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Note

Determination of epichlorohydrin in blood by gas chromatography and selected ion monitoring

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Epichlorohydrin (3-chloro-1,2-epoxypropane) is a monomer widely used in the manufacture of epoxy resins[1]. It has been shown to be mutagenic *in vitro* and *in vivo*[2–4], and the possibility of it having carcinogenic effects has been raised[5,6].

Epichlorohydrin has been determined by a series of different techniques such as spectrophotometry[7,8], infrared spectroscopy[9,10], gas chromatography[11–13] and mass fragmentography[14]. The methods so far described, however, lack specificity or sensitivity, or both, and are not applicable to *in vivo* determinations.

Interest in the biochemical mechanisms of the toxicity of compounds used in the plastics and rubber industry led us to develop a procedure involving gas chromatographic separation and selected ion monitoring for the determination of nanogram concentrations of epichlorohydrin in complex matrices such as biological materials.

EXPERIMENTAL

Chemicals

Epichlorohydrin was kindly supplied by the Division of Occupational Health, Montedison (Milan, Italy). Epibromohydrin (3-bromo-1,2-epoxypropane), used as internal standard for quantitation, was obtained from Merck (Darmstad, G.F.R.). All solvents were of analytical-reagent grade.

Animals

Male CD_2F_1 mice (body weight 20–22 g) were obtained from Charles River Italy (Calco, Como, Italy). Animals were given a single intraperitoneal injection of epichlorohydrin (200 mg/kg dissolved in corn oil). Groups of ten mice were killed by decapitation 1, 3, 5, 7, 10, 15, 20 and 30 min after this treatment and blood samples were collected and immediately processed for epichlorohydrin determination.

Micro-extraction procedure

To 1 ml of blood, 2 ml of 1.15% potassium chloride solution and 200 μl of methylene chloride containing epibromohydrin were added (10 μg/ml, depending on

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the expected concentration range for quantitation and the detector utilized). The appropriate amount of epibromohydrin to be added to the 3-ml biological sample was determined from a preliminary experiment to establish a suitable ratio between the peak areas. The tubes were capped, shaken on a rotary shaking system for 15 min and centrifuged at 1000 *g* for 2 min. The methylene chloride phase was transferred with a Pasteur pipette into capillary-ended glass tubes, which were further centrifuged at 4000 *g* for 10 min. Any traces of biological material and water were removed by aspiration and 2–5 μl of the methylene chloride phase were injected on to the gas chromatographic column. Addition of epichlorohydrin to epichlorohydrin-free blood at concentrations from 50 ng/ml to 100 μg/ml resulted in an extraction recovery of 65.0 $\pm$ 3.9%.

Gas chromatography

Gas chromatography was carried out on a Carlo Erba Model G1 instrument with a flame-ionization detector. The column was a glass tube, 2 m $\times$ 4 mm I.D., packed with 100–120-mesh Gas-Chrom Q coated with 3% OV-17 (Applied Science Labs., State College, PA, U.S.A.). All newly prepared columns were conditioned at 280°C for 1 h without carrier gas and then for 12 h with a carrier gas flow-rate of 15 ml/min. During analysis, nitrogen was used as the carrier gas at a flow-rate of 35 ml/min; the air and hydrogen flow-rates were adjusted to give maximal detector

