Biochemical Pharmacology, Vol. 33, No. 13, pp. 2021–2027, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

NOVEL METABOLITES OF TRICHLOROETHYLENE THROUGH DECHLORINATION REACTIONS IN RATS, MICE AND HUMANS*

WOLFGANG DEKANT, MANFRED METZLER and DIETRICH HENSCHLER
Institute of Toxicology, University of Würzburg, Versbacher Strasse 9, D-8700 Würzburg, Federal Republic of Germany

(*Received* 2 *November* 1983; *accepted* 3 *January* 1984)

Abstract—The excretion and biotransformation of [^{14}C]trichloroethylene (Tri) has been studied in female rats and mice. Seventy-two hours after a single oral dose of 200 mg/kg, rats exhaled 52% and mice 11% of the recovered radioactivity as unchanged Tri, and 1.9% and 6%, respectively, as $^{14}CO_2$. Rats excreted 41.2% of the recovered radioactivity in the urine, in contrast to mice where urinary activity amounted to 76%. The isolation of urinary metabolites was accomplished by reversed-phase HPLC, using a water–methanol gradient. After chemical derivatization, a combination of radio-GC and GC/MS was used for identification. The metabolites identified in rat urine were: trichloroacetic acid (15.3%); trichloroethanol, free (11.7%) and as the glucuronide (61.9%); dichloroacetic acid (2.0%); oxalic acid (1.3%) and *N*-(hydroxyacetyl)-aminoethanol (HAAE) (7.2%). In mice, trichloroethanol (free and in several conjugated forms) is the main metabolite of Tri (94.3%), but small amounts of HAAE (4.1%) and oxaiic acid (0.7%) are also excreted. Only traces of dichloro- and trichloroacetic acids were found in this species. In human male subjects, HAAE was also identified as a urinary metabolite of Tri after exposure of two volunteers to 200 ppm Tri for 6 hr. The identification of HAAE and oxalic acid as metabolites indicates hydrolytic dechlorination reactions in the metabolism of Tri.

1,1,2-Trichloroethylene (Tri)† has been widely used as an industrial solvent for the past 50 years; an estimated 3.5 million workers are presently exposed to Tri. Suspicion that Tri exerts a tumorigenic effect was aroused by the results of several long-term carcinogenicity studies [1–3]. However, these data are considered to be inadequate for a conclusive determination of a carcinogenic effect exerted by Tri [4]. Numerous studies on the mutagenicity of Tri after metabolic activation in different strains of bacteria have been conducted, revealing that Tri is either non-mutagenic or only weakly mutagenic [4, 5].

All data available on the metabolism show that Tri is metabolically converted by cytochrome P-450 dependent monooxygenases to an intermediate electrophilic epoxide [6], which may rearrange to chloral. All metabolic end-products of Tri identified under normal exposure conditions are explained by this rearrangement [7]. So far, trichloroethanol, its glucuronide and trichloroacetic acid have been identified. This rearrangement to non-toxic 1,1,1-trichloro compounds, if complete conversion of the epoxide is achieved, may be considered to be a deactivation reaction, since no intermediate electrophile would be available for the alkylation of cellular macromolecules. In mice, Hathaway [8] detected traces of dichloroacetic acid only after oral application of very high doses; this author proposes a 'spill over' model, whereby an excess of trichlorooxirane would saturate the above-mentioned deactivation mechanism and thus become available for reactions outside the microsomal system. The epoxide is also suggested to be responsible for the covalent binding of radioactivity to lipids after application of [^{14}C]Tri both *in vivo* and *in vitro* [9–11]. CO_2 was recently reported to be a metabolite of Tri in mice and rats. A very slight interaction of [^{14}C]Tri with DNA *in vivo* was reported by several authors, but no indication of the formation of specific DNA adducts was obtained after hydrolysis of the DNA [12–14]. These results indicate that the metabolic fate of Tri intermediates is still unsettled. In the present study, we have re-evaluated the metabolism of Tri in different species using more advanced methodology in order to identify previously unknown metabolites, which might shed light on the conditions for the possible carcinogenic effect of Tri.

MATERIALS AND METHODS

Chemicals. 1,2-[^{14}C]trichloroethylene was purchased from New England Nuclear (Dreieich, F.R.G.). The sample received contained several radioactive impurities and was diluted with pure Tri to a sp. act. of 0.1 mCi/mmole.

