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DETERMINATION OF *cis*- AND *trans*-1,3-DICHLOROPROPENE IN WHOLE RAT BLOOD BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–CHEMICAL IONIZATION MASS SPECTROMETRY WITH SELECTED ION MONITORING

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SUMMARY

An analytical method was developed for quantitating low concentrations of the isomers *cis*- and *trans*-1,3-dichloropropene in whole rat blood by gas chromatography and gas chromatography–chemical ionization mass spectrometry with selected ion monitoring.

INTRODUCTION

The *cis*- and *trans*-isomers of 1,3-dichloropropene* are found in several preplant soil fumigants which are used to control plant parasitic nematodes, certain insects and diseases in agricultural cropland. The dichloropropene isomer mixture is a clear, pale straw-colored liquid that reacts readily with aluminum, aluminum alloys, other active metals and metal salts. Tests on laboratory animals have shown that exposure to this mixture at elevated concentrations results in irritation of the eyes and mucous membranes. It also penetrates common rubber and vinyl protective equipment[1].

In support of toxicology studies in animals, an analytical method was required to quantitate low concentrations of *cis*- and *trans*-1,3-dichloropropene in whole rat blood. This paper describes a rapid and simple analytical method that is both specific and sensitive to blood levels of dichloropropene as low as 5.88 ng/ml and 5.35 ng/ml for the *cis*- and *trans*-isomers, respectively, using gas chromatography (GC), and 51.8 ng/ml and 47.1 ng/ml for the *cis*- and *trans*-isomers, respectively, using gas chromatography–chemical ionization mass spectroscopy (GC–CI-MS) with selected ion monitoring (SIM).

* 1,3-Dichloropropene is the active ingredient in Telone II soil fumigant, a trademark of The Dow Chemical Company.

MATERIALS AND METHODS

Materials

Production grade Telone II soil fumigant was supplied by Dow Chemical (Midland, MI, U.S.A.). The composition of the test material was determined by GC to be 92.0 ± 0.1% 1,3-dichloropropenes with the balance being other chlorinated hydrocarbons; of the 1,3-dichloropropenes, 48.2% existed as the *cis*- and 43.8% as the *trans*-isomer. Distilled-in-glass UV hexane was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Healthy, adult male Fischer 344 rats were purchased from the Charles River Breeding Labs. (Wilmington, MA, U.S.A.). Following cervical dislocation, blood was withdrawn from these rats by open chest heart puncture into a heparinized syringe.

Instrumentation

A Varian 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) was used for quantitative analysis of the blood standards. The isomers were separated on a 3.5 m × 2 mm I.D. column containing 20% Carbowax 20M on Chromosorb W AW (80–100 mesh) (Supelco, Bellefonte, PA, U.S.A.). The column was conditioned overnight at 150°C with a nitrogen flow of 20 ml/min. During the analysis the GC column was maintained at 120°C with a nitrogen flow of 20 ml/min. The injection port and detector temperatures were 150°C and 250°C, respectively. External standard calculations were used in quantitating the dichloropropene blood standards.

A Finnigan Model 3200 chemical ionization quadrupole mass spectrometer in conjunction with a Model 6100 data system was also used for quantitative analysis of the blood standards by selected ion monitoring. GC separation of the dichloropropene isomers was obtained with a 2.1 m × 2 mm glass column containing HNU Synerg C-IH (80 mesh) (HNU Systems, Newton, MA, U.S.A.). Prior to use the column was conditioned overnight at 125°C with a helium flow of 4 ml/min. During the analysis it was maintained at 110°C. The injection port and transfer line temperatures were 150 and 210°C, respectively. Methane was used as reactant gas at a flow-rate yielding an ion source pressure of 500 μm. The GC–MS instrument parameters were as follows: ion source temperature, 70°C; source pressure, 500 μm at $5 \cdot 10^{-4}$ Torr; ionization energy, 102 eV; filament emission current, 1.01 mA. The mass spectrometer was programmed to monitor selected fragment ions of *cis*- and *trans*-1,3-dichloropropene: $[C_3H_4{}^{35}Cl]^+$, m/z 75; $[C_3H_4{}^{37}Cl]^+$, m/z 77. Peak areas were determined by electronic integration with background subtraction. External standard calculations were used in quantitating the dichloropropene blood standards. Peak area determinations were performed on m/z 77 because this ion exhibited less background noise and was of sufficient intensity for low ng/ml quantitation.

