

THE BIOLOGICAL FATE OF VINYLIDENE CHLORIDE IN RATS

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SUMMARY

The main eliminative route for [^{14}C]vinylidene chloride ([^{14}C]DCE) after intragastric, i.v. or i.p. administration to rats is pulmonary; both unchanged DCE and DCE-related CO_2 are excreted by that route and other DCE metabolites via the kidneys. Part of the urinary ^{14}C is of biliary origin. After intragastric dosing, the plot of the pulmonary output of unchanged DCE against the logarithm of reciprocal doses is biphasic. Pulmonary elimination of DCE and CO_2 and urinary excretion of DCE metabolites after an intragastric dose occupy 3 days. In comparison, 80% of a small i.v. dose is excreted unchanged within 1 h of injection; more than 60% within 5 min.

Biotransformation of DCE affords thiodiglycollic acid, and an *N*-acetyl-S-cysteinyl-acetyl derivative as major urinary metabolites together with substantial amounts of chloroacetic acid, dithioglycollic acid and thioglycollic acid. It is probable that chloroacetic acid, which is a DCE metabolite per se, lies on a main metabolic pathway for DCE, since it affords several metabolites in common with DCE. Furthermore, electrolysis of one molecular proportion of the [^{14}C]thiodiglycollate metabolite from [$1\text{-}^{14}\text{C}$]DCE or [$1\text{-}^{14}\text{C}$]chloroacetic acid gives 1 equivalent of $^{14}\text{CO}_2$, and this evidence is consistent with the transformation of DCE into chloroacetic acid by a mechanism involving the migration of one Cl atom and the loss of the other one. CO_2 (and hence urea) may be produced through the action of epoxide hydratase on 1,1-dichloroethylene oxide or by a minor oxidative pathway for chloroacetic acid. The *N*-acetyl-S-cysteinyl-acetyl derivative is probably formed via the reaction of 1,1-dichloroethylene oxide and glutathione S-epoxide transferase.

INTRODUCTION

The widespread usage of the polymer of vinylidene chloride (1,1-dichloro-

Abbreviations: DCE, vinylidene chloride, TFAA, trifluoroacetic anhydride.

ethylene, DCE) for packaging film and for coating other packaging materials, and the fact that the structurally-related vinyl chloride is a frank carcinogen in animals and in man (see ref. 1, which contains a summary of the position of this subject), make an understanding of the biological fate of DCE in mammals desirable. This paper describes the results of such an investigation and their possible interpretation.

The scientific background to this problem has grown during the time that the present experiments were being made. Thus, *in vitro*, DCE was shown to be mutagenic in the tissue-mediated Ames test [2,3]. A much higher mutagenic response was observed in mice than in rats with kidney and liver fractions [2], but the mutagenicity of DCE was very much lower than that of vinyl chloride [3]. A high incidence of an unusual kidney tumour, the kidney adenocarcinoma, has now been found [4] in the Swiss mice, but not in the Sprague-Dawley rats, that had been exposed chronically to DCE. However, so far no case of DCE carcinogenicity has been reported in man.

MATERIALS AND METHODS

Chemicals

Vinylidene chloride was supplied by Imperial Chemical Industries Ltd., Mond Division, Runcorn, Cheshire. Before each experiment, stabiliser was removed by washing with alkali and water.

Dithioglycollic acid (Aldrich Chemical Co.), and thiodiglycollic acid and thioglycollic acid (both obtained from the Sigma Chemical Co.) were all of grade 1 quality with a purity exceeding 99.5%.

S-(2-Carboxymethyl)-L-cysteine was prepared [5] from chloroacetic acid plus L-cysteine hydrochloride, and the structure of the crystalline product (m.p. 175–176° C, decomp.) was confirmed by mass spectrometry. 4-Acetyl-5-carboxy-3-oxo-tetrahydro-1,4-thiazine was prepared from *N*-acetyl-*S*-(2-carboxymethyl)-L-cysteine by the method of Ozawa [6].

All reagents and solvents were of AnalaR grade or of the next best quality available.

Radioactive chemicals

[1-¹⁴C]DCE and [2-¹⁴C]DCE, with a specific activity of 1.0 mCi/mol and with a chemical and radiochemical purity exceeding 99.0%, were synthesized from ¹⁴CO₂ by our colleagues, Mr. D.C. Greenslade and Dr. J.A. Heslop, of Imperial Chemical Industries Ltd., Petrochemicals Division, Billingham, Cleveland. Whereas in the case of [1-¹⁴C]DCE, ¹⁴CO₂ was converted into [1-¹⁴C]acetic acid by the Grignard reaction with methyl iodide, in that of [2-¹⁴C]DCE, ¹⁴CO₂ was reduced to [¹⁴C]methanol, which was transformed by a reaction sequence involving [¹⁴C]methyl iodide and [2-¹⁴C]acetonitrile into [2-¹⁴C]acetic acid.

