TWO RAPID METHODS FOR THE SIMULTANEOUS GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF CARBON TETRACHLORIDE AND CHLOROFORM IN BIOLOGICAL MATERIAL AND EXPIRED AIR*

COLIN J. REDDROP**, WALTER RIESS and TREVOR F. SLATER

Biochemistry Department, Brunel University, Uxbridge UB8 3PH, Middlesex (Great Britain) and Pharmacological Chemistry Department, Ciba-Geigy Ltd., CH-4002 Basle (Switzerland)

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SUMMARY

Two simple gas–liquid chromatographic techniques were developed for the simultaneous determination of CCl_4 and $CHCl_3$ in biological material and expired air and principally for use in the well-known CCl_4-induced hepatotoxicity model: a non-extractive head-space analysis by flame ionization detection (FID) and a single-step toluene extraction using electron-capture detection (ECD).

For head-space analysis, blood or liver homogenate is incubated with buffer in sealed reaction vials and the head-space vapour sampled for FID determination. Absolute signal response to CCl_4 and $CHCl_3$ was used for calibration in the range 5–500 μg per gram of biological material. The method is reasonably accurate, *e.g.* CCl_4 in liver homogenate 98 $\pm$ 21.8 (S.D.) %, in blood 94 $\pm$ 13.3%, but the precision is poor (rel. S.D. 10–20%). Air samples in volumes of up to 2 ml may be determined by direct FID injection.

The ECD sensitivity of to CCl_4 and $CHCl_3$ permits determination of micro-samples (50–500 μl) of blood and liver homogenate by extraction with buffer into toluene containing an internal standard (propyl iodide). The linear range of the detector allowed calibration by peak area ratio in the concentration range 10–1500 ng of CCl_4 or $CHCl_3$ per millilitre of toluene. The accuracy of the method is high, *e.g.* in blood $CHCl_3$ 101 $\pm$ 9.5 (S.D.)%, CCl_4 100 $\pm$ 15.2%, as is the precision: rel. S.D. *ca.* 5% for both CCl_4 and $CHCl_3$. For elimination studies, CCl_4 and $CHCl_3$ in air may be trapped in toluene and determined by ECD. Recovery of known amounts of CCl_4 and $CHCl_3$ from an air chamber was high: 100 $\pm$ 4.7 (S.D.)% and 111 $\pm$ 10.9%, respectively, and reduction of CCl_4 to $CHCl_3$ by the trapping system negligible (<0.01%).

Cross-checking of the methods and application to the commonly used CCl_4 hepatotoxicity model is demonstrated.

* These studies form part of the Ph.D. thesis of C.J.R.

** To whom correspondence should be addressed, c/o Pharmacological Chemistry Department, Ciba-Geigy Ltd., CH-4002 Basle, Switzerland.

INTRODUCTION

The measurement of chlorinated hydrocarbons has become increasingly important to a wide range of scientific investigation, *e.g.* environment, industry, food technology, agriculture, medicine. There consequently exists a large body of information on the general occurrence of these substances in our environment and their hazards (see McConnell[1] for a review). It is now clear that many of these otherwise relatively inert chemicals undergo biological "activation" to form highly reactive intermediates (*e.g.* free radicals, epoxides) capable of eliciting a variety of toxic effects (see Slater[2] for a review). In the study of the biochemical mechanisms of these toxic activations, the measurement of the reactive intermediates[3,4] (or their products[5–9]) is therefore an area of primary interest.

Biochemical aspects of the carbon tetrachloride (CCl_4) hepatotoxicity rat model, for example, have been studied intensively for 40 years[10,11]. However, little work has been conducted on the distribution and metabolism of CCl_4 *in vivo*[5,6,12,13]. This situation arose historically because of the difficulty in developing methods for measurement of trace amounts of volatile materials (including metabolites) in biological tissues[12]. A recent comprehensive review[14] shows that a large number of highly specific and sensitive gas–liquid chromatographic (GLC) techniques are now available for determination of CCl_4 in water and air using four basic sample handling methods: direct aqueous injection, solvent extraction, gas stripping and head-space sampling (see Dietz and Singley[15] for recent examples of each). However, still only very few methods deal specifically with biological media, and the techniques used are often impractical for analysis of large numbers of samples (complex sampling or clean-up procedures, *e.g.* distillation[6], purge/trap[9]).