Standards

Standard solutions were prepared by adding an accurately measured volume of the 1,3-dichloropropene isomer mixture to hexane and diluting concentrations within the calibrated range of the GC detector; *i.e.*, 5.88–58.8 ng/ml *cis*-dichloropropene and 5.35–53.5 ng/ml *trans*-dichloropropene. The calibrated SIM response range of the mass spectrometer was 25.9–12,900 ng/ml *cis*-dichloropropene and

23.5–11,800 ng/ml *trans*-dichloropropene. Both modes of analysis, GC and GC–CI-MS, gave a linear response within the calibrated ranges of their detectors.

Solutions for spiking blood were prepared by adding an accurately measured volume of the dichloropropene isomer mixture to 95% ethanol, yielding $1.17 \cdot 10^3$–$1.17 \cdot 10^6$ ng/ml *cis*-isomer and $1.07 \cdot 10^3$–$1.07 \cdot 10^6$ ng/ml *trans*-isomer. Blood standards were prepared by spiking an accurately weighed sample of approximately 200 mg fresh rat blood with an aliquot of the appropriate spiking solution. After spiking, the standard was vigorously shaken by hand for approximately 15 sec to ensure mixing.

Extraction method

The blood standards were extracted with 200 μl of hexane added with a syringe through the septum cap of the vial. The blood standard was then vortexed for 1 min and centrifuged at 800 *g* for 1 min. A 2-μl volume of the organic layer was injected directly into the GC or GC–MS system. All blood standard concentrations above the calibrated detector range were diluted in hexane prior to GC analysis. Blood standards analyzed by chemical ionization GC–MS were not diluted prior to analysis.

RESULTS AND DISCUSSION

To determine the per cent recovery, blood standards were prepared by adding known amounts of *cis*- and *trans*-1,3-dichloropropene to approximately 200 mg whole rat blood. The standards were then extracted with hexane prior to analysis by GC–ECD and/or chemical ionization GC–MS with selected ion monitoring.

TABLE I

RECOVERY OF 1,3-DICHLOROPROPENE ISOMERS FROM WHOLE RAT BLOOD BY GAS CHROMATOGRAPHY

ND = No dilution.

Concn. (ng/ml) in whole rat blood		*Dilution*	*Mean* recovery (%)*		*Standard coefficient of*			
Cis	*Trans*		*Cis*	*Trans*	*Deviation*		*Variation*	
					Cis	*Trans*	*Cis*	*Trans*
$1.17 \cdot 10^4$	$1.07 \cdot 10^4$	1/201	91.1	92.6	5.7	3.5	6.3	3.8
$1.17 \cdot 10^3$	$1.07 \cdot 10^3$	1/21	91.0	81.3	5.6	2.8	6.2	3.4
$5.88 \cdot 10$	$5.35 \cdot 10$	ND	86.9	92.7	1.9	3.3	2.2	3.6
$1.17 \cdot 10$	$1.07 \cdot 10$	ND	80.8	87.5	4.2	3.8	5.2	4.3
5.88	5.35	ND	98.5	98.2	4.8	6.9	4.9	7.0
			89.7 ±6.5**	90.5 ±6.4**	±4.7***	4.3***	5.2§	4.6§

* Each data point is the mean from three unique samples, two determinations each.

** Standard error of the mean.

*** Average standard deviation, $x = [\Sigma(x)^2/n]^{1/2}$.

§ Average coefficient of variation, $y = [\Sigma(y)^2/n]^{1/2}$.