Whence [1-¹⁴C] and [2-¹⁴C]acetic acids respectively were transformed through the variously labelled forms of the reaction sequence, dichloro-

acetic acid, 2,2-dichloroethanol and 1,1,2-trichloroethane, into the pure liquids [$1\text{-}^{14}\text{C}$]DCE and [$2\text{-}^{14}\text{C}$]DCE, b.p. 37°C , which were stabilized by addition of 10 ppm of hydroquinone. (1,2-Elimination of 1,1,2-trichloroethane was specific, and gave DCE exclusively, but the attempted 1,2-elimination of 2,2-dichloroethanol furnished intractable polymer.) On account of the risk of polymerization, it was convenient to store [^{14}C]DCE as a solution in peroxide-free corn oil at -20°C . (Preparative details will be included in a forthcoming paper by D.C. Greenslade and J.A. Heslop, on the synthesis of these substances.)

[$1\text{-}^{14}\text{C}$]Chloroacetic acid (52 mCi/mmol), [$2\text{-}^{14}\text{C}$]chloroacetic acid (42 mCi/mmol), L-[$U\text{-}^{14}\text{C}$]cysteine hydrochloride (24.5 mCi/mmol) and [$U\text{-}^{14}\text{C}$]glycine (10 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks.

Experiments with animals

Adult male rats (approx. 2 months old, 200 g body wt.) were used [Alderley Park strain (Wistar-derived), specific pathogen-free], and kept on a standard pellet diet.

(a) For the purpose of excretion-retention experiments, groups of 4 animals were administered single doses of [$2\text{-}^{14}\text{C}$]DCE (either 500 $\mu\text{g/kg}$ or 350 mg/kg; 1 μCi) as a corn-oil solution by the intragastric, i.v. and i.p. routes. The animals were housed singly in glass metabolism cages for 72 h after dosing, and ^{14}C was measured in the urine, faeces and exhaled air, which was drawn successively through trichloroethylene at -70°C and CO_2 absorbers (for details, see ref. 1).

(b) 3 rats, equipped with a biliary fistula, were each given an intragastric dose of [$1\text{-}^{14}\text{C}$]DCE (350 mg/kg; 15 μCi) as a corn-oil solution, and they were maintained in restraining cages for 48 h, during which time bile and urine were collected. The 48-h urine was also collected from control animals, which had been given the same dose.

For the identification of urinary metabolites [1,7], groups of 4 animals were administered intragastrically:

(c) [^{14}C]DCE (350 mg/kg; 10 μCi per animal) in corn-oil solution.

(d) [$1\text{-}^{14}\text{C}$]chloroacetic acid (50 mg/kg; 6.5 μCi) in aq. solution.

(e) [$2\text{-}^{14}\text{C}$]chloroacetic acid (50 mg/kg; 11.4 μCi) in aq. solution.

(f) 2 rats were each given 2 i.p. injections of an aq. solution of L-[$U\text{-}^{14}\text{C}$]cysteine hydrochloride with a 17-h interval (total dose, approx. 50 μCi). 2 h after the second injection, each animal was given a single intragastric dose of DCE (350 mg/kg) in corn-oil solution, and the 48-h urine was collected.

(g) 2 rats were each administered 2 i.p. injections of an aq. solution of 20 μCi of L-[$U\text{-}^{14}\text{C}$]cysteine hydrochloride and 20 μCi of [$U\text{-}^{14}\text{C}$]glycine with an 18-h interval. 1 h after the second injection, each animal was given a single intragastric dose of DCE (350 mg/kg) in corn-oil solution, and the 48-h urine was collected.

(h) To each young rat (approx. 80 g body wt.), there was administered an

intragastric dose of 20 μ Ci of [14 C]DCE. 30 and 60 min, and 2 days after dosing, the animals were deeply anaesthetised and were rapidly frozen by immersion in solid CO_2 . Longitudinal sagittal sections, 20 μ in thickness, were cut with a mechanically operated Cambridge microtome, in a Bright cryostat at -20°C ; some were cut through the vertebrae and others through a kidney of each animal. Apposition autoradiograms were prepared from the freeze-dried sections [1].

Measurement of radioactivity

An automated and computerized Intertechnique Model SL30 liquid scintillation spectrometer was used for measurement of ^{14}C , making use of standard channels-ratio quench-correction curves. Liquid samples were admixed with standard scintillator and radio-assayed direct, and samples of faeces were burnt in an Intertechnique "Oxymat" solid-sample oxidizer.

Systematic separation of the urinary metabolites into fractions of chemically similar substances

Urine from each group of rats, (c) to (g), was separated into 3 fractions by anion-exchange chromatography in a manner analogous to that described by Green and Hathway [1]. The 3 N HCl fractions were evaporated separately to dryness under reduced pressure, and the various residues were methylated with an ethereal solution of diazomethane. Methanolic solutions of the resulting *O*-methyl esters were analysed on GC columns and in the GC-mass spectrometer system. The neutral fractions and the 3 N acetic acid fractions were also evaporated separately to dryness under reduced pressure, and the different residues were examined by TLC. After esterification with diazomethane or butan-1-ol (Fischer-Speier) and acylation with trifluoroacetic anhydride (TFAA) [1,7], the individual compounds, in the residues resulting from the evaporation of the acid hydrolyates of the last two fractions, were analysed on gas chromatography (GC) columns and in the GC-mass spectrometer system.