Homolysis to give the highly reactive $CCl_3^{\cdot}$ free radical is generally accepted as the starting point of CCl_4 metabolism and toxicity[10,11], indirect evidence being provided by the detection of chloroform ($CHCl_3$) and hexachloroethane in the livers of CCl_4-treated rats and rabbits[5,6]. We therefore recognized that the development of simple GLC methods for simultaneous determination of CCl_4 and $CHCl_3$ would provide rapid means of monitoring the kinetics of metabolism of CCl_4 *in vivo* and of screening the effect of various pre-treatments and "hepato-protective" agents on the toxic metabolism. Simple methods considered most suitable for development were a flame ionization detector (FID) head-space analysis of volatile solvents in whole blood[16] and an electron-capture detector (ECD) determination of CCl_4 and $CHCl_3$ extracted by toluene from liver microsome suspensions[17].

MATERIALS AND METHODS

Reagents and glassware

CCl_4 (for spectroscopy) and $CHCl_3$ (analytical grade) (Merck, Darmstadt, G.F.R.) and propyl iodide (analytical grade) (Fluka, Buchs, Switzerland) were used throughout. All other reagents were of analytical grade. Organic solvents were tested for purity and possible GLC interference by carrying out blank runs.

No special treatment of glassware is required.

Gas chromatography

The instruments used for FID and ECD analysis were Perkin-Elmer Models 900 and 3920, respectively, the latter employing ^{63}Ni as the ionizing source. Each gas chromatograph was attached to an Infotronics CRS 208E electronic integrator with angular baseline corrector and a W + W 1100 recorder.

20% Supelco SP-2100 plus 0.1% Carbowax 1500 on Supelcoport 80–100 mesh was chosen as a suitable phase for the separation and determination of chlorinated solvent mixtures, and a 3.0 m × 2 mm I.D. Pyrex glass column employed. Optimization of peak separation and retention times was achieved in both instruments by temperature control alone. Final GLC operating conditions adopted were as follows:

		FID	*ECD*
Temperatures (°C):	Injector	100	100
	Column	95	75
	Interface	—	200
	Detector	140	300
Gas flow-rates (ml/min):	N_2	20	15 + 30
	H_2	equiv. 2.4 kg/cm²	—
	Air	equiv. 3.5 kg/cm²	—
Attenuation:		×8	×512
Standing current (nA):		—	1.0

The choice of propyl iodide (PI) as internal standard for the ECD method was governed by the existing chromatogram peaks: a retention time of 6–9 min under these conditions was required.

The linear range of the ECD was estimated by injection of standard toluene solutions of CCl_4 and $CHCl_3$ using PI as internal standard, and was found to be equivalent to *ca.* 8–80 pg CCl_4 at attenuation ×512. An internal standard concentration of 0.1 nl PI/ml toluene was chosen as one allowing wide variation of GLC injection volume (*e.g.* 0.2–5.0 μl) without danger of leaving the linear range of the detector.

Head-space analysis method (FID)

Liver homogenate. The method chosen for optimization was of the general concept described by Premel-Cabic *et al.*[16] for blood analysis: 1 g homogenate (for preparation, see below) weighed into a glass reaction vial, vial sealed with septum and gas-tight PTFE screw closures. Add 1 ml diluent and × μl organic solvents (both injected through the septum). Mix for 10 sec on a vortex mixer. Incubate at 30° for 30 min. Remove aliquot of head-space vapour for GLC injection. Only homogenate from CCl_4-treated rats was used for method development.

Ultrasonic treatment (50 kHz for 30 sec) did not improve signal response. Diluted samples were found to give greater signal response but the nature of the diluent appears to be unimportant. Phosphate buffer (pH 7.0) was therefore employed as this is used in the initial liver homogenization.

Several similar solvents were tested for use as internal standards to CCl_4 and $CHCl_3$ but rejected as the response of the system using the peak area or height ratio calibration method was non-linear. A complex interplay of solubility, partition and

vapour pressure properties was probably involved. However, the absolute signal responses of CCl_4 and $CHCl_3$ (electronic integration of signal peak, rel. units) in the same experiments were linear and calibration was possible.

More consistent signal response was obtained by raising the incubation temperature from 30° to 35°. At this temperature an incubation time of 30 min appeared to be satisfactory for establishing a head-space equilibrium.

