.; Riedel, D.; Muir, N. I.; Greenhalgh, R.; Hladka, A. A New GC On-Column Methylation Procedure for Analysis of DMTP (*O,O*-Dimethyl Phosphorothioate) in Urine of Workers Exposed to Fenitrothion. *J. Agric. Food Chem.* **1985**, *33*, 464–467.

Morgan, D. P.; Hetzler, H. L.; Slach, E. F.; Lin, L. I. Urinary Excretion of Paranitrophenol and Alkyl Phosphates Following Ingestion of Methyl and Ethyl Parathion by Human Subjects. *Arch. Environ. Contam. Toxicol.* **1977**, *6*, 159–173.

Muan, B.; Skare, J. U. A Method for the Determination of the Main Metabolites of Malathion in Biological Samples. *J. Agric. Food Chem.* **1989**, *37*, 1081–1085.

Reid, S. J.; Watts, R. R. A Method for the Determination of Dialkyl Phosphate Residues in Urine. *J. Anal. Toxicol.* **1981**, *5*, 125–132.

Shafik, M. T.; Enos, H. F. Determination of Metabolic and Hydrolytic Products of Organophosphorus Pesticide Chemicals in Human Blood and Urine. *J. Agric. Food Chem.* **1969**, *17*, 1186–1189.

Shafik, M. T.; Bradway, D. E.; Enos, H. F. A Cleanup Procedure for the Determination of Low Levels of Alkylphosphates, Thiophosphates, and Dithiophosphates in Rat and Human Urine. *J. Agric. Food Chem.* **1971**, *19*, 885–889.

Shafik, M. T.; Bradway, D. E.; Enos, H. F.; Yobs, A. R. Human Exposure to Organophosphorus Pesticides. A Modified Procedure for the Gas-Liquid Chromatographic Analysis of Alkyl Phosphate Metabolites in Urine. *J. Agric. Food Chem.* **1973**, *21*, 625–629.

Wojeck, G. A.; Nigg, H. N.; Stamper, H. J.; Bradway, D. E. Worker Exposure to Ethion in Florida Citrus. *Arch. Environ. Contam. Toxicol.* **1981**, *10*, 725–735.

Wolfe, N. L.; Cox, R. H.; Gordon, J. A. Synthesis and Carbon-13 Studies of Malathion Acid Derivatives. *J. Agric. Food Chem.* **1975**, *23*, 1212–1215.

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