Thin-layer chromatography

The material eluted from the anion exchange column with 3 N acetic acid was applied as bands on 500 μg SiO_2 gel GF thin-layer plates which were developed with butan-1-ol-acetic acid-water (4 : 1 : 2). Zones of ^{14}C were located with a Panax TLC scanner, excised and eluted with methanol.

Gas chromatography

Fractions containing radioactive metabolites were examined with a Pye Model 104 instrument that was equipped with flame-ionization detection and that was coupled to an E.S.I. Nuclear 504 Radiogas detector. The column effluent was split in the ratio of 10:1 between the Radiogas detector and the flame-ionization detector. This gas chromatograph was fitted with glass columns (2.1 m long $\times$ 4 mm int. diam.), which were packed with 6%

(w/w) OV-101 on Supelcoport (80–100 mesh size), and run at 100°C and 180°C. GC analysis was also used for the identification of unchanged DCE, which had been collected from the exhaled air in cold traps containing 1,1,2-trichloroethylene [1]. In that case, the gas chromatograph was fitted with glass columns (1.5 m long × 4 mm int. diam.), which were packed with 10% (w/w) squalene on Chromosorb P (80–100 mesh size), and run at 80°C. All of the columns were operated at a 40 ml/min flow-rate of a (95 : 5 v/v) Ar–CO₂ mixture.

Radioactive peaks were located, and the corresponding samples were analysed by GC-mass spectrometry, using the same columns under identical operating conditions.

GC-mass spectrometry

An LKB2091 gas chromatograph-mass spectrometer system was used for the E.I. spectra of the compounds described.

RESULTS

For much of the work, either of the labelled forms of DCE, viz. 1,1-dichloro-[1-¹⁴C] and [2-¹⁴C]ethylenes ([1-¹⁴C]DCE and [2-¹⁴C]DCE) was used, since CO₂ and urea were the only DCE metabolites with a single C-atom, and they were produced in similar low proportions from the 2 labelled forms.

Excretion of radioactivity

The total excretion data obtained from rats after 500-μg and 350-mg intragastric, i.v. and i.p. doses of [2-¹⁴C]DCE per kg are detailed in Table I. In every case except one, almost all of the radioactivity was recovered during the first 72 h after dosing, but after low intragastric administration, small amounts of ¹⁴C were still being excreted during 72–108 h after dosing. At the different dose levels, there is a marked change in excretion pattern after intragastric administration (Table I). Whereas with the higher dose, nearly 70% as unchanged DCE and 1% of CO₂ are excreted via the lungs, with the lower dose, urinary excretion accounts for 80%, and less than 1% of unchanged DCE together with 4–6% of CO₂ are eliminated by the pulmonary route. However, about 210 times more DCE was metabolized at the higher dose level than at the lower one. The change in excretion pattern appears to be due to a saturable drug metabolism and to an efficient arterial-alveolar transfer of unchanged DCE from systemic blood that leaves a relatively low concentration of material for biotransformation in successive passes through the liver. Thus, 80% of a small i.v. dose of DCE is excreted unchanged from systemic blood within an hour of injection; more than 60% within 5 min. Predictably, the excretion pattern after a small i.p. injection is intermediate between that resulting from i.v. injection and that from intragastric administration (Table I); some of the DCE is taken up into systemic blood and is excreted unchanged via the lungs and some is absorbed into the hepatic-

TABLE I

EXCRETION OF RADIOACTIVITY IN RATS, GIVEN A SINGLE DOSE OF [2-¹⁴C]DCE

Doses of 500 µg or 350 mg of [2-¹⁴C]DCE per kg in corn-oil solution were given by the i.g. or i.p. routes respectively to individual rats, comprising 4 groups of 4 animals, and the 4 animals belonging to a 5th group were injected with 500 µg/kg in a tail vein.