In expectation of encountering samples with different relative concentrations of $CHCl_3$ (present as a metabolite of CCl_4), the effect of presence or absence of $CHCl_3$ on CCl_4 signal response was investigated but no significant differences were apparent up to CCl_4 concentrations of 640 μg/g liver.

Losses incurred during homogenization and handling of CCl_4-containing tissues are inevitably large[12]. Attempts to homogenize small liver samples (0.1–0.5 g) inside sealed reaction vials by sonication or use of solubilizers were unsuccessful. Estimation of losses on homogenization was carried out by injecting known volumes of CCl_4 and $CHCl_3$ (or equilibrated aqueous solutions of CCl_4 and $CHCl_3$ (ref. 12)) directly into rat livers, and homogenizing with phosphate buffer pH 7.0 (1:1, w/w) using a Bühler HO sealed-chamber, ice-cooled homogenizer run for exactly 30 sec. Losses were large but reproducibility good (Table I), and the signal response *versus* concentration graphs were linear.

TABLE I

FID HEAD-SPACE ANALYSIS

Recovery of CCl_4 and $CHCl_3$ from rat liver homogenate prepared with a Bühler HO sealed-chamber, ice-cooled homogenizer; CCl_4 and $CHCl_3$ injected into freshly excised liver immediately prior to homogenization. Recovery is defined as the GLC signal response from analysis of fresh homogenate samples expressed as a percentage of the signal response obtained from equivalent concentrations injected into blank liver homogenate in sealed reaction vials.

Conc. CCl_4, $CHCl_3$ in homog. (μg/g)	*Day*	*Recovery (%)*	
		$CHCl_3$	*CCl_4*
600	1	36.7	28.9
	2	36.6	34.4
	3	34.9	33.9
300	1	38.8	33.5
	2	29.4	34.3
	3	38.0	37.5
150	1	31.1	26.3
	2	39.7	41.8
	3	34.8	39.6
75	1	32.3	29.1
	2	35.9	40.1
	3	37.3	32.9
30	1	30.4	24.6
	2	42.1	46.2
	3	40.5	31.9
	$\bar{x}$	35.9	34.3
	S.D.	3.79	5.93
	S.E.M.	0.98	1.53
	C.V. (%)	10.6	17.3

The effect of storage and handling on homogenate samples was investigated in order to avoid the impractical necessity of analysing samples immediately after homogenization. Maximum, consistent signal response was obtained by snap-freezing sample tubes of freshly prepared homogenate on dry ice (−78°) prior to normal deep-freeze storage (−20°), then thawing the samples quickly (water bath, 35°) prior to analysis. This procedure increased signal response (*versus* fresh samples) significantly, presumably by assisting solvent release through improved cell lysis.

The following whole-process (homogenization, storage, sampling) final procedure was therefore adopted: 1 g liver homogenate. Seal reaction vial. Inject 1 ml phosphate buffer (pH 7.0) through the septum. Mix for 10 sec on a vortex mixer. Incubate at 35° for 30 min. Remove 200 μl of head-space vapour for GLC injection. Calibration graphs were prepared in the concentration range 15–500 μg CCl_4 or $CHCl_3$ per gram of liver (Fig. 1).

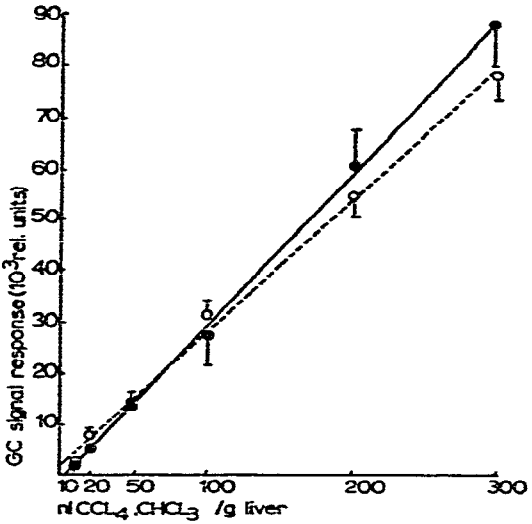

Fig. 1. FID head-space analysis. Whole-procedure calibration graphs for (●) CCl_4 and (○) $CHCl_3$ in rat liver homogenate. CCl_4 and $CHCl_3$ injected directly into rat liver prior to homogenization. After deep-freeze storage, samples analysed without addition of toluene. Injection volume, 200 μl head-space vapour; GC attenuation ×8. Mean ± S.D. ($n = 4$). Regression equations: (●) CCl_4, $y = -1347 + 301.51x$ ($r = 0.990$); (○) $CHCl_3$ (×10), $y = 1602 + 260.51x$ ($r = 0.994$).

