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Note

Gas chromatographic–mass spectrometric method to assess residues of 2-methyl-4-chlorophenoxyacetic acid in human urine

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2-Methyl-4-chlorophenoxyacetic acid (MCPA) and chlorophenoxyacetic acid-based herbicides in general are extensively used in forestry and agriculture. However, in man they have been associated with a significant increase in the soft-tissue sarcoma rate in three population-based case-control studies [1–4]. The aim of this study was to establish suitable analytical methods for the biological monitoring of occupationally exposed spraymen through the assessment of 2,4-dichlorophenoxyacetic acid (2,4-D) and especially MCPA levels in their urine [5]. Urine has been widely utilized for monitoring exposure to 2,4-D, MCPA and related compounds and is recognized as the most important clearance route of such chemicals from the body [6,7]. In 24-h urine samples, MCPA has been reported to exhibit levels even more than 50 times greater than pertinent background values [8–12] and a clearance half-life of 12–48 h [13,14].

EXPERIMENTAL

Chemicals and glassware

2-Butanone, diethyl ether and isooctane were high-purity solvents and used as received from Carlo Erba (Milan, Italy) and Fluka (Buchs, Switzerland). Solid MCPA (>97%) and N-nitrosotoluene 4-sulphomethylamide for the production of diazomethane [15] were obtained from Fluka and anhydrous sodium sulphate from BDH Italia (Milan, Italy). Solid 2,4-D (gas chromatographic-grade standard) (used without further purification) and methanol–boron trifluoride and methanol–boron trichloride methylating mixtures were obtained from Supelco (Bellefonte, PA, U.S.A.).

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Human urine was collected in polyethylene containers, sealed with leak-proof screw caps and stored at −20°C. Transfer of specimens from the sampling location to the laboratory was performed in a dry-ice box.

Pyrex glassware was used throughout; it was carefully cleaned and heated at 250°C for several hours before utilization. Regular capped 35-ml scintillation vials were employed for hydrolysis. When required, test-tubes (<10 ml) were used and sealed with PTFE-lined screw caps.

Instrumentation

A Hewlett-Packard Model 5992/A gas chromatographic–mass spectrometric (GC–MS) unit, modified with a Model 5993 GC–MS data system, was employed. The GC section was equipped with an HP-5 fused-silica macrobore capillary column (10 m×0.53 mm I.D.). The flow-rate of the carrier gas (helium) was approximately 2 ml/min, of which an average of 15% [coefficient of variation (C.V.) ≤5%] entered the ion source owing to the presence of a GC–MS splitting interface. The GC conditions were as follows: injection block, 220°C; oven, initially 5 min isothermal at 150°C, programmed at 5°C/min to 200°C and held there for 5 min; and interface, as oven temperature.

Sampling

Blank urine specimens were obtained from unexposed healthy subjects. Spraymen's urine specimens were collected 0.5 h before the start of the work shift (pre-exposure sampling) and during the next 24 h (post-exposure sampling) [5]. However, the different sampling times appeared not to affect the MCPA levels detected.

Analysis

For recovery studies, 2,4-D and MCPA standard solutions were added to blank urine samples to cover the range 10–5000 μg/l (ppb).

Herbicide residues and standards were analysed as follows: a 25-ml aliquot from a urine specimen was mixed with sulphuric acid (pH 1) in a scintillation vial, heated to 90°C in a thermostated bath and hydrolysed for 1 h. After cooling, the hydrolysed sample was extracted six times with diethyl ether. The extracts were combined and the pool was dehydrated overnight with anhydrous sodium sulphate, transferred quantitatively into a test-tube (<10 ml) and reduced to a small volume (<0.1 ml).

The free acids were methylated within the sealed tube by adding 0.5 ml of methylating mixture and heating at 60°C for 0.5 h. Alternatively, 2 ml of ethereal diazomethane were added to the dehydrated extract and the mixture was allowed to react for 10 min at 0°C. In both instances the final solution was gently evaporated to a small volume (<0.1 ml).

After methylation, each concentrated sample was mixed with 5 ml of 10% sodium chloride solution and 1 ml of isooctane and cooled to 0°C. The sealed test-tube was shaken vigorously for a few minutes to extract the esters into the organic phase, then the system was allowed to rest and separate while chilled. Methyl

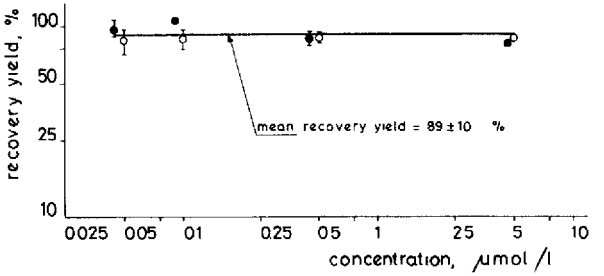

Fig. 1. Analytical recoveries of 2,4-D (○) and MCPA (●) as methyl esters from blank urine matrices spiked with the free acids. Mean yields were always >80% with C.V. ≤15% over the entire 10–1000 μg/l range tested. Each data point is the mean of at least two independent determinations. Weighted means: (a) for 2,4-D data subset, 93 ± 11%; (b) for MCPA data subset, 85 ± 8%; (c) for the whole (combined) set, 89 ± 10%.

derivatives in isooctane (upper layer) were submitted to GC–MS analysis. The mean recoveries were always >80% (Fig. 1).