Purification of the sample by preparative GC (30% Squalane on Chromosorb WAW 60/80, Carlo Erba Fractovap) yielded Tri with a radiochemical purity of >99.9% as checked by radio-GC: *N*-(Hydroxyacetyl)-aminoethanol (HAAE) was prepared as follows: 2-aminoethanol was added dropwise to molten glycolic acid. After 1 hr at 80°, the reaction mixture was allowed to cool and the resulting colourless needles were recrystallized from isopropanol–water (5:1, v/v). Its identity was confirmed

* Preliminary communications have been published in: *Toxic. Letters* **18** (Suppl. 1), 121 (1983); *Naunyn-Schmiedeberg's Archs Pharmac.* **319** (Suppl. 1), 16 (1982).

† Abbreviations: HAAE, *N*-(hydroxyacetyl)-aminoethanol; TCE, 1,1,1-trichloroethanol; Tri, 1,1,2-trichloroethylene.

by GC/MS and ^{1}H NMR. All other chemicals used were obtained from EGA-Chemic (Steinheim, F.R.G.).

Animals and treatment. Female Wistar rats (220–260 g) and female NMRI-mice (24–27 g) (Institut für Versuchstierkunde, Hannover, F.R.G.) were used. Single doses of 200 mg/kg [^{14}C]Tri in corn oil (2.5 g/kg) were administered by stomach tube between 9 and 10 a.m. The animals were transferred to an all-glass metabolic cage immediately after application. Dry air was drawn through the cage at a constant rate (400 ml/min). Standard diet (Altromin®) and water were supplied *ad libitum*.

Sampling and measurement of radioactivity. Excreted ^{14}C-activity was monitored for 72 hr. Urine and faeces were collected at 6 hr intervals, the cage being rinsed each time with a total volume of 50 ml of water in several portions. Exhaled [^{14}C]Tri and $^{14}CO_2$ were collected by drawing the air from the metabolic cage through a series of four consecutive traps. Before entering the traps, moisture in the air was removed with Drierite (W. H. Hammond, Drierite Co., Xenia, U.S.A.). The first two traps each contained 100 ml of hexane maintained at −78° in a dry-ice bath. Each of the two remaining traps was filled with a solution of 100 ml of diethanolamine and water (1:1, v/v) for absorption of $^{14}CO_2$, and was maintained at room temperature. Samples from the traps and the urine were transferred into Quickszint 112 (Zinsser, Frankfurt, F.R.G.) and counted in a liquid scintillation counter (Packard, Downers Grove, U.S.A., Tricarb 2650). The carcass was digested in methanolic KOH. Portions of the digest were also dissolved in Quickszint.

Separation of urinary metabolites. Aliquots of the urine were injected directly into a Waters (Milford, U.S.A.) liquid chromatography system, consisting of two M 6000A pumps, a U6K injector and a Model 660 solvent programmer. Separation of urinary metabolites was achieved by running a linear gradient with an initial concentration of 5% methanol in redistilled water (acidified by the addition of 1 per ml v/v formic acid) and a final concentration of 95% methanol (v/v). The columns (25 cm × 0.8 cm i.d.) were filled with LiChrosorb® C 18 (Merck, Darmstadt, F.R.G.) of a particle size of 10 μm. The two most polar fractions eluting from the HPLC column were further separated for quantification of metabolites by ion-pair chromatography (PIC A, Waters, in water–methanol, 70:30, v/v, LiChrosorb® C 18). Aliquots of the fractions (1.5 ml out of 3 ml) were dissolved in 10 ml Quickszint. To check the separation efficiency, the eluate was also monitored by u.v. spectroscopy at 225 nm.

Derivatization of urinary metabolites and reference compounds. Silylation of the respective metabolites and reference compounds was performed by treating the samples with bis-trimethylsilyl acetamide (BSA) and heating the mixture to 80° for 2 hr. For esterification of carboxylic acids, the samples were dissolved in BCl_3–methanol or BF_3–propanol according to Brian [15].

Identification of urinary metabolites. The radioactive fractions obtained by HPLC were divided into two parts for derivatization followed by radio-GC. Esterified metabolites were separated on 6 ft glass columns filled either with 20% OV-101 on Chromosorb WHP 100–120 or 30% Carbowax 20 M on Chromosorb WAW 80–100. Silylated metabolites were also separated on 6 ft columns (3% OV-101 or 3% OV-225 on Chromosorb WAW 100–120). A Packard Instruments Model 427 gas chromatograph connected with a Packard Instruments Model 894 proportional counter was used for detection and separation of radioactive peaks.