Size of dose	Time (h)	Radioactivity excreted (% of dose)*																	
		Intragastric						Intravenous						Intraperitoneal					
		Exhaled air		Urine		Faeces		Exhaled air		Urine		Faeces		Exhaled air		Urine		Faeces	
		DCE	CO ₂					DCE	CO ₂					DCE	CO ₂				
500 µg/kg	0-24	0.6 ± 0.2	3.9 ± 0.7	71.3 ± 1.2	5.1 ± 0.7	80.0 ± 4.0	3.5 ± 0.6	14.4 ± 3.6	0.3	11.4 ± 2.8	2.4 ± 0.7	65.8 ± 2.5	14.2 ± 4.4						
	24-48	0.06	0.5 ± 0.1	5.3 ± 0.9	2.7 ± 0.9	0	0	0.7 ± 0.2	0.1	0.2	0.5 ± 0.1	2.0 ± 0.1	1.6 ± 1.1						
	48-72	0.08	0.5 ± 0.2	3.6 ± 1.7	0.6 ± 0.2	0	0	0	0	0.1	0.5 ± 0.2	1.2 ± 0.3	0.4						
	Total	0.7 ± 0.1	4.8 ± 1.3	80.2 ± 1.4	8.3 ± 0.1	80.0 ± 4.0	3.5 ± 0.6	15.0 ± 3.9	0.4	11.7 ± 2.8	3.6 ± 0.7	69.0 ± 2.8	16.2 ± 4.5						
350 mg/kg	0-24	62.4 ± 4.3	0.3	17.6 ± 4.4	0.4			90.5 ± 2.9	0.7 ± 0.4	7.1 ± 2.1	0.5 ± 0.2								
	24-48	1.8 ± 2.8	0.1	10.0 ± 4.0	0.5 ± 0.3			0.6 ± 0.3	0.5 ± 0.2	0.3	0.1								
	48-72	0.1	0.3	1.9 ± 1.0	0.1			0	0.1	0.3	0.1								
	Total	64.3 ± 4.3	1.0 ± 0.1	29.5 ± 6.7	1.3 ± 0.4			91.1 ± 3.2	1.3 ± 0.6	7.9 ± 2.1	0.7 ± 0.3								

*Values shown are the means ± S.D. of those means.

TABLE II

EXCRETION OF ^{14}C IN THE URINE AND BILE AFTER AN INTRAGASTRIC DOSE OF $[1\text{-}^{14}\text{C}]\text{DCE}$

3 normal rats and 3 animals equipped with a biliary fistula were each dosed intragastrically with 350 mg of $[1\text{-}^{14}\text{C}]\text{DCE/kg}$ in corn-oil solution; urine and urine and bile were collected as appropriate over a 48-h period.

	Recovery of ^{14}C (% of dose)			
	0-3 h	3-6 h	6-24 h	24-48 h
<i>Normal rats</i>				
Urine	0	2.9 ± 0.9	15.3 ± 1.8	3.1 ± 1.0
<i>Rats equipped with biliary fistula</i>				
Urine	0	0.2 ± 0.1	6.5 ± 0.5	2.4 ± 0.4
Bile	0.9 ± 0.7	2.1 ± 0.3	5.9 ± 1.7	2.4 ± 0.6

portal system and is metabolized by the liver. After intragastric dosing, the rate of pulmonary excretion of unchanged DCE and CO_2 parallels that of the 3-day excretion of urinary metabolites. Hence, the matching formation of CO_2 probably belongs to the same metabolic pathway as that for the urinary metabolites. GC analysis established that, apart from CO_2 , unchanged DCE was the only product, related to the original substance, which was excreted via the lungs.

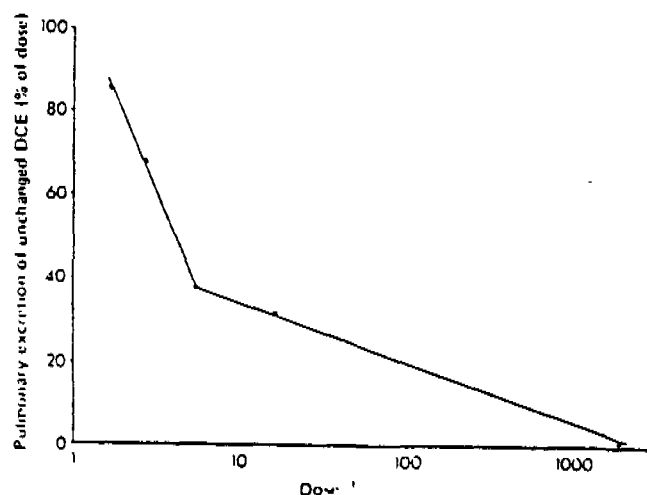


Fig. 1. Linear-log plot of the pulmonary excretion of unchanged DCE against the reciprocal doses. 5 groups of 4 rats were dosed i.g. with DCE at dose levels in the range of 0.5 to 650 mg/kg; each point represents 4 independent values.

The fact that the cumulative urinary excretion of ^{14}C in whole animals after a single intragastric dose of $[1\text{-}^{14}\text{C}]\text{DCE}$ is roughly equal to the sum of the separate cumulative biliary and urinary eliminations, in animals equipped with a biliary fistula, suggests that the origin of at any rate part of the urinary radioactivity was biliary (Table II). This finding and its possible enterohepatic implication are consistent with the relatively slow excretion of the water-soluble $[^{14}\text{C}]$ urinary metabolites from intact animals.

For groups of rats that had been administered various dose levels within a 1300-fold dose range, the plot of the pulmonary excretion of unchanged DCE (expressed in terms of percentage of the dose) against the logarithm of reciprocal doses is biphasic (Fig. 1), and this relationship reflects a "pool phenomenon" associated with a saturable drug metabolism. Whereas the initial steep slope represents the fast release of DCE, which had not been absorbed by the body organs and tissues or which exceeded the saturation of the body pool, the subsequent gentle slope relates to the slow release of DCE from the body pool, which in the case of this lipid-soluble, water-insoluble substance is presumably adipose tissue plus liver.