Each urine specimen was examined by assaying three independent 25-ml samples.

2,4-D and MCPA methyl esters were determined by the multiple ion detection (MID) technique using the external standard method. The ion masses scanned were (ions for quantitation in italics) *m/z* 199, *234* and 236 for 2,4-D and *m/z* 141, *214* and 216 for MCPA; little interference from the background was experienced for MCPA and 2,4-D levels greater than the detection threshold (Fig. 2). The MS detector response was linear over the entire concentration range tested (Fig. 3). The detection threshold was 25 μg/l [signal-to-noise ratio (S/N) = 10] for both compounds with the capability of identification via ion intensity ratios, and as low as 10–15 μg/l (S/N ≥ 2.5, based on the most intense signal) with the loss of such a capability.

In each set of determinations, aliquots (≤2 μl) of the same sample and standard were injected several times. To determine the amount of an analyte, its mean response from repeatedly analysed triplicate samples was evaluated against the mean value for the pertinent standard. Final standard deviations (S.D.) were obtained by propagation error analysis.

RESULTS AND DISCUSSION

Table I gives MCPA levels determined with different derivatization techniques for several pre- and post-exposure urine specimens. 2,4-D levels were always negligible owing to the specific MCPA-based herbicides used [5] and are not reported. For a given specimen, determinations with different methylating agents yielded quantitative values that appear to be substantially equal, within experimental error. Hence it may be concluded that any of the methylating agents investigated is adequate for the analysis. However, handling was easier with methanol-based reagents.

Table II gives the lowest herbicide figures assessed. From these values, all ≤50 μg/l, it may be elicited that even at or near the detection threshold the analytical