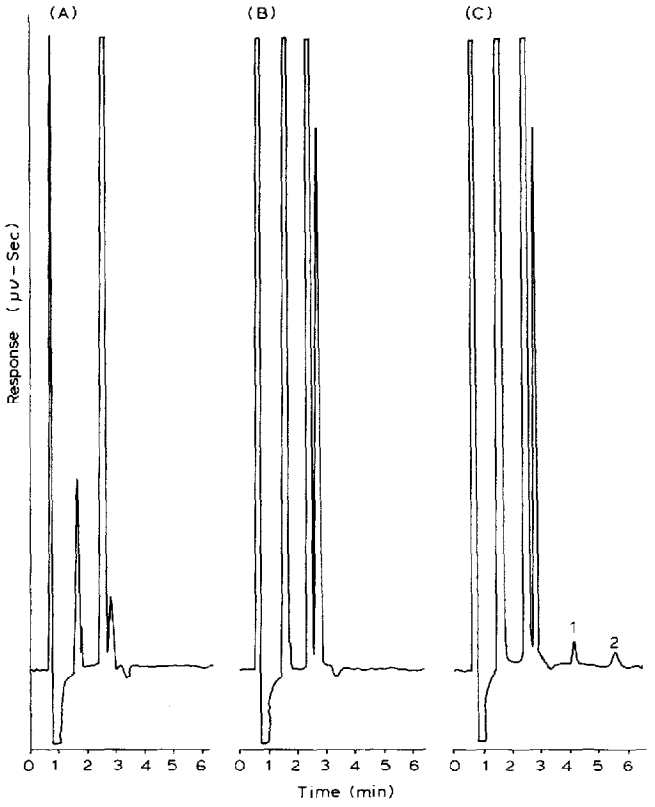

Fig. 1. Representative GC chromatograms of 1,3-dichloropropenes: A, blank solvent; B, blank blood standard; C, blood standard. Peaks: 1 = 5.88 ng/ml *cis*-1,3-dichloropropene; 2 = 5.35 ng/ml *trans*-1,3-dichloropropene.

TABLE II

RECOVERY OF 1,3-DICHLOROPROPENE ISOMERS FROM WHOLE RAT BLOOD BY CHEMICAL IONIZATION GC–MS WITH SELECTED ION MONITORING

Quantitated by external standard calculations on *m/z* 77. Other details as in Table I.

Concn. (ng/ml) in whole rat blood		*Mean recovery (%)*		*Standard deviation*		*Coefficient of variation*	
Cis	*Trans*	*Cis*	*Trans*	*Cis*	*Trans*	*Cis*	*Trans*
$1.29 \cdot 10^4$	$1.18 \cdot 10^4$	92.0	96.1	4.1	4.5	4.5	4.7
$6.47 \cdot 10^3$	$5.88 \cdot 10^3$	83.1	88.7	3.2	2.9	3.9	3.3
$1.29 \cdot 10^3$	$1.18 \cdot 10^3$	85.1	92.3	4.1	4.5	4.8	4.9
$5.18 \cdot 10$	$4.71 \cdot 10$	94.9	98.8	3.2	7.4	3.4	7.5
	Average	88.8 ± 5.6	94.0 ± 4.4	3.7	5.1	4.2	5.3

The recovery of the *cis*-isomer from whole rat blood by GC ranged from 80.8 to 98.5% and the mean per cent recovery for the concentration range of 5.88–1.17 · 10^4 ng/ml was 89.7 ± 6.5% (see Table I). The GC recovery of the *trans*-isomer from whole rat blood ranged from 81.3 to 98.2% and the mean recovery for the concentration range of 5.35–1.07 · 10^4 ng/ml was 90.5 ± 6.4%. The coefficient of variation for five blood concentrations analyzed by GC ranged from 2.2% at 58.8 ng/ml to 6.3% at 1.17 · 10^4 ng/ml for the *cis*-isomer and 3.4% at 1.07 · 10^3 ng/ml to 7.0% at 5.35 ng/ml for the *trans*-isomer.

No peaks which would interfere with the quantitative GC analysis of the dichloropropene isomers were noted with either blank UV hexane or blank blood standard solutions. GC chromatograms of blank solvent, a blank blood standard and a blood standard are presented in Fig. 1. The retention times of the *cis*- and *trans*-isomers of 1,3-dichloropropene were 4.1 and 5.6 min, respectively.