Blood. Fresh 1 g samples from CCl_4-treated rats were used for studies on the release of CCl_4 and $CHCl_3$ from blood using the final procedure for liver homogenate. However, conflicting results obtained by substitution of diluents of various pH in the reaction vials led to the choice of borax buffer (pH 10.0) as the optimal diluent for obtaining complete haemolysis under the mildest possible pH conditions.

Calibration graphs in the range 7.5–80 μg/g blood were obtained by analysis of samples injected with 5.0 μl of toluene solutions of CCl_4 and $CHCl_3$ through the vial septum (Fig. 2).

The effect of storage and sampling on blood samples was investigated by comparing the signal responses of fresh blood and deep frozen/thawed samples

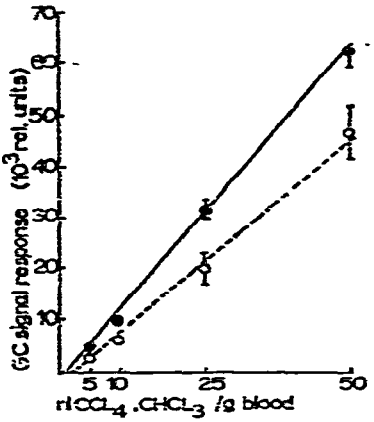

Fig. 2. FID head-space analysis. Blood calibration graphs for (●) CCl_4 and (○) $CHCl_3$ in the whole blood of rats. Each tube was injected with 5 μl of a toluene solution of CCl_4 and $CHCH_3$ to the required concentration. Injection volume, 200 μl head-space vapour; GLC attenuation ×8. Mean ± S.D. ($n = 6$). Regression equations: (●) CCl_4, $y = -1250 + 1290.47x$ ($r = 0.995$); (○) $CHCl_3$ (×10), $y = -3069 + 957.66x$ ($r = 0.977$).

(storage as for liver homogenate, above). Recoveries averaging 71 ± 5.8 (S.D.)% of the fresh blood values were recorded for CCl_4. A correction factor of 1/0.7 was therefore applied to the CCl_4 signal responses obtained from samples handled in this way.

Expired air. The FID system as described is suitable for direct injection of large volumes (up to 2 ml) of air drawn from a sealed breathing chamber of the type described by Lindstrom and Anders[18].

As FID calibration relies on absolute signal response and no internal standard is used, daily GLC calibration is required. Machine response at various attenuations was found to be constant over a 2-month period.

Toluene extraction method (ECD)

Liver homogenate and blood. Extraction of CCl_4 and $CHCl_3$ from biological material by toluene was investigated using samples of liver homogenate and blood from a CCl_4-treated rat and found to be satisfactory and constant at pH > 7. Therefore, in consideration of the variation in blood-lysing properties of buffers (referred to above), borax buffer (pH 10.0) was chosen as aqueous extractant. The effect of this pH on CCl_4 and $CHCl_3$ signal response after long periods of shaking was negligible. The following general procedure was adopted for development and calibration purposes: Liver homogenate or blood 50–100 μl. Add 2.0 ml borax buffer (pH 10.0), 0.025–0.500 nl CCl_4, $CHCl_3$ abs. in toluene, 0.500 nl PI abs. in toluene (internal standard) and toluene to 5.0 ml. Shake at 120 rpm for 10 min. Centrifuge, remove organic phase and inject 1.0 μl into ECD .

The efficiency of extraction of the toluene–buffer system was tested on calibration samples and samples from CCl_4-treated rats by analysis of toluene fractions from repeated extractions of the same aqueous/biological layer. Table II shows that the extraction efficiency of a single extraction may thus be estimated to be *ca.* 99% for 50–100 μl samples and greater than 90% for volumes up to 500 μl. In calibration

TABLE II

ECD ANALYSIS

Efficiency of extraction of CCl_4 and $CHCl_3$ from biological material using a toluene–borax buffer (pH 10) extraction system. Recovery (measured as signal response) of CCl_4 and $CHCl_3$ on *re-extraction* of biological material, expressed as a percentage of original signal response.

