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Note

Quantitative determination of 1,2-dibromo-3-chloropropane in whole rat blood and drinking water by gas chromatography

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1,2-Dibromo-3-chloropropane (DBCP) is an amber-colored liquid that has been used as a soil fumigant for nematodes since 1957 in agricultural cropland. Formulations containing DBCP have been primarily sold under the trademarks Fumazone (Dow Chemical, Midland, MI, U.S.A.) and Nemagon (Shell, The Hague, The Netherlands). Recent reports have associated exposure to DBCP with disruption of spermatogenesis[1] and azoospermia or oligospermia in male workers[2]. Tests on laboratory animals have indicated that DBCP has an adverse effect on spermatogenesis and leads to testicular atrophy[3].

In support of DBCP studies in animals, an analytical method was developed to determine low-level concentrations of DBCP in whole rat blood. Previous analytical methods for DBCP in blood required extensive sample preparation[4] and steam distillation[5] which limits the number of analysis per time period. This paper describes a simple gas chromatographic–electron-capture detection (GC–ECD) method that is both specific and sensitive to DBCP blood levels at concentrations as low as $2.28 \cdot 10^{-1}$ ng/ml DBCP. In addition, a quantitative analytical method was developed to measure levels of DBCP in drinking water which parallels the method in blood.

MATERIALS AND METHODS

Materials

Production grade DBCP (Lot No. M03196) and hexachloroethane (HCE) were supplied by Dow Chemical (Midland, MI, U.S.A.). Distilled-in-glass toluene was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Healthy, adult male Fischer 344 rats were purchased from the Charles River Breeding Laboratories (Wilmington, MA, U.S.A.).

Instrumentation

A Varian 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and CDS-111 integrator was used for quantitative analysis of the samples. Separation was obtained on a 1.4 m × 2 mm I.D. glass column containing 10% OV-330 on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The column was conditioned overnight at 160°C with a nitrogen flow of 25 ml/min. During the analysis the GC column was maintained at 120°C with a nitrogen flow-rate of 25 ml/min. The injection port and detector temperatures were 150°C and 250°C, respectively.

Extraction method

The DBCP blood standards were extracted with 200 μl of toluene stock solution containing the internal standard, HCE. The blood standard was then vigorously vortexed for 1 min and centrifuged for 1 min. A 2-μl volume of the organic layer was injected directly onto the GC column. All blood standards above $1.14 \cdot 10^2$ ng/ml DBCP were diluted to below this level with the appropriate toluene stock solution to stay within the calibrated range of the detector.

The DBCP water standards were extracted with 5 or 10 ml toluene depending on the DBCP concentration in water. The water standard was shaken for 30 min on a wrist-action shaker. A 2-μl volume of the organic layer was injected directly onto the GC column. One water standard, $1.14 \cdot 10^5$ ng/ml DBCP, was diluted into the calibrated range of the detector prior to analysis.

Standards

DBCP standard solutions were prepared by adding an accurately measured amount of DBCP to toluene stock solutions and diluting into the linear range of the electron-capture detector; $2.28 \cdot 10^{-1}$ ng/ml to $1.14 \cdot 10^2$ ng/ml DBCP. The toluene stock solutions contained hexachloroethane as internal standard.

DBCP blood spiking solutions were prepared by adding accurately weighed amounts of DBCP to 95% aqueous ethanol to yield solutions of $1.14 \cdot 10^2$ ng/ml to $1.14 \cdot 10^6$ ng/ml DBCP. DBCP blood standards were prepared by spiking an aliquot of the appropriate DBCP spiking solution into an accurately weighed sample of approximately 200 mg fresh blood. After spiking, the standard was shaking by hand for approximately 15 sec to insure mixing. Blood was obtained from Fischer rats via open chest heart puncture and a syringe containing heparin. Quantitation of the blood standards was determined by internal standard calculations.

DBCP water spiking solutions were prepared by adding accurately weighed amounts of DBCP to methanol to yield solutions of $1.14 \cdot 10^2$ ng/ml to $1.14 \cdot 10^7$ ng/ml DBCP. DBCP water standards were prepared by spiking an aliquot of the appropriate DBCP spiking solution into an accurately measured amount of water (100–250 ml). The water standards were quantitatively determined by external standard calculations.

RESULTS AND DISCUSSION

To determine the % recovery, blood standards were prepared by adding known amounts of DBCP and HCE to 200 mg blood. All standards were extracted with toluene and analyzed by GC–ECD. Recoveries of DBCP from blood ranged from 92.6 to 102.4% and the mean % recovery for the entire concentration range of $2.28 \cdot 10^{-1}$ to $2.28 \cdot 10^4$ ng/ml was 96.7 ± 3.0% (see Table I). The coefficient of variation for 8 blood concentrations ranged from 0.8% at $1.14 \cdot 10^2$ to 6.1% at $2.28 \cdot 10^{-1}$ ng/ml DBCP. No peaks which would interfere with the DBCP analysis were noted with either the toluene stock solutions or blank toluene; however, a small impurity peak from the toluene eluted at the same time as the HCE. This impurity peak was present at only a 0.4% level in the lowest toluene stock solution containing HCE and accordingly no adjustments were made for its presence. Blank blood standards exhibited no background peaks which would interfere with the quantitative analysis of DBCP or HCE.