GC/MS was performed on a Finnigan (San Jose, U.S.A) 4510 GC/MS system. Mass spectrometric data were processed by a Finnigan Incos MAT 2100 data system. Mass spectra were recorded at 70 eV.

Identification of N-(hydroxyacetyl)-aminoethanol (HAAE) as a metabolite of Tri in man. Two healthy male volunteers were exposed for 6 hr to 200 ppm of pure Tri in a dynamic exposure chamber as described previously [16]. Urine was collected for 48 hr from the beginning of the exposure. The 24 hr urine was concentrated to a small volume under reduced pressure and extracted with methanol. The methanolic extract was then subjected to preparative HPLC (Lichroprep® C 18, 50 cm × 3.2 cm i.d.) and fractionated. The fractions with retention volumes from 60 to 160 ml were collected. A second purification step by HPLC (LiChrosorb® C 8, 25 cm × 0.8 cm i.d., 10% methanol in water) was conducted and the fractions with retention volumes of 10–17 ml were pooled. The bis-trimethylsilyl-ether was prepared by treating the dry residue with bis-trimethylsilyl acetamide for 2 hr at 80°. HAAE was identified by GC/MS using different capillary columns (OV-1 and Se-54). Quantification was done with the silylated metabolite by GC comparison with the synthetic reference compound (Packard Instruments, Model 430, 6 ft 20% OV-101 on Chromosorb WAW).

RESULTS

Elimination pathways of ^{14}C-radioactivity after oral administration of trichloroethylene to rats and mice

The amounts of ^{14}C-radioactivity eliminated by different routes 72 hr after a single oral dose of labelled Tri (200 mg/kg) in corn oil to rats and mice are compiled in Table 1. No volatile radioactive

Table 1. Elimination routes of ^{14}C-radioactivity in rats (n = 2) and mice (n = 3) after administration of [^{14}C]Tri (data represent % of recovered activity, which was 93–98% of the administered radioactivity)

Sample	Time (hr)	Rats	Mice
Exhaled air			
[^{14}C]Tri	0–72	52.0	11.0
$^{14}CO_2$	0–72	1.9	6.0
Total		53.9	17.0
Urine	0–12	22.0	30.0
	12–24	17.0	39.0
	24–48	2.0	6.0
	48–72	0.2	1.0
Total		41.2	76.2
Faeces	0–72	1.8	4.9
Carcass, tissues		2.9	2.0
Final cage rinse		0.2	0.1

compounds other than [^{14}C]Tri and $^{14}CO_2$ could be detected by radio-GC in the exhalates. In contrast to rats, which exhaled about half of the dose as unchanged [^{14}C]Tri, mice, with their higher microsomal enzyme activity and capacity, metabolized 89% of the recovered radioactivity. In mice, a greater proportion of ^{14}C-radioactivity was converted to CO_2; this indicates that the biotransformation of Tri-metabolites progresses further in this species than in rats.

Isolation and identification of urinary metabolites in rats and mice

The HPLC chromatogram of the total 72 hr urine from rats revealed four clearly separated radioactive peaks, as shown in Fig. 1a. All the radioactive compounds were isolated through repeated chromatographic runs. When aliquots of peaks 1 and 2 were subjected to ion-pair chromatography, peaks 1 and 2 each subdivided further into two radioactive compounds. Radio-GC showed that one of the radioactive metabolites in peak 1 cochromatographed, after treatment with BCl_3–methanol or bis-trimethylsilyl acetamide, with the methyl and trimethylsilyl esters of oxalic acid, respectively. The mass spectrum of the other active compound after silylation indicated that the active material was HAAE. Therefore the reference compound was synthesized (see Materials and Methods). The synthetic substance and the metabolite had identical retention times on GLC columns with different stationary phases (OV-225 and OV-101). Moreover, the mass spectra of the metabolite and the synthetic reference compound, after derivatization to the bis-TMS-ether, were in good agreement with respect to typical fragments (see Fig. 2). Thus the proposed structure can be assigned unequivocally to this metabolite.