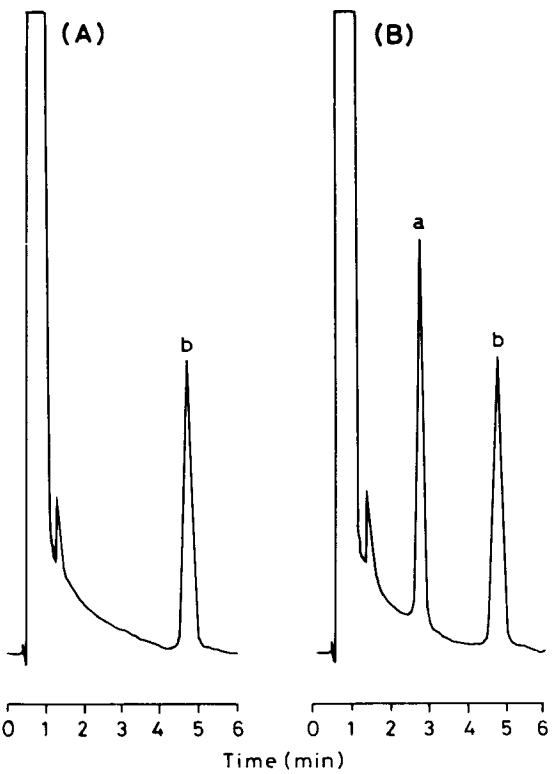

Fig. 1. Gas chromatographic analysis with flame-ionization detection of the blood of (A) an untreated mouse and (B) an epichlorohydrin treated mouse. Peaks: a = epichlorohydrin; b = epibromohydrin.

response. The column oven temperature was 60°C, injection port heater temperature 150°C and flame-ionization detector temperature 250°C.

Mass spectrometry

An LKB 2091 mass spectrometer was used, equipped with a Model 2130 computer system for data acquisition and calculation. The gas chromatographic conditions were as above, except that helium was used as the carrier gas. Selected ion monitoring was performed at 70 eV, focusing the instrument on the ions at *m/e* 62 and 64 for epichlorohydrin, *m/e* 106 and 108 for epibromohydrin and on the ion at *m/e* 57 which is common to both compounds.

RESULTS AND DISCUSSION

Fig. 1 shows typical gas chromatograms, recorded with flame-ionization detection, for the determination of epichlorohydrin in the blood of control mice (A) and treated mice (B). Epibromohydrin was chosen as the internal standard for quantitative purposes because of its similarity in structure, its suitable retention time and its pattern of fragmentation under electron impact. The chemical natures of peaks a and b were checked by mass spectrometry and the resulting mass spectra are shown in Fig. 2.

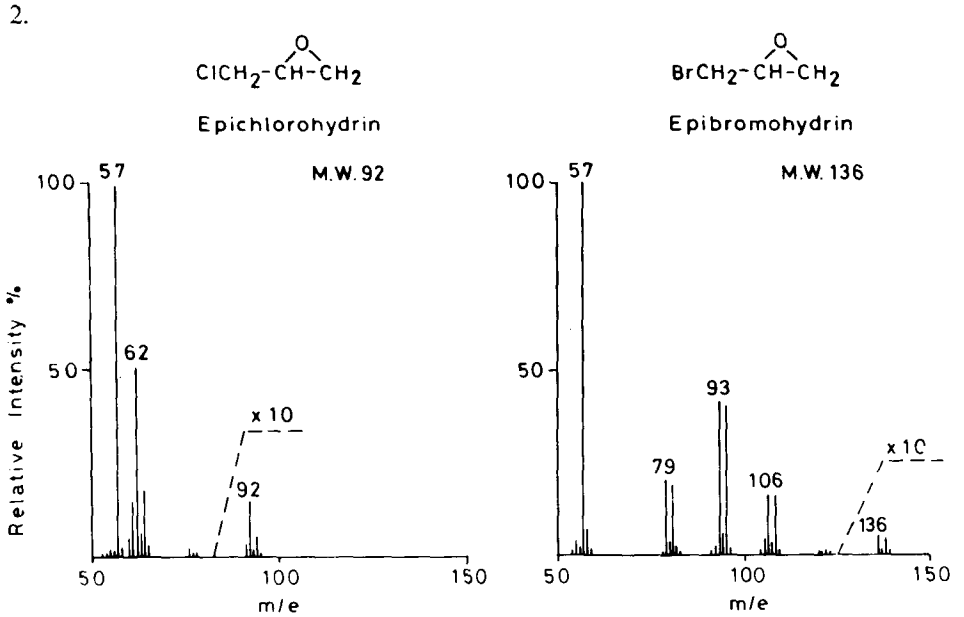

Fig. 2. Mass spectra of epichlorohydrin and epibromohydrin obtained at 70 eV.

