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Note

Gas chromatographic–mass spectrometric method for the determination of perchloroethylene and its major metabolites in urine

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Perchloroethylene (PCE), a volatile, non-flammable solvent has been used extensively in dry-cleaning and metal-cleaning industries. At high exposure levels, it has been reported to cause loss of muscular coordination, light-headedness, and reversible hepatic dysfunction (Stewart and co-workers[1,2]). Although some data on the metabolism of PCE are available (Yllner[3], Bonse *et al.*[4]), a pharmacokinetic profile of PCE as a function of dose and route of exposure is not available. In addition, it is desirable to determine possible alterations in the metabolic patterns or saturation of detoxification mechanisms resulting from accidental high doses of PCE. However, before these objectives can be met, a method is needed to measure PCE and its major metabolites, trichloroacetic acid (TCA), 2,2,2-trichloroethanol (TCE) and oxalic acid (OXA) (Ikeda *et al.*[5]) in body fluids.

EXPERIMENTAL

Standard solutions

As reference standard, a methanolic solution of PCE, TCE, TCA, and OXA with a 100 μg/ml concentration of each compound was prepared gravimetrically.

Urine standards were prepared from blank rat urine which was "spiked" with the appropriate volume of the reference standard to generate standards of 10, 3, 1.0, 0.3, 0.1, and 0.0 μg/ml of each compound.

Sample preparation

The PCE and TCE concentrations in urine were determined without any sample preparation by gas chromatography–mass spectrometry (GC–MS) as described below. To determine the TCA and OXA concentrations, 0.5 ml of urine was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 0.5 ml of methanol and exhaustively methylated with diazomethane. After methylation, the methanol was evaporated to near dryness under a nitrogen stream and the residue was immediately redissolved in 0.5 ml of methanol. (If the samples are left under the nitrogen stream for extended periods, some loss of the volatile esters of TCA and OXA is observed. This problem can be completely eliminated by careful observation of the samples and removing them from the nitrogen immediately after the solvent evaporates).

GC–MS (selected ion monitoring) was used to determine separately the response from each compound of interest in the urine sample.

A Finnigan Quadrupole Model 3200 equipped with a Model 6110 Data System was used. The column was a 6 ft. × 2 mm I.D. glass tube, packed with Chromosorb 101, 80–100 mesh: the carrier gas was methane. The temperatures were: injector, 220°; column, 185°; transfer line, 240°; and ion source, 140°. The pressures were: ion source, 450 μmHg; analyzer, $8 \cdot 10^{-5}$ torr. Chemical ionization was performed with methane. The following ions were monitored: PCE, 165 *m/e* (P+1); TCE, 149 *m/e* (P+1); TCA, 177 *m/e* (P+1) of methyl ester and OXA, 119 *m/e* (P+1) of dimethyl ester.

RESULTS AND DISCUSSION

Fig. 1 is a selected ion chromatogram of PCE, TCE, TCA (methyl ester), and OXA (dimethyl ester) obtained from the 100-ppm reference standard. Note that PCE and its major metabolites (TCE, TCA and OXA) are well separated. The only ion overlap observed was between the P+29 of TCE and the P+1 of TCA; however, with the different retention times of the two compounds, no interference is encountered.

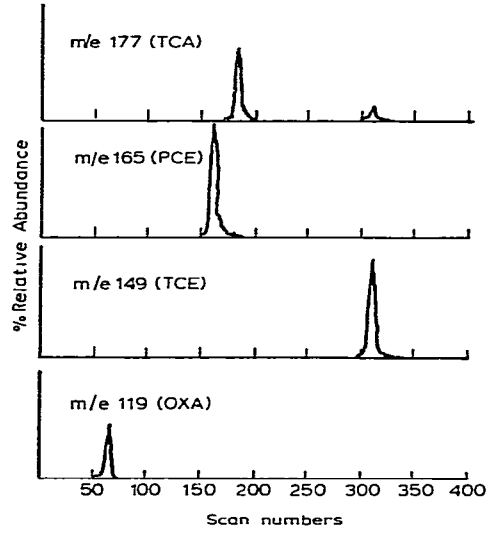

Fig. 1. Selected ion chromatogram of 100 ppm reference standard of PCE, TCE, TCA, and OXA.

Fig. 2 is a plot of the response in mV *vs.* concentrations of PCE, TCE, TCA, and OXA in the range from 0.1 to 10 ppm. The data used to generate Fig. 2 are present in Table I as the mean ± standard deviation for four determinations. Note that the response (mV) changes linearly with changes in concentration when equal volumes are injected.

Since chemical ionization has been reported to be troubled by matrix effects, the response of standards prepared in methanol were compared with the response of standards prepared in urine. In the concentration range of 0.1 to 10 μg/ml no matrix effects were observed for PCE, TCE, TCA, or OXA.