Peak 2 was also subdivided into two radioactive compounds by ion-pair chromatography: one component eluted with dichloroacetic acid, which was run as a reference; the other eluted with trichloroacetic acid. Moreover, the mass spectra of the

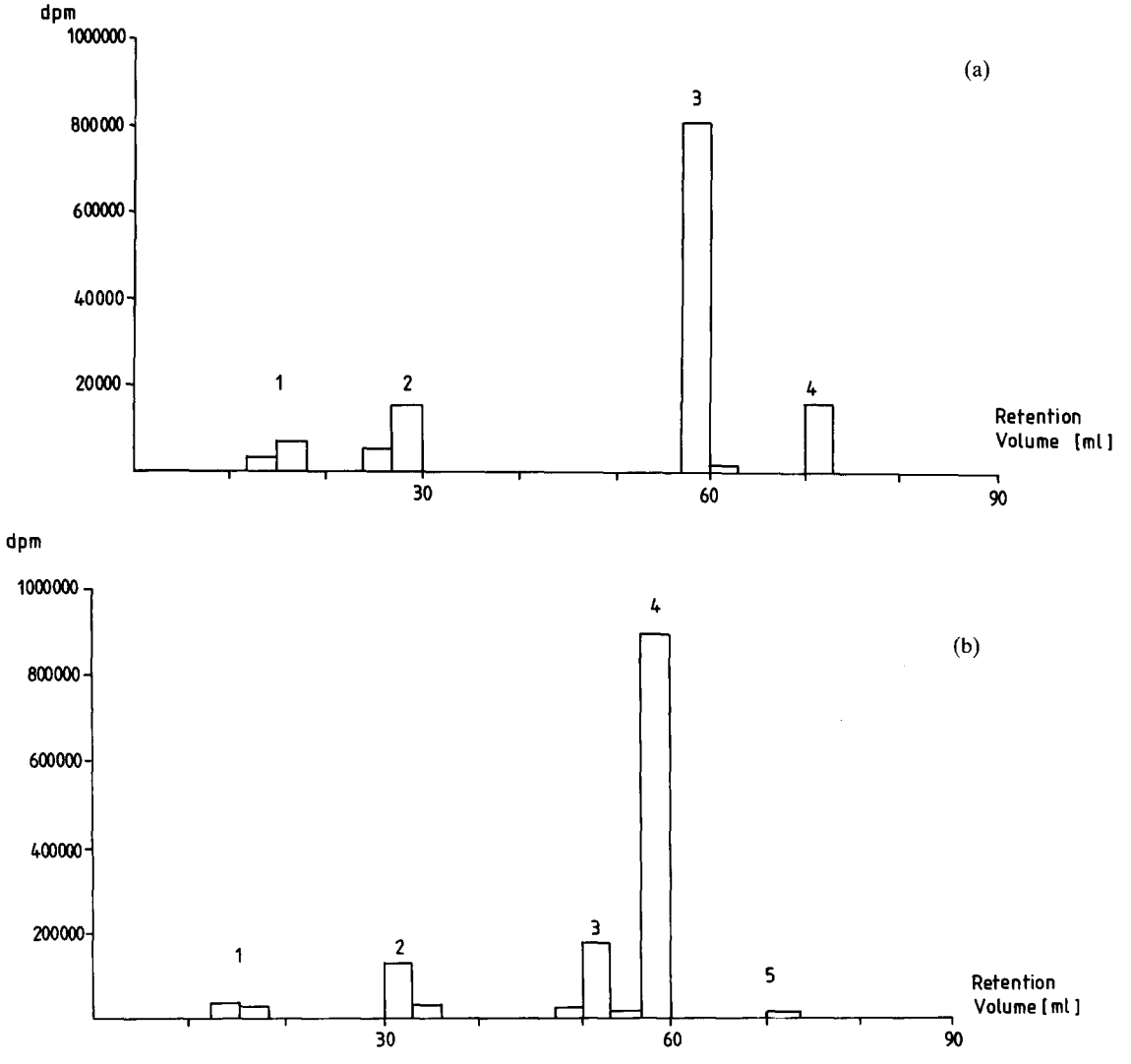

Fig. 1. HPLC radioactivity elution profile of pooled 72 hr urine from two rats (a) and three mice (b) after oral administration of [^{14}C]Tri. For peak numbers, see text.