Sample		*Re-extraction peak area ratio (:PI) (% of original)*	
		$CHCl_3$	*CCl_4*
Calibration samples			
Blood	100 µl	< 0.1	< 0.1
	500 µl	1.8	< 0.1
Liver	100 µl	< 0.1	< 0.1
	500 µl	0.8	< 0.1
		0.9	< 0.1
Biological samples			
Blood	100 µl	< 0.4	< 0.2
		< 0.3	< 0.1
		< 0.1	< 0.1
	500 µl	< 0.5	< 0.1
		< 0.1	0.5
		< 5.0	6.3
Liver	100 µl	< 0.5	0.6
		< 0.1	0.4
	500 µl	2.6	8.2
		< 2.0	2.9

samples, biological material volume variations in the range 50–500 µl had no major effect on GLC signal response. Further evidence for extraction efficiency is also provided by comparison of the regression equations to the calibration graphs of unextracted toluene solutions with those prepared from the same solutions extracted from whole blood and liver homogenate (Table III, Fig. 3).

TABLE III

LINEAR REGRESSION EQUATIONS FOR ECD DETERMINATION OF CCl_4 AND $CHCl_3$ IN THE CONCENTRATION RANGE 0.005–0.100 nl/ml TOLUENE IN THE PRESENCE OF PI 0.1 nl/ml (INTERNAL STANDARD)

	Medium	*Regression equation, $y = a \cdot x + b$**		*Regression coefficient*	
		a	*b*	*r*	*n*
$CHCl_3$	Toluene	42.624	0.072	0.999	21
	Blood	40.143	0.172	0.995	18
	Liver homog.	43.018	0.196	0.997	18
CCl_4	Toluene	38.122	−0.071	0.998	19
	Blood	38.385	−0.084	0.997	18
	Liver homog.	42.151	−0.160	0.993	18

* Where y = peak area ratio (CCl_4, $CHCl_3$ × 10:PI) and x = CCl_4, $CHCl_3$ concentration (nl/ml).

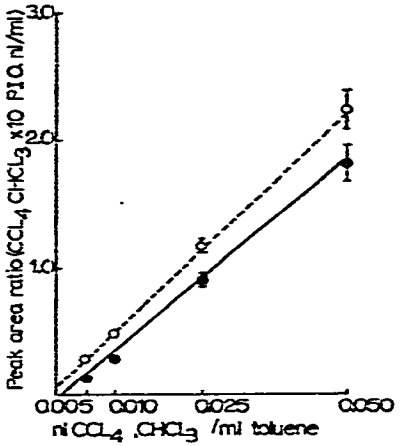

Fig. 3. ECD analysis. Calibration graphs for CCl_4 and $CHCl_3$ in toluene. Mean ± S.D. ($n = 4$). Regression equations: (●) CCl_4, $y = 0.072 + 42.624x$ ($r = 0.999$); (○) $CHCl_3$ (×10), $y = -0.071 + 38.122x$ ($r = 0.998$).

The general procedure for ECD calibration described above was therefore revised as follows for determination of biological samples: 2.0 ml borax buffer (pH 10.0). Add 1.0–5.0 ml toluene containing PI (0.1 nl/ml). Weigh tube and contents. Add 50–500 μl liver homogenate or blood. Reweigh tube to obtain exact sample weight. Shake at 120 rpm for 10 min. Centrifuge, remove organic layer for ECD determination diluting as required with 0.1 nl PI/ml toluene.

Expired air. A flow-through breathing chamber system of the type commonly used for expired $^{14}CO_2$ measurement was converted and optimized for expired CCl_4 and $CHCl_3$ determination.

Air for determination is drawn from a breathing chamber through glass tube bubble traps containing toluene maintained at 0° by ice-filled Dewar flasks. Internal standard PI is then added and the collected samples determined by direct ECD injection.

RESULTS

Chromatograms

Typical chromatograms recorded during FID and ECD analysis are shown in Fig. 4.