For analysis at the nanogram level a selected ion monitoring procedure was applied, and Fig. 3 shows a typical recording for mouse blood. The mass spectrometer was focused on the ions at *m/e* 106 (isotopic 108) and 62 (isotopic 64), arising from the loss of formaldehyde from the molecular ions of epibromohydrin and epichlorohydrin, respectively, as shown in Fig. 4, and on the ion at *m/e* 57 (CH_3–CH_2–$C\equiv O^+$), the base peak in the spectra of both compounds. During the *in vivo* determinations

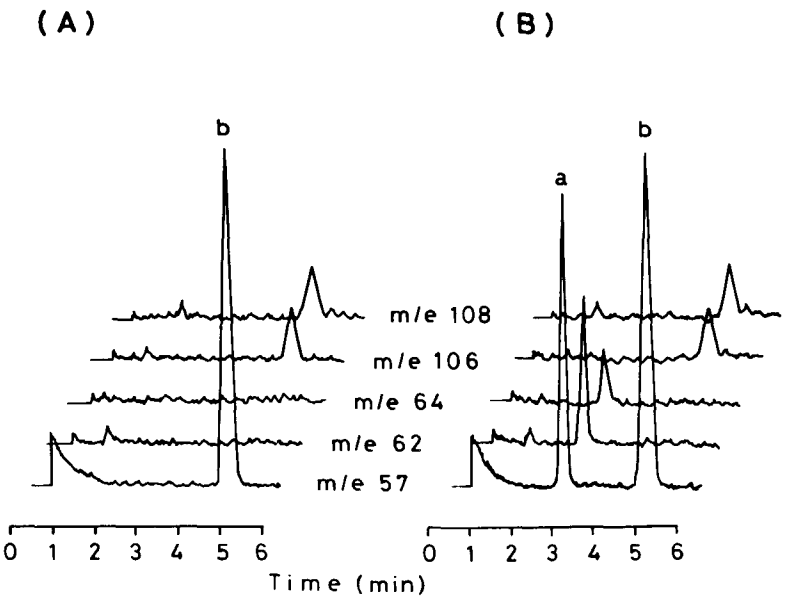

Fig. 3. Selected ion monitoring of (A) analysis of the blood of an untreated mouse and (B) analysis of the blood of an epichlorohydrin-treated mouse. Peaks: a = epichlorohydrin; b = epibromohydrin.

interferences from endogenous compounds were never observed and the detector response (flame-ionization or selected ion monitoring) was linear over a range of epichlorohydrin concentrations from 50 ng/ml to 100 μg/ml in blood.

The precision of this procedure, expressed as the coefficient of variation (C.V.), was also determined. Table I reports the results with both flame-ionization and selected ion monitoring. The concentrations selected ranged from values near the sensitivity limit of the procedure up to 100 μg/ml in blood. The method can be considered precise, as shown by the low coefficients of variation.

A kinetic study of epichlorohydrin in mouse blood was also performed to check the validity of the method for determining this compound in biological material. Fig. 5 shows a semi-logarithmic plot of the time course of epichlorohydrin concentrations in mouse blood after i.p. administration of 200 mg/kg. The monomer

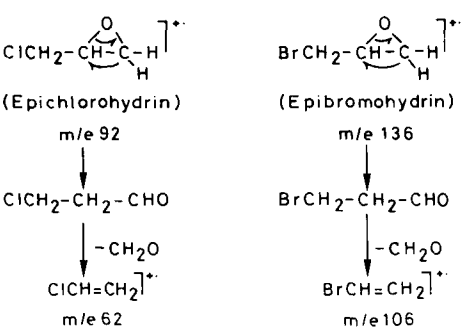

Fig. 4. Rationale for the loss of formaldehyde from the molecular ions of epichlorohydrin and epibromohydrin.