The recovery of *cis*-isomer from whole rat blood when analyzed by GC–CI-MS with SIM ranged from 83.1 to 94.9% and the mean per cent recovery for the concentration range of 51.8–1.29 · 10^4 ng/ml was 88.8 ± 5.6% (see Table II). The recovery of the *trans*-isomer from whole rate blood ranged from 88.7 to 98.8% and the mean recovery for the concentration range of 47.1–1.18 · 10^4 ng/ml was 94.0 ± 4.4%. The coefficient of variation for the four blood concentrations ranged from 3.4% at 51.8 ng/ml to 4.8% at 1.29 · 10^3 ng/ml for the *cis*-isomer and 3.3% at 5.88 · 10^3 ng/ml to 7.5% at 47.1 ng/ml for the *trans*-isomer.

No peaks which would interfere with the quantitative GC–MS analysis of either dichloropropene isomer were noted in the blank solvent or the blank blood standards with selected ion monitoring. Fig. 2 presents the observed selected ion chromatograms of a blank blood standard, a solution standard and a dichloropropene isomer blood standard. The total GC–CI-MS analysis time per injection was less than 2 min.

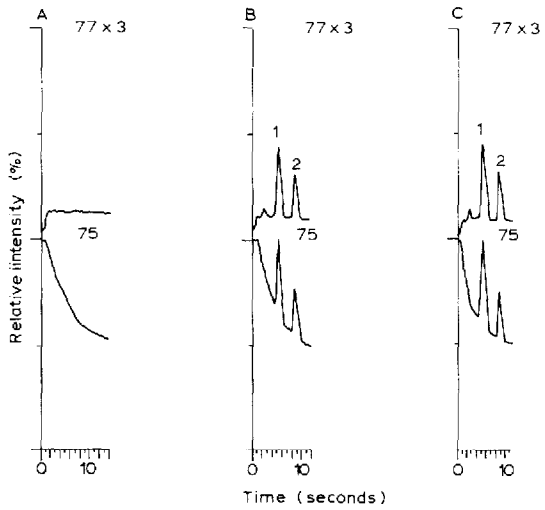

Fig. 2. Representative selective ion chromatograms: A, blank blood standard; B, 1,3-dichloropropene isomer solution standard; C, 1,3-dichloropropene isomer blood standard. Peaks: 1 = 51.8 ng/ml *cis*-1,3-dichloropropene; 2 = 47.1 ng/ml *trans*-1,3-dichloropropene.

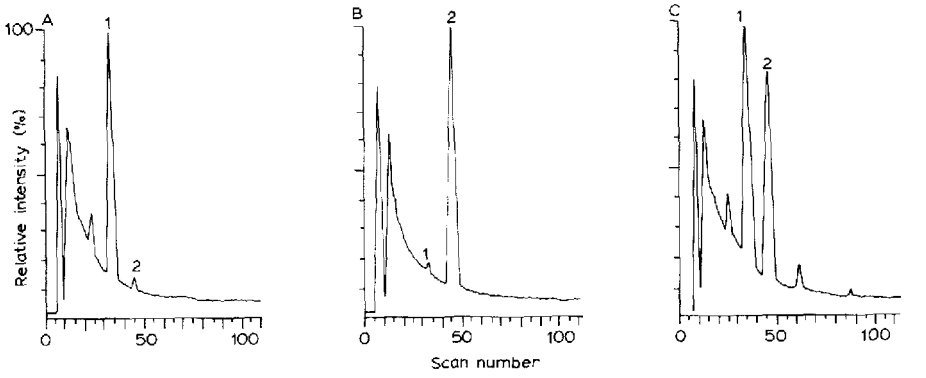

Fig. 3. Representative positive ion reconstructed gas chromatograms: A, *cis*-1,3-dichloropropene; B, *trans*-1,3-dichloropropene; C, mixture of *cis*- and *trans*-1,3-dichloropropene (Telone II soil fumigant). Peaks: 1 = *cis*-1,3-dichloropropene; 2 = *trans*-1,3-dichloropropene.