Whole-animal autoradiography

Sequential autoradiograms of longitudinal sagittal sections through whole animals, which had been dosed intragastrically with $[^{14}\text{C}]\text{DCE}$ show large ^{14}C concentrations in the kidneys and liver after 30 min and a more general distribution of ^{14}C throughout the soft organs of the body at 1 h. The kidneys and liver retain ^{14}C for the longest times after dosing.

Separation and identification of urinary DCE metabolites

The fraction of the bulked urines of treated animals, which was displaced

TABLE III

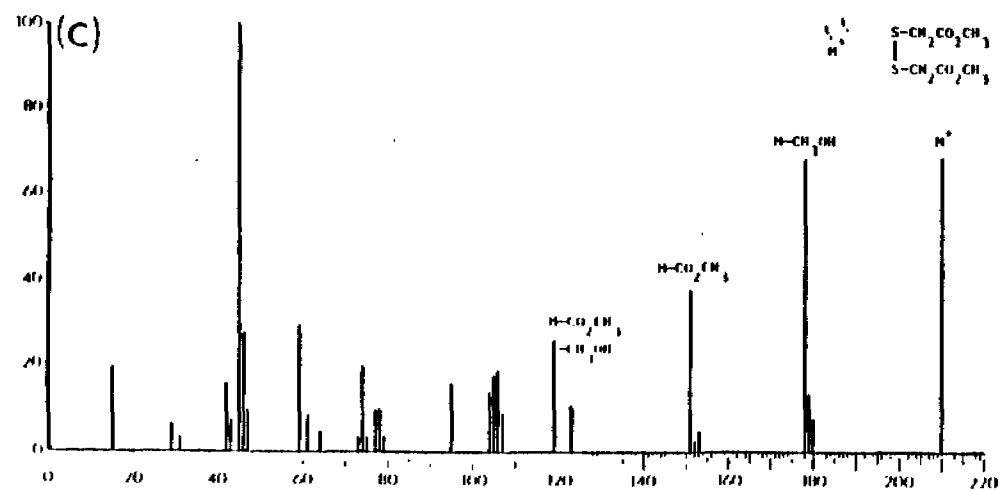
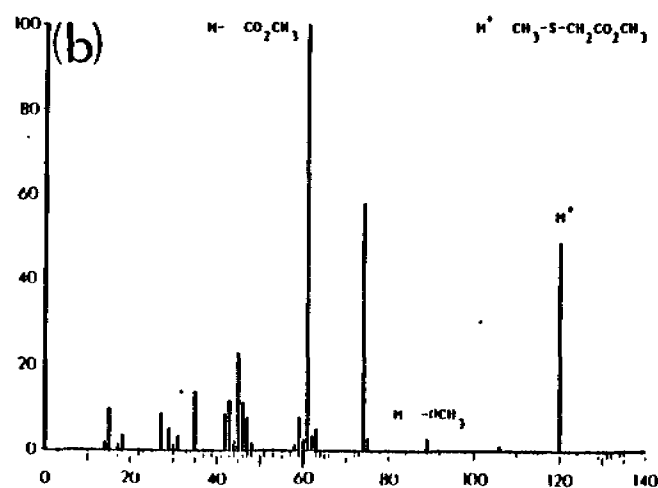
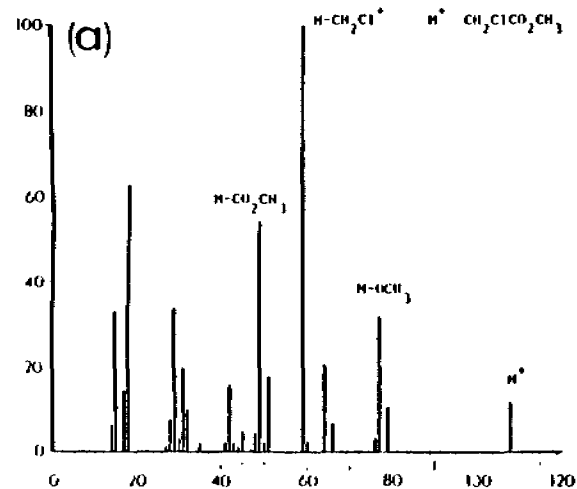
GAS CHROMATOGRAPHIC CHARACTERISTICS OF THE METHYL AND *n*-BUTYL ESTERS OF SOME DCE METABOLITES AND RELATED SUBSTANCES