TABLE I

RECOVERY OF DBCP FROM WHOLE BLOOD

S.D. = Standard deviation; C.V. = coefficient of variation; NS = no sample; ND = no dilution.

Concentration (ng/ml) DBCP in blood	*Dilution*	*Recovery for Replicate Samples (%)**				*Mean recovery (%)*	*S.D.*	*C.V.*
		No. 1	*No. 2*	*No. 3*	*No. 4*			
$2.28 \cdot 10^4$	1/201	92.6	94.5	94.5	NS	93.9	1.3	1.4
$1.14 \cdot 10^4$	1/101	92.6	97.2	95.9	NS	95.2	2.2	2.3
$1.14 \cdot 10^3$	1/11	93.2	93.1	93.8	90.4	92.6	1.5	1.6
$1.14 \cdot 10^2$	ND	97.4	98.9	97.8	NS	98.0	0.8	0.8
$1.14 \cdot 10^1$	ND	98.0	96.4	97.7	NS	97.4	1.5	1.5
1.14	ND	98.1	95.4	93.7	NS	95.7	4.2	4.4
$5.70 \cdot 10^{-1}$	ND	98.6	95.8	96.9	100.6	98.0	2.1	2.1
$2.28 \cdot 10^{-1}$	ND	95.4	100.7	103.4	110.2	102.4	6.2	6.1
					Mean:	96.7	3.0	3.1

* Each data point is the mean of 2 determinations minimum.

HCE was 100% recoverable from blank blood but due to the constraints of the method ethanol was required to incorporate DBCP into blood. The mean % recovery of HCE from blood with an ethanol concentration of 1% (v/v) total was determined to be 93.8 ± 1.7%, hence, the HCE areas for the non-diluted blood standards were normalized to 100%. The HCE areas for the diluted blood standards ($1.14 \cdot 10^3$, $1.14 \cdot 10^4$ and $2.28 \cdot 10^4$ ng/ml DBCP) were not adjusted. The HCE loss in these blood standards was negligible since the dilutions were done with the appropriate toluene stock solutions.

Representative chromatograms of a blank blood standard and a DBCP blood standard are presented in Fig. 1. The retention times of DBCP and HCE are 3.95 and 1.75 min, respectively.

DBCP water standards were prepared by spiking an aliquot of methanol containing DBCP into an accurately measured amount of tap water. The water standards were extracted with toluene, shaken 30 min on a wrist-action shaker and analyzed by GC–ECD. The mean % recovery for 30 determinations was 95.9 ± 2.6% (see Table II). The concentration range evaluated was 1.14 to $1.14 \cdot 10^5$ ng/ml DBCP in water.

No peaks which would interfere with the analysis were noted to be present. Blank water standards exhibited no background peaks that would interfere with the quantitative analysis of DBCP.

Representative chromatograms of a blank water standard and a DBCP water standard are presented in Fig. 2.

A plot of detector response *vs.* concentration of DBCP and HCE standards in toluene is shown in Fig. 3. The responses for both DBCP and HCE were linear for a concentration range of $2.28 \cdot 10^{-1}$ to $1.14 \cdot 10^2$ ng/ml and 1.0 to $1.73 \cdot 10^2$ ng/ml, respectively. Linearity was indicated by the linear regression correlation coefficients of 0.9999 and 0.9871 for DBCP and HCE, respectively.