TABLE I

PRECISION OF THE METHOD FOR THE DETERMINATION OF EPICHLOROHYDRIN IN BLOOD

Parameter	*Concentration (μg/ml)*					
	Selected ion monitoring			*Flame-ionization detection*		
	0.05	*0.25*	*1*	*5*	*25*	*100*
$\bar{x}$	0.048	0.261	1.034	4.91	23.4	98.9
S.D.	0.006	0.011	0.091	0.57	1.2	3.6
C.V. (%)	12.5	4.2	8.8	11.6	5.1	3.6
n	5	5	5	5	5	5

is rapidly absorbed, reaching a peak concentration of 3.7 μg/ml within the first few minutes, after which it disappears so fast that after 15 min it is only just detectable.

The parameters describing the kinetics of epichlorohydrin in mouse blood are reported in the legend to Fig. 5 and were calculated by the method of residuals[15]. The extremely short *in vivo* half-life of epichlorohydrin provides evidence of its biological lability. Apart from its chemical reactivity towards cellular molecules and/or macromolecules, epichlorohydrin may also be a good substrate for epoxide hydrolase and glutathione-S-epoxide transferase, two ubiquitous enzymatic systems.

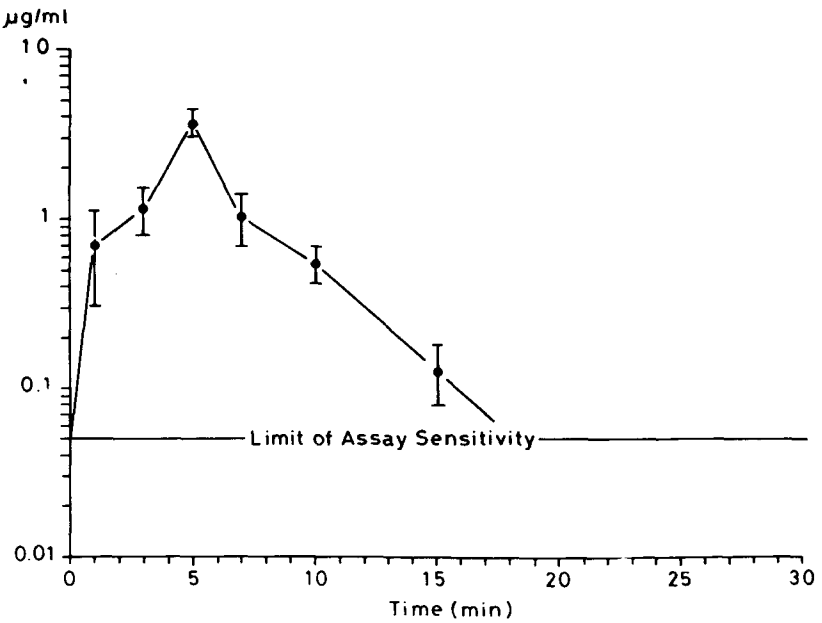

Fig. 5. Blood concentrations of epichlorohydrin at different times after intraperitoneal injection of 200 mg/kg. Each point is the mean value ± standard deviation of ten determinations. Kinetic parameters: K_a, rate constant of absorption = 0.405 min^{-1}; K_{el}, rate constant of elimination = 0.518 min^{-1}; $T_{\frac{1}{2}}$, half-life = 1.3 min; C_0, concentration extrapolated at time zero = 13.6 μg/ml; V_d, apparent distribution volume = 68.4 l/kg; AUC, area under the curve = 9.2 μg/ml · min.

In conclusion, the simple and rapid method described here offers better specificity and sensitivity than the procedures previously published, and should lend itself to application in experimental toxicology to help clarify the molecular mechanisms of epichlorohydrin toxicity.

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