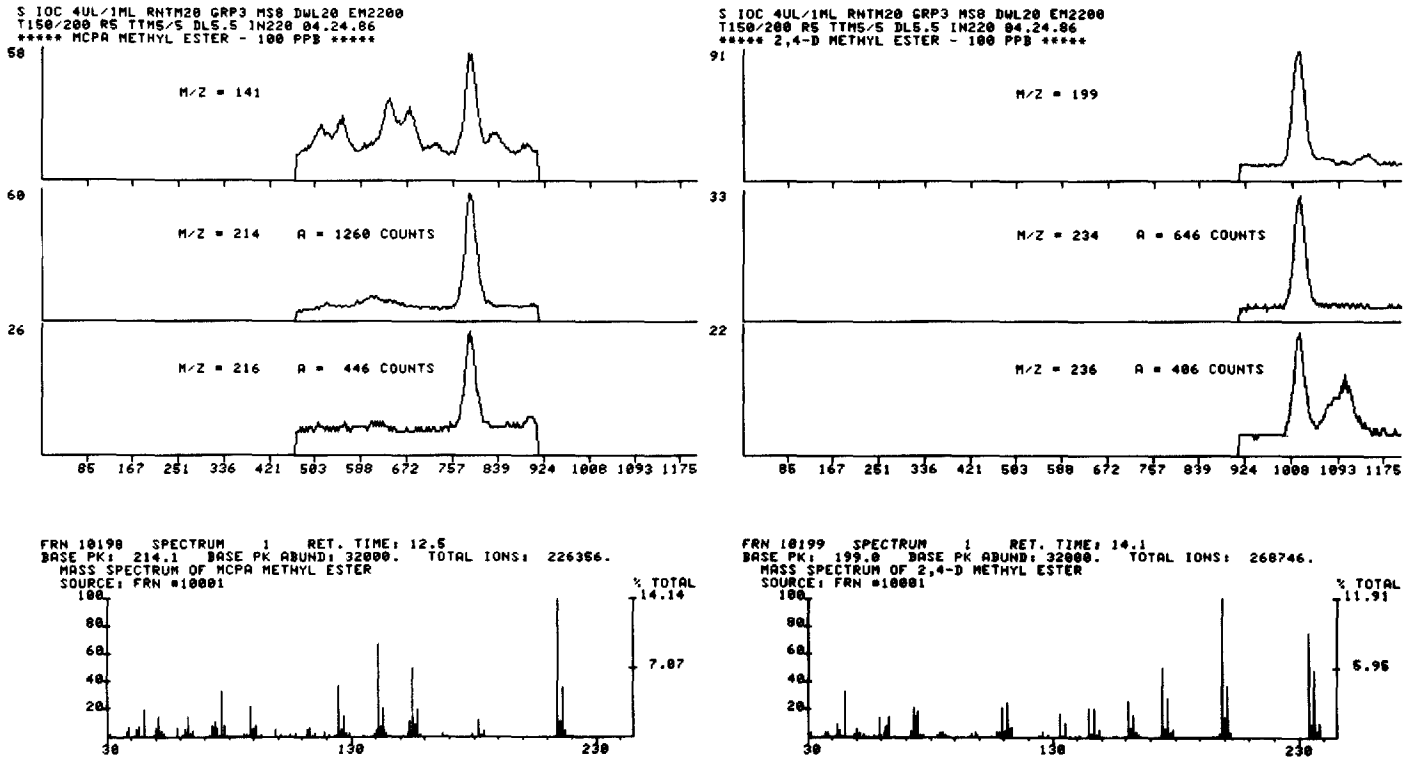

Fig. 2. Mass chromatograms (above) and mass spectra (below) of MCPA (left) and 2,4-D (right). Mass chromatograms were obtained from chemicals (100 μg/l) dissolved in blank urine samples; to provide standard spectra, MCPA and 2,4-D were added to a water medium, extracted therefrom and derivatized to methyl esters. GC conditions are described in the text. General conditions for MS: amount of each compound reaching ion source, ca. 3.2 ng; electron energy, 50–70 eV; electron multiplier, 1800–2200 V; threshold, 10; scan range, 30–330 a.m.u.; scan delay, 5 min; total run time, 20 min.

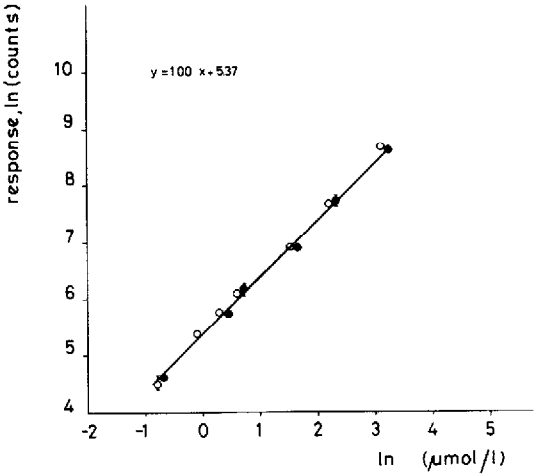

Fig. 3. GC–MS responses of 2,4-D (○) and MCPA (●) methyl esters, which are linear over the whole 100–5000 μg/l range tested. The linear regression performed on the whole logarithmically transformed data set exhibits a significant correlation coefficient (> 0.999); the existence of a linear trend for the original untransformed data is proved, as is well known, by the slope (indistinguishable from unity) in the regression equation $y = 1.00x + 5.37$. Linear regressions performed on the original data yield the equations $y = 241x - 47.7$ for 2,4-D and $y = 199x + 21.2$ for MCPA; both regressions exhibit significant correlation coefficients (> 0.999).

TABLE I

COMPARISON OF DIFFERENT METHYLATION TECHNIQUES UTILIZED TO CONVERT MCPA FREE ACID EXTRACTED FROM URINE INTO ITS METHYL ESTER

Specimen[a]	MCPA concentration (μg/l)				
	CH_3OH–BCl_3 (*A*)	CH_3OH–BF_3 (*B*)	*B/A*	CH_2N_2 (*C*)	*C/A*
1	31.4 ± 6.1	Not assessed		26.7 ± 2.2	0.85
2	36.3 ± 6.3	43.7 ± 7.5	1.20	36.4 ± 4.4	1.00
3	64.2 ± 5.1	61.9 ± 14.0	0.96	50.8 ± 3.7	0.79
4	175 ± 15	168 ± 20	0.96	134 ± 18	0.76
5	341 ± 51	293 ± 24	0.86	267 ± 32	0.78
6	513 ± 54	433 ± 81	0.84	373 ± 15	0.73

[a]Specimens 1 and 5 from pre-exposure sampling; others from post-exposure sampling.

procedure tested has a good degree of reproducibility. In Tables I and II, the coefficients of variation are always < 20%.

The results presented here together with the previous results [5] validate the use of GC–MS described for the assessment of MCPA, and also 2,4-D and similar herbicides, in urine specimens.

Finally, the pre-exposure values (specimens 1, 5, 8, 9, 10 and 13) seem to indicate that the time elapsed since the previous work shift was insufficient to allow complete body clearance of MCPA. It cannot be ruled out that the observed pre-

TABLE II

NEAR-DETECTION-THRESHOLD LEVELS OF MCPA AND 2,4-D IN SELECTED URINE SPECIMENS

Specimen[a]	Analyte	Concentration (mean ± S.D.) (μg/l)	Coefficient of variation (%)
7	MCPA	23.6 ± 3.1	13
8	MCPA	33.1 ± 5.8	18
9	2,4-D	⩽15	
	MCPA	33.9 ± 3.5	10
10	2,4-D	⩽15	
	MCPA	38.9 ± 4.9	13
11	2,4-D	⩽26	
	MCPA	39.5 ± 3.7	9.3
12	MCPA	43.7 ± 7.5	17
13	2,4-D	⩽24	
	MCPA	50 8 ± 6.7	13

[a]Specimens 8, 9, 10 and 13 from pre-exposure sampling; others from post-exposure sampling.

exposure values are a measure of MCPA background residues, and this aspect seems to require further investigation.

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