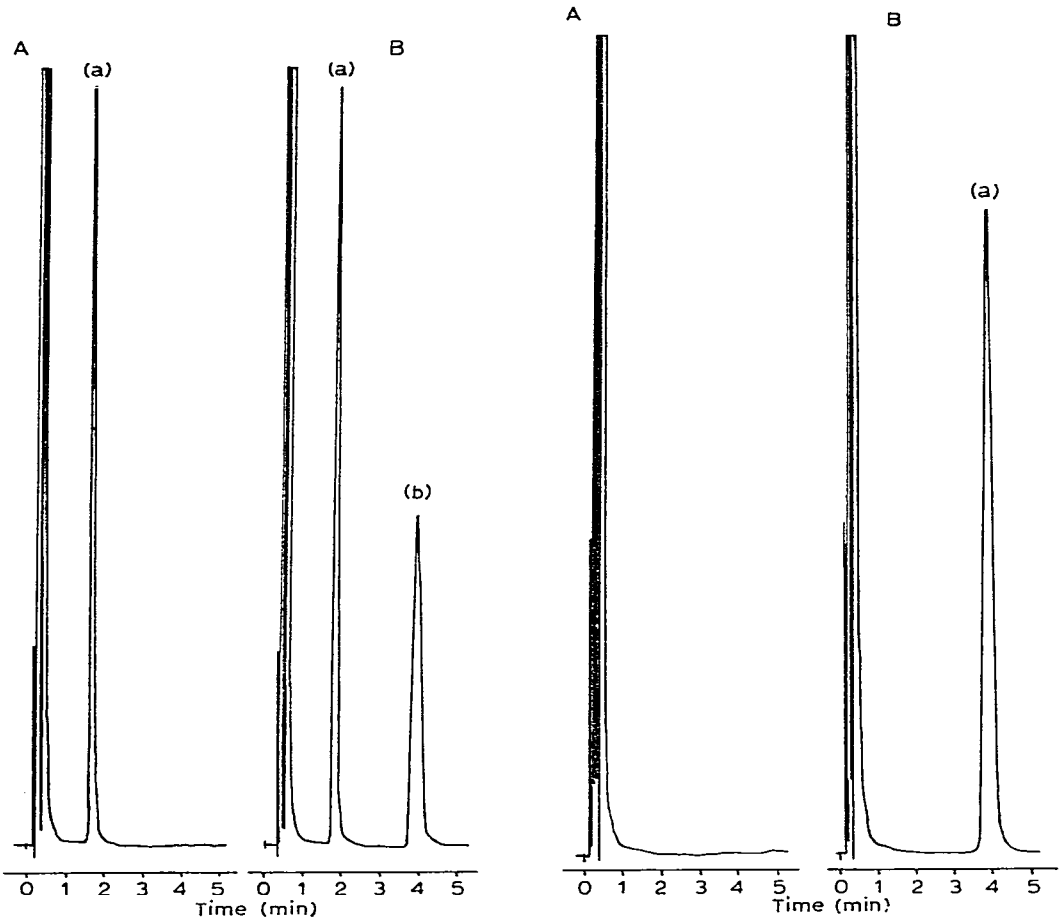

Fig. 1. Representative chromatograms of DBCP in whole blood. (A) Chromatogram corresponding to analysis of blank blood standard. (B) Chromatogram corresponding to analysis of DBCP blood standard. Peaks: a = HCE; b = DBCP.

Fig. 2. Representative chromatograms of DBCP in water. (A) Chromatogram corresponding to blank water standard. (B) Chromatogram corresponding to DBCP water standard. Peak: a = DBCP.

CONCLUSION

The method described in this paper is simple, sensitive and specific for the determination of low level DBCP concentrations in rat blood and water. DBCP concentrations above the calibrated detector range of $2.28 \cdot 10^{-1}$ to $1.14 \cdot 10^{2}$ ng/ml DBCP were diluted into the linear range.

TABLE II

RECOVERY OF DBCP FROM WATER

S.D. = Standard deviation; ND = no dilution.

DBCP concentration in water (ng/ml)	*No. of water standards*	*Extraction ratio (parts water/ parts toluene)*	*Methanol in water (%)*	*Dilution**	*Recovery ± S.D. (%)*
$1.14 \cdot 10^5$	3	1/10	1.0	1/100	97.6 ± 2.9
$1.14 \cdot 10^4$	6	1/100	1.0	ND	96.7 ± 2.5
$1.14 \cdot 10^3$	6	1/10	1.0	ND	92.9 ± 3.7
$4.56 \cdot 10^2$	6	1/4	0.4	ND	95.0 ± 3.3
$1.14 \cdot 10^1$	3	1/1	1.0	ND	92.9 ± 1.8
$4.56 \cdot 10^0$	3	1/1	1.0	ND	96.0 ± 1.5
$1.14 \cdot 10^0$	3	1/1	1.0	ND	100.0 ± 9.4
					Mean: 95.9 ± 2.6

* Dilution prior to analysis.

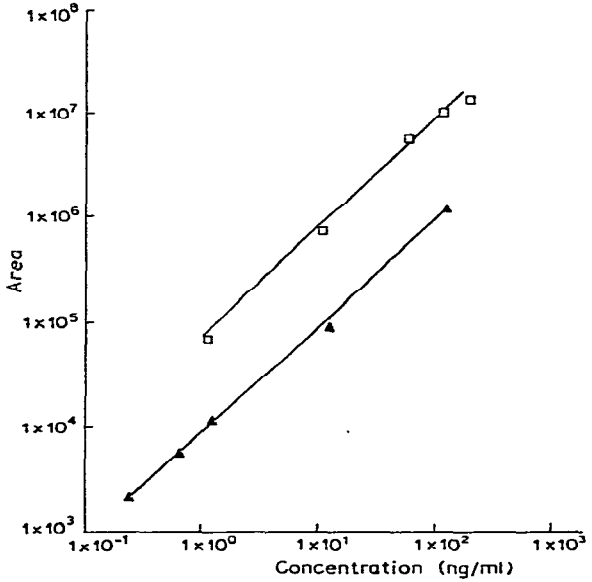

Fig. 3. log/log plot of detector response *vs.* concentration in toluene. □ = HCE; ▲ = DBCP.

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