Esterified DCE metabolites	GC-retention times (min) on columns coated with OV-101	
	Run at 100°C	Run at 180°C
Methyl chloroacetate	2.0	
Methyl thioglycollate	3.9	
Dimethyl thiodiglycollate		2.9
Dimethyl dithioglycollate		5.9
Methyl <i>S</i> -(2-carboxymethyl)cysteine	8.3	
<i>n</i> -Butyl chloroacetate	6.3	
<i>n</i> -Butyl thioglycollate	10.2	
Di- <i>n</i> -butyl thiodiglycollate		6.1
Di- <i>n</i> -butyl dithioglycollate		13.5
<i>n</i> -Butyl <i>S</i> -(2-carboxymethyl)cysteine	10.7	

by 3 N HCl from anion-exchange resin in the acetate form, was esterified with diazomethane, and an aliquot portion was analysed on GC columns of 6% OV-101 for peaks containing appreciable amounts of radioactivity. (GC retention times are given in Table III). Mass spectra of the substances in those peaks were obtained with another aliquot portion, which had been injected on to the similar column of a GC-mass spectrometer system. Chloroacetic acid, dithioglycollic acid, thioglycollic acid and thiodiglycollic acid were found to be the principal DCE metabolites in this fraction; conventional mass spectra of the corresponding methyl and *n*-butyl esters were identical with those of authentic materials. (Mass spectra of methyl esters of the first three substances are shown in Figs. 2 (a-c), whereas that of di-*n*-butylthiodiglycollate was illustrated previously [1].)

A single major DCE metabolite was weakly absorbed on anion-exchange resin in the acetate form (*v. supra*), but not on cation-exchange resin, and was displaced from the former either by 3 N acetic acid or by deionised water that had been allowed to become acidic in the atmosphere. Its identification was exacerbated by difficulties of derivative formation and by the unavailability of the compound or its (possible) derivatives to GC methods. Thus, the fraction, which was displaced by 3 N acetic acid, was shown by TLC to contain a single ^{14}C -compound, which gave a yellow colour reaction with ninhydrin. The product from attempted esterification of this compound with diazomethane did not afford an interpretable mass spectrum, when it was injected on to the probe of the mass spectrometer. However, hydrolysis of this ^{14}C -compound with boiling 3 N HCl for 1 h, followed by esterification of the product with butan-1-ol and acylation with trifluoroacetic anhydride (TFAA), gave the pure di-*n*-butyl ester, *N*-TFA derivative of *S*-(2-carboxymethyl) cysteine, the mass spectrum of which was identical with that of authentic material. Moreover, a few minutes acid hydrolysis of the ^{14}C -compound with boiling N HCl gave *N*-acetyl-*S*-(2-carboxymethyl)-cysteine; the mass spectrum [Fig. 2(d)] of the dimethyl ester was identical in all respects with that of authentic material. All of the radioactivity associated with the DCE metabolite was accountable in terms of a single hydrolytic product [^{14}C] *N*-acetyl-*S*-(2-carboxymethyl)cysteine. In addition, the GC and mass spectrometric properties of 4-acetyl-5-carboxy-3-oxo-tetrahydro-1,4-thiazine, prepared from *N*-acetyl-*S*-(2-carboxymethyl)cysteine [6], were shown to differ from those of the DCE metabolite. The absence of glycine from the DCE metabolite was found in experiments with unlabelled DCE in rats in which the cysteine-cystine and glycine pools had been labelled adequately with ^{14}C , and in fact *S*-(^{14}C)(2-carboxymethyl)cysteine, but not [^{14}C]glycine, was identified in the acid hydrolysate of the resulting [^{14}C]DCE metabolite by mass spectrometry.

Since these experiments exclude the possibility of internally-compensated lactam or glutathione-derived *S*-(2-carboxymethyl)cysteinylglycine dipeptide structures for the major DCE metabolite concerned, there is a reasonable supposition that it may have been formed via glutathione-derived *N*-acetyl-*S*-cysteinyl-acetyl chloride. However, on that assumption, an *N*-acetyl-*S*-



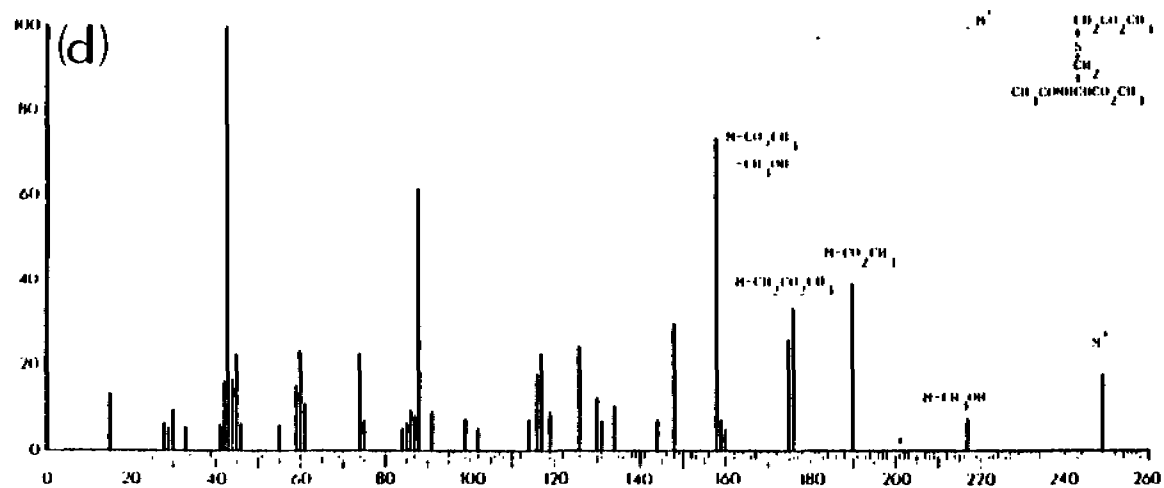


Fig. 2 Mass spectral data of methyl chloroacetate (a), methyl S-methylthioglycollate (b), dimethyl dithioglycollate (c) and di-O-methyl, N-acetyl S-(2-carboxymethyl)cysteine (d).