A stock standard solution of Telone II soil fumigant ($1.91 \cdot 10^6$ ng/ml) and standards of the individual 1,3-dichloropropene isomers ($9.74 \cdot 10^5$ ng/ml *cis* and $9.79 \cdot 10^5$ ng/ml *trans*) were monitored by positive ion GC–MS to confirm the identity of the observed peaks. Separation of the isomers was obtained by temperature programming the GC column from 80°C (hold 50 scans) to 150°C (110 scans total) at 20°C per min. The positive ion reconstructed gas chromatograms of these solution standards are presented in Fig. 3. The *cis*-isomer was eluted at scan number 36 in the temperature program and the *trans*-isomer at scan number 46. Two minor impurity peaks were eluted after the dichloropropene isomers when the Telone II soil fumigant standard was analyzed. Fig. 4 presents the observed mass spectra for the standards of the individual *cis*- and *trans*-1,3-dichloropropene isomers. As observed, the base peak in the mass spectra of each isomer was a fragment ion with *m/z* 75.

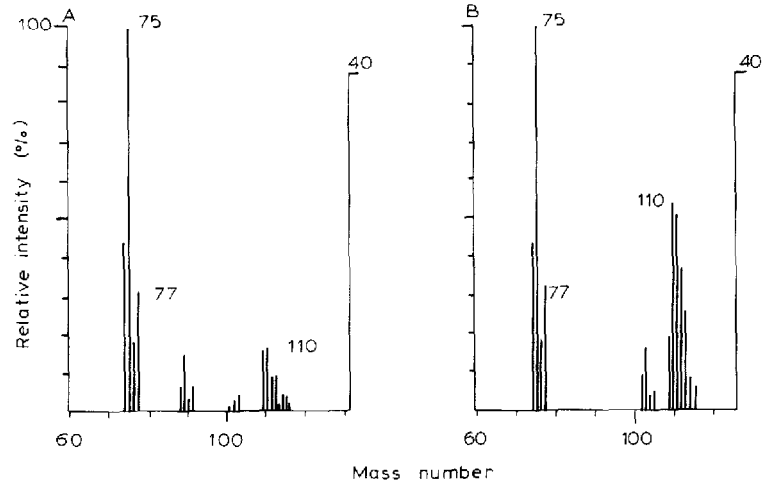

Fig. 4. Representative positive ion mass spectra: A, *cis*-1,3-dichloropropene (mass numbers greater than 85 multiplied by 10); B, *trans*-1,3-dichloropropene (mass numbers greater than 90 multiplied by 10).

This mass number and the resulting isotope ratio correspond with the loss of chlorine ($^{35}Cl^{\cdot}$) from the molecular ion (M). The molecular ion (m/z = 110) and the M + 1 addition ion were observed in the mass spectra of each dichloropropene isomer but neither had sufficient intensity for quantitative purposes. The methane addition ions, M + 29 and M + 41, were not observed.

CONCLUSIONS

The method described in this paper is both sensitive and specific for the GC determination of the *cis*- and *trans*-isomers of 1,3-dichloropropene in whole rat blood at concentrations ranging from 5.88 to 1.17 · 10^4 ng/ml and 5.35 to 1.07 · 10^4 ng/ml, respectively. Blood standards having concentration above the linear range of the GC detector were diluted in hexane prior to analysis. Although it is an order of magnitude less sensitive, the more rapid mode of analysis, GC–CI-MS with selected ion monitoring, was employed for analyzing the dichloropropene isomer blood standards at concentrations ranging from 51.8 to 1.29 · 10^4 ng/ml *cis*-isomer and 47.1 to 1.18 · 10^4 ng/ml *trans*-isomer. In this case the standards were not diluted prior to analysis.

ACKNOWLEDGEMENTS

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REFERENCE

1 T. R. Torkelson and F. Oygen, *Amer. Ind. Hyg. Ass., J.*, 38 (1977) 217.