Sensitivity, accuracy and precision

The FID method allows accurate determination of concentrations of *ca.* 5 μg (30 nmol) of CCl_4 or $CHCl_3$ per gram of biological material. This limit may be lowered by increasing the volume of head-space vapour subjected to analysis (*e.g.* to 1.0 ml) and/or by decreasing the signal attenuation used.

The ECD method allows accurate determination of concentrations of *ca.* 10 ng (75 pmol) of CCl_4 or $CHCl_3$ per millilitre of toluene. In consideration of the tested volume range, this corresponds to *ca.* 15 ng (100 pmol) of CCl_4 or $CHCl_3$ per gram of biological material. Extension of the valid sample volume range and establish-

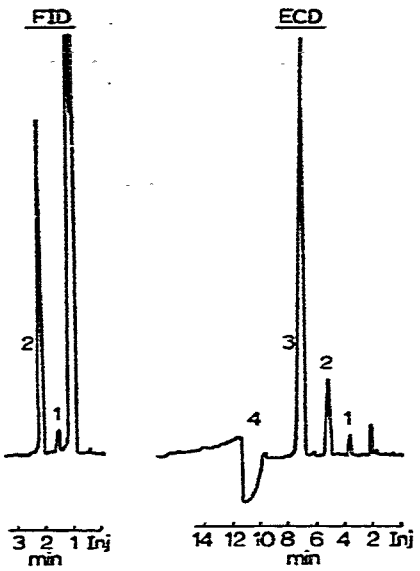

Fig. 4. Typical chromatograms recorded during FID and ECD analysis of whole blood from CCl_4-treated rats. FID peaks: 1 = $CHCl_3$; 2 = CCl_4; injection volume, 200 μl. ECD peaks: 1 = $CHCl_3$ (equivalent to 7.5 pg absolute injected); 2 = CCl_4 (8.0 pg abs.); 3 = propyl iodide (internal standard, 175 pg abs.), 4 = toluene (solvent). Injection volume, 1.0 μl. For complete GLC conditions, see the text.

ment of detector linearity at lower attenuations ($< \times 512$) would permit determination of lower concentrations.

The accuracy of the methods was established by determination of concentrations within the calibration ranges but unknown to the analyst. For liver homogenate, CCl_4 and $CHCl_3$ were injected into freshly excised rat livers prior to homogenization. For blood, solutions of CCl_4 and $CHCl_3$ were added directly. The results (Table IV) indicate that both methods are sufficiently accurate for concentration–time measurements. However, the recovery variations are on the limit of acceptability, and efforts to increase the sensitivity of the methods would also require investigation of recoveries at the lower end of the calibration scale.

TABLE IV

ACCURACY OF THE METHODS

Determination of concentrations within the calibration range but unknown to the analyst.

Method	*Sample*	*Recovery (%) $\bar{x} \pm$ S.D. (n)*	
		$CHCl_3$	*CCl_4*
FID	Blood	114 ± 18.0 (9)	94 ± 13.3 (13)
	Liver homog.	108 ± 14.4 (11)	98 ± 21.8 (13)
ECD	Blood	101 ± 9.5 (7)	101 ± 15.2 (7)
	Liver homog.	98 ± 18.2 (6)	103 ± 12.2 (7)

As progressive loss of CCl_4 and $CHCl_3$ from biological material is rapid, FID method precision could only be estimated from analysis of multiple calibration samples over a period of weeks (Table V). The precision of the ECD method was established with fresh whole-blood samples. Using a retro-orbital serial sampling method, consecutive series of 5–6 drops of blood from a CCl_4-treated rat were collected in five tared tubes containing buffer–toluene and the CCl_4 and $CHCl_3$ concentrations determined against PI. A whole-process coefficient of variation of *ca.* 5% was achieved (Table V).

TABLE V

PRECISION OF THE METHODS

Method	*Sample*	*Rel. standard deviation (%)*		
		$CHCl_3$	*CCl_4*	*n*
FID	Blood (25 nl/g)	15.9	4.2	8
	Liver homog. (200 nl/g)	11.2	4.5	4
	Liver homog. (10 nl/g)	21.1	19.1	4
ECD	Blood series A	2.3	5.5	5
	series B	2.0	3.3	5
	series C	9.7	10.1	5

Stability

Standard solutions of CCl_4 and $CHCl_3$ in toluene (concentrations 1–30 μl/ml) used for daily FID calibration and preparation of FID and ECD calibration samples were stable for at least 10 days at room temperature. However, as a very slight decrease in the relative signal responses was observed, it is recommended that all extracted toluene samples be analysed within 48 h of preparation.