TABLE IV

RELATIVE PROPORTIONS OF THE URINARY METABOLITES OF DCE AND CHLOROACETIC ACID OF RATS, DOSED INTRAGASTRICALLY EITHER WITH $[1-^{14}\text{C}]$ DCE OR WITH $[^{14}\text{C}]$ CHLOROACETIC ACID

DCE and chloroacetic acid metabolites	% of Urinary radioactivity	
	DCE	Chloroacetic acid
Thiodiglycollic acid	37.0	90.0
<i>N</i> -Acetyl-S-cysteinyl-acetyl derivative	48.0	0
Dithioglycollic acid	5.0	3.0
Thioglycollic acid	3.0	3.0
Chloroacetic acid	3.0	0
Urea	0.5	0.5
<i>S</i> -(Carboxymethyl)cysteine	0	2.0

cysteinyl-acetyl-S-cysteine structure would be unacceptable, because experiments with unlabelled DCE in rats, in which the cysteine-cystine pool had been labelled adequately with ^{14}C , yielded the di-*n*-butylester, *N*-TFA derivative of *S*- $[^{14}\text{C}]$ (2-carboxymethyl) cysteine, but not of $[^{14}\text{C}]$ cysteine, in the hydrolysate of the resulting $[^{14}\text{C}]$ metabolite. Hence, while the pertinent structural features of the *N*-acetyl-S-cysteinyl acetyl (DCE) metabolite are now known, more work is required to establish its exact structure.

$[^{14}\text{C}]$ Urea was shown by isotope-dilution analysis to be a relatively minor $[^{14}\text{C}]$ DCE metabolite in the neutral fraction from the urine of treated rats. The *N*-TFA derivative was crystallized (with the naturally-occurring unlabelled urea) to a constant specific activity, and the purified product was shown by mass spectrometry to be identical with that of authentic material.

Urinary chloroacetic acid metabolites

The fact that more than 100 times as much chloroacetic acid was excreted from rats dosed with DCE than had been found in a similar investigation with vinyl chloride, where chloroacetic acid was a minor or negligible metabolite [7], and that in the present work chloroacetic acid may have been formed *in vivo* through spontaneous rearrangement of 1,1-dichloroethylene oxide into chloroacetyl chloride [8], suggested that the metabolism of DCE and chloroacetic acid may be interrelated. This supposition is strongly supported by the fact that several of the urinary metabolites of DCE were found by the previously-described methods to be excreted from animals dosed with chloroacetic acid *per se* (Table IV).

DISCUSSION

The foregoing evidence suggests a scheme (Fig. 3), which is economical in

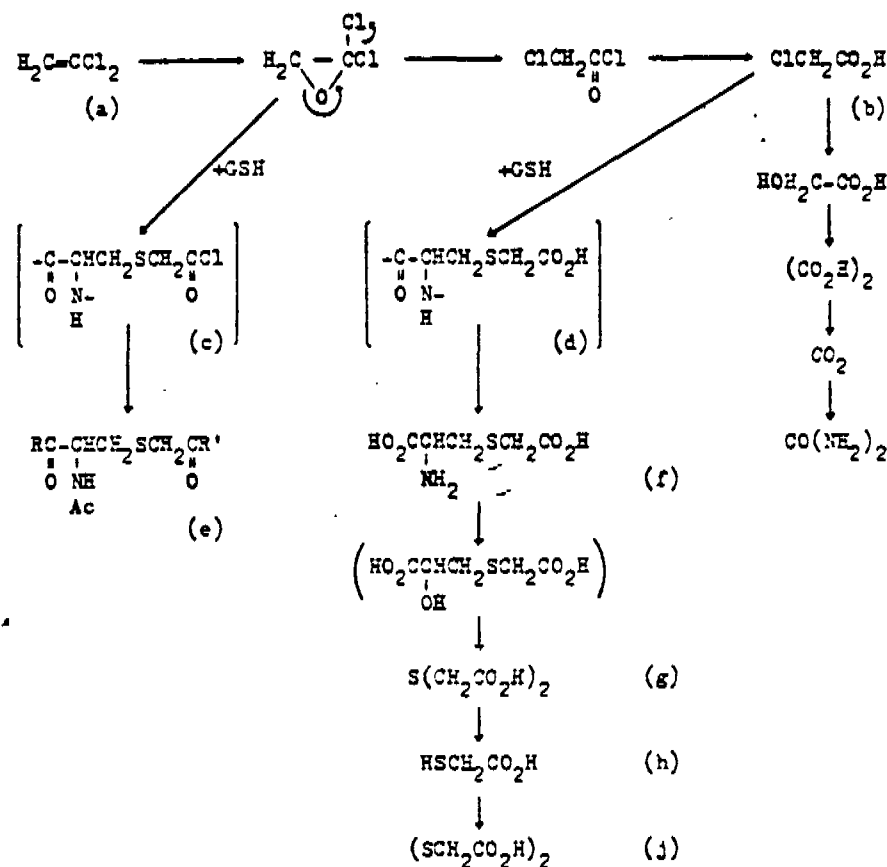


Fig. 3. Scheme for the metabolism of vinylidene chloride in rats.