TABLE I

STANDARDS OF PCE, TCE, TCA, AND OXA IN URINE *VERSUS* RESPONSE* IN mV

Concentration (μg/ml)	*Amount injected (ng)*	*PCE (mV)*	*TCE (mV)*	*TCA (mV)*	*OXA*	
					Uncorrected for blank (mV)	*Corrected for blank (mV)*
0	0	0	0	0	$(1.16 \pm 0.20) \cdot 10^4$	0
0.1	0.1	$(4.10 \pm 0.52) \cdot 10^2$	$(3.95 \pm 0.47) \cdot 10^2$	$(2.96 \pm 0.16) \cdot 10^2$	$(1.20 \pm 0.16) \cdot 10^4$	(400)***
0.3	0.3	$(1.26 \pm 0.16) \cdot 10^3$	$(1.07 \pm 0.14) \cdot 10^3$	$(7.82 \pm 1.03) \cdot 10^2$	$(1.09 \pm 0.11) \cdot 10^4$	(−700)***
1.0	1.0	$(3.90 \pm 0.52) \cdot 10^3$	$(3.12 \pm 0.37) \cdot 10^3$	$(2.73 \pm 0.40) \cdot 10^3$	$(1.38 \pm 0.13) \cdot 10^4$	$2.16 \cdot 10^3$
3.0	3.0	$(1.17 \pm 0.77) \cdot 10^4$	$(9.54 \pm 0.63) \cdot 10^3$	$(8.50 \pm 0.74) \cdot 10^3$	$(1.91 \pm 0.32) \cdot 10^4$	$7.53 \cdot 10^3$
10.0	10.0	$(3.82 \pm 0.17) \cdot 10^4$	$(3.13 \pm 0.26) \cdot 10^4$	$(2.96 \pm 0.16) \cdot 10^4$	$(3.39 \pm 0.46) \cdot 10^4$	$2.23 \cdot 10^4$
Slope $= \frac{\Sigma XY}{\Sigma X^2}$		$3.83 \cdot 10^3$ mV/ng	$3.13 \cdot 10^3$ mV/ng	$2.95 \cdot 10^3$ mV/ng		$2.25 \cdot 10^3$ mV/ng
Relative standard error (%)§		9.9	10.3	4.5		11.3**

* Response for 1-μl injections.

** Standard error (%) for OXA determination calculated from 1.0, 3.0, and 10 ppm standards only.

*** Below detection limit due to endogenous OXA.

§ % Relative standard error $= \frac{2\,(\text{S.D.})}{\bar{x}} \cdot 100\%$.

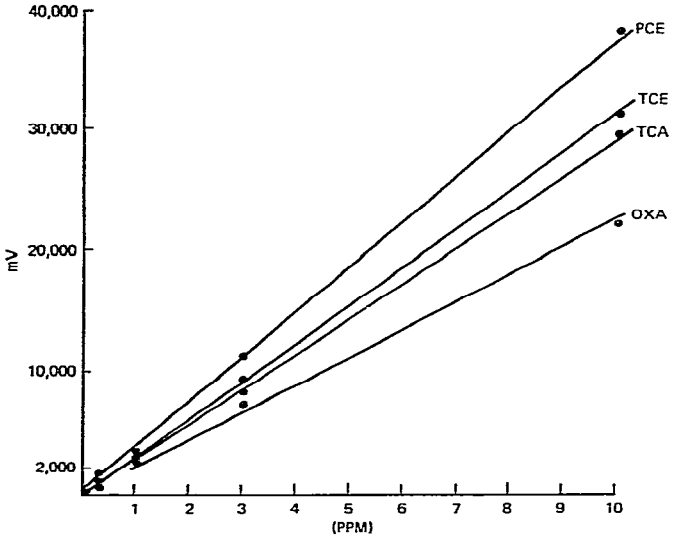

Fig. 2. Plot of PCE, TCE, TCA, and OXA urine standards *versus* response in mV. (We routinely use a log-log scale. Data points become roughly equidistant.)

The blank rat urine contained 5.4 ppm OXA but no other interferences were observed in the other ions monitored. The limiting factor in the analysis of exogenous OXA, *i.e.*, that formed by metabolism of PCE, will be the concentration and variability of endogenous (background) OXA in the urine. The background of OXA in the rat urine will vary considerably from laboratory to laboratory, however, by using a blank correction, the concentration of the exogenous urinary OXA could be determined at 1.0 ppm in spiked urine. For experimental urines, the detection limit for OXA will depend upon the variability of OXA in the rat urine of that laboratory. The detection limits (signal-to-noise ratio = 2) of PCE, TCA, and TCE were calculated from the signal-to-noise ratio at 0.1 ppm to be 0.025, 0.022, and 0.011, respectively. The standard errors calculated from the data in Table I were 9.9, 10.3, 9.5, and 11.3% for PCE, TCE, TCA, and OXA, respectively.

CONCLUSION

The method described is simple and rapid. It is extremely specific and sensitive for PCE and two major metabolites, TCE and TCA. The usefulness in determining the OXA resulting from PCE metabolism will be limited by the concentration and variability of endogenous urinary OXA.

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