The inevitable losses incurred between collection and analysis of biological samples can be reduced and determined under the controlled handling conditions described in the FID method above. Loss on storage of blood samples is extensive and occurs rapidly and inconsistently, especially when sample tubes are only partly filled. Liver homogenate in well-filled tubes is more stable: calibration samples were stable for up to 6 weeks following freezing at −78° and storage at −20°. It is nevertheless recommended that after snap-freezing, biological samples be analysed within 72 h.

Recovery from expired air

Amounts of CCl_4 and $CHCl_3$ in the ranges 3–7.5 mg (*ca.* 20–50 μmol) and 60–375 μg (*ca.* 0.5–3.0 μmol), respectively, were injected into the flow-through breathing chamber of the expired air apparatus, withdrawn in the chamber air and collected in toluene bubble traps. The recovery, expressed as percentage of injected amount, indicates the efficiency of the trapping method: CCl_4: $100.4 \pm 4.7\%$ ($\bar{x} \pm$ S.D., $n = 5$); $CHCl_3$: $110.7 \pm 10.9\%$. The slightly higher values obtained for $CHCl_3$ are due to background from adsorption on to the glass walls of the apparatus. To ensure that further contribution to $CHCl_3$ levels did not arise through reduction of CCl_4 by the system itself, large volumes of CCl_4 alone were injected into the breathing

chamber and, following recovery in the toluene traps, analysed for $CHCl_3$ content. Three separate tests indicated that such a reduction of CCl_4 by the system is negligible (<0.01 %).

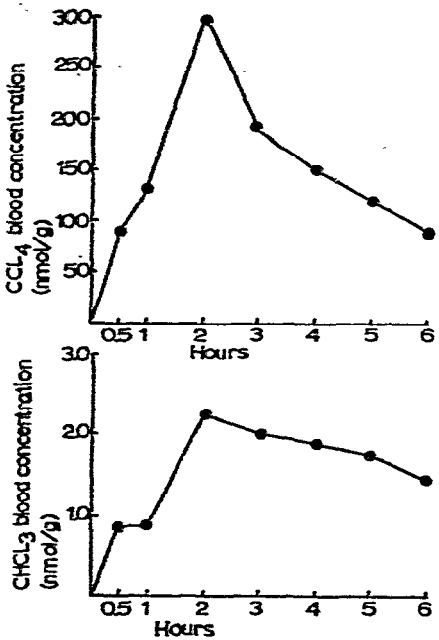

Fig. 5. ECD analysis. Simultaneous determination of CCl_4 and $CHCl_3$ in micro-samples (*ca.* 100 μl) of whole blood following administration of CCl_4 13 mmol/kg body weight by intragastric canula to a single, male, fasted, 200 g Tif RAI f(SPF) rat. Blood samples were collected at each time point using a retro-orbital capillary sampling technique.

TABLE VI

FID/ECD CROSS-CHECK

CCl_4 blood concentrations (nl/g) 2.0 h after administration of CCl_4 13.0 mmol/kg body weight by intra-gastric canula to Tif RAI f (SPF) male, fasted, 200 g rats. FID blood samples: rats sacrificed at 2.0 h (Group I). ECD blood samples: micro-samples taken at 2.0 h from a separate set of rats (Group II)*

	FID analysis Group I rats (n = 7)	*ECD analysis Group II rats (n = 6)*
	15.3	30.5
	10.6	40.0
	26.4	30.3
	46.2	12.2
	11.6	14.6
	36.6	27.8
	35.0	
$\bar{x}$	26.0	25.9
± S.D.	13.91	10.57

* This is an historical comparison only: FID and ECD methods were not operating simultaneously.

APPLICATION

Comparison of the CCl_4 blood concentration data produced by the FID and ECD methods and presented in Table VI provides confirmation of their accuracy, despite the fact that different blood sampling techniques were used for each method.

The power of the ECD method is displayed in Fig. 5. Using a retro-orbital serial sampling method, CCl_4 and $CHCl_3$ blood concentrations may be followed in a single rat.

It is concluded that these methods are suitable for detailed investigation of kinetic and metabolic aspects of CCl_4 intoxication in experimental animals.

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