respect of primary biotransformations, for the metabolism of DCE(a) (Fig. 3), in which chloroacetic acid (b) occupies a key position. There is a strong supposition that detoxification of chloroacetic acid is effected through the enzyme-catalysed reaction with glutathione and ensuing degradative sequence for the resulting carboxymethylglutathione (d), and that this represents the principal metabolic pathway for chloroacetic acid and a major one for DCE. Thioglycolic acid (g) is the ultimate detoxification product, and previous work [7] has established the biotransformation of S-(2-carboxymethyl)-cysteine (f) into thioglycolic acid (g) by a pathway involving transamination and oxidative decarboxylation. (In the present investigation, some evidence was found for the formation of the intermediary lactic and pyruvic acids.) Since thioglycolic acid (h) may be formed through CS bond fission

of the symmetrical thiodiglycollic acid (g), (h) and dithioglycollic acid (j) might be appended from (g). A feasible metabolic pathway of thiodiglycollic acid from chloroacetic acid and involving cysteine desulphhydrase [EC 4.4.1.1] has now been shown to be unacceptable in experiments with unlabelled DCE in rats, in which the cysteine-cystine pools had been labelled adequately with ^{14}C . Since labelled thiodiglycollic acid resulted, a part of the C-skeleton must be derived in fact from cysteine. Whilst the formation of a small amount of [^{14}C]dithioglycollic acid (and hence of the intermediate [^{14}C]thioglycollic acid) is reconcilable with the predictable action of an unspecific thionase [5], which would lyse a small proportion of the preponderating [^{14}C]thiodiglycollic acid to give some [^{14}C]thioglycollic acid, these substances were undetected in a similar experiment with vinyl chloride [7], in which thiodiglycollic acid was also the major metabolite. However, had unlabelled thioglycollic acid and dithioglycollic acid been produced from DCE via the cysteine desulphhydrase pathway, they would not have been identifiable in the presence of their labelled counterparts.

Irrespective of whether [$1\text{-}^{14}\text{C}$]DCE or [$1\text{-}^{14}\text{C}$]chloroacetic acid was administered to rats, one molecular proportion of the resulting [^{14}C]thiodiglycollic acid gave 1 equivalent of $^{14}\text{CO}_2$ in the micro-scale [9] Kolbe electrolysis reaction [10]. This evidence is utterly consistent with the transformation of (a) into (b) (Fig. 3), which involves migration of one Cl atom and the loss of the other one. Hence, the metabolic pathway (Fig. 3), which was tentatively proposed for the biotransformation of vinylidene chloride into thiodiglycollic acid, does in fact operate in rats.

In view of the established metabolic pathway for thiodiglycollic acid, the formation of the *N*-acetyl-S-cysteinyl-acetyl derivative (e) (Fig. 3) by the metabolism of DCE, but not of chloroacetic acid (Table IV), is rather puzzling. However, this evidence must mean that this compound, (e) arises from an earlier metabolite of DCE than chloroacetic acid. Present work suggests, but does not prove, that this compound may be formed in fact from 1,1-dichloroethylene oxide through the agency of glutathione S-epoxide-transferase [EC 4.4.1.7], to afford S-glutathionyl acetyl chloride (c) (Fig. 3), and its subsequent reactions (v. supra). This supposition is important, since the reactivity displayed by 1,1-dichloroethylene oxide may be particularly relevant to the possible interaction of reactive DCE metabolite(s) with mouse-kidney DNA (v. supra). If this should prove to be the case, then such interaction would be analogous to that of vinyl chloride with rat-liver DNA *in vivo* which, we have found [11], leads to the production of 6 β -D-ribofuranosyl-5-oxo-5,6-dihydroimidazo-[1,2-*c*]pyrimidine and 3 β -D-ribofuranosyl-imidazo-[2,1-*i*]purine residues and subsequent depurination [11].

Another point about the scheme (Fig. 3) concerns the origin of up to 6% of CO_2 in DCE metabolism. Whether this CO_2 arises (i) through the action of epoxide hydratase [EC 4.2.1.63] on 1,1-dichloroethylene oxide and subsequent degradation of resulting intermediates or (ii) through the oxidation of chloroacetic acid is equivocal. Metabolic studies have provided evidence of a minor oxidative pathway for chloroacetic acid, which leads to CO_2 (4% of

the dose) via glycollic acid and oxalic acid; both intermediates have been found in mice [12] and a trace of oxalic acid was detected in rat material in the present work.

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