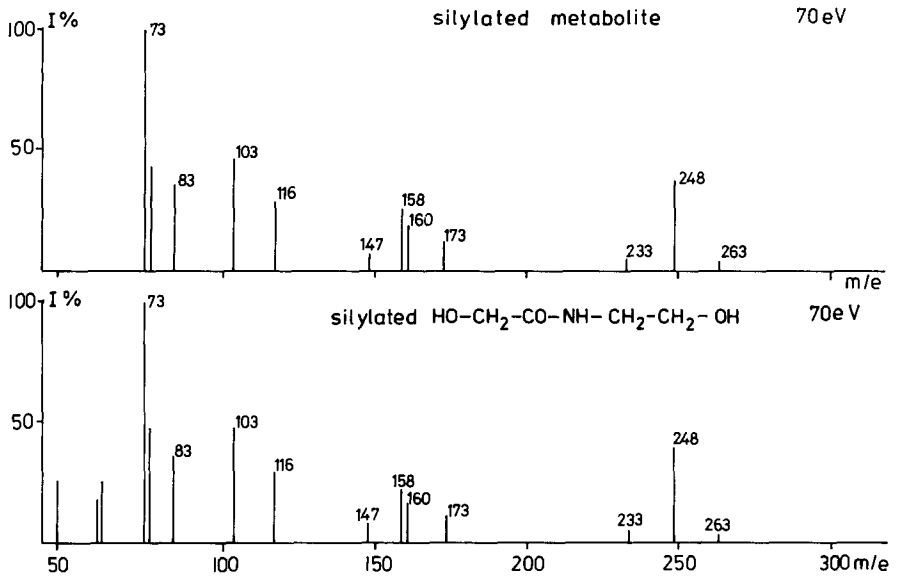

Fig. 2. Mass spectra of synthetic HAAE and the metabolite after silylation.

two radioactive metabolites, after methylation or propylation, proved to be identical to those of the authentic methyl- and propyl esters of dichloro- and trichloroacetic acids. This indicates that the metabolites in peak 2 are dichloro- and trichloroacetic acids.

Hints about the chemical nature of HPLC peaks 3 and 4 (Fig. 1) were obtained when an aliquot of the urine was treated with β-glucuronidase–arylsulfatase. In the HPLC chromatogram of the enzymatically treated urine, peak 3 was markedly decreased and peak 4 increased, suggesting that peak 3 contains a conjugate of the metabolite in peak 4. The metabolite represented by peak 4 was subsequently identified as trichloroethanol (TCE) by GC/MS after extraction from the urine with hexane.

When the metabolite in peak 3 was silylated and analysed by GC/MS, a mass spectrum with typical fragments of silylated polyhydroxy compounds and a fragment of a 1,1,1-trichloromethyl group was obtained. Upon cleavage of peak 3 with BCl_3–methanol, TCE was released and identified by GC/MS. These data indicate that the metabolite in peak 3 is TCE glucuronide.

HPLC separation of mice urine, collected after a single oral dose of 200 mg/kg Tri, revealed five peaks of radioactivity (Fig. 1a; two of these differed in their retention volumes, however, from those seen in rat urine). In peak 1, a small amount of oxalic acid was detected by ion-pair chromatography and identified by GC/MS as the TMS ester and the methyl ester. The main ^{14}C-labelled compound in peak 1 was identified as HAAE by GS/MS after treatment with bis-trimethylsilyl-acetamide. Less than 0.1% of the total urinary radioactivity was eluted in the retention volumes expected for dichloro- and trichloroacetic acids. The next two major metabolites of Tri (peaks 2 and 3) in mice (R_V = 30 ml and R_V = 51 ml) were dissolved in BCl_3–methanol. After this treatment, TCE was identified by GC/MS and radio-GLC as the only volatile ^{14}C-labelled compound in both fractions, indicating that the active substances consist of acid-labile conjugates of TCE rather than the glucuronide. No volatile radioactive material was detected by radio-GC after attempted silylation of the adducts, thus the exact nature of the conjugates could not be determined. TCE and its glucuronide (peaks 5 and 4) were identified as described in the experiment with rats. Table 2 summarizes the identified urinary metabolites of Tri in rats and mice.

Table 2. Identified urinary metabolites of Tri in two and three mice and their relative portions of ^{14}C-radioactivity in pooled 72 hr urine

Metabolite	% of urinary activity in rats	mice
Oxalic acid	1.3	0.7
Dichloroacetic acid	2.0	0.1
N-(Hydroxyacetyl)-aminoethanol (HAAE)	7.2	4.1
Trichloroacetic acid	15.3	0.1
Trichloroethanol (free)	11.7	0.1
Trichloroethanol (conjugated)	61.9	94.2

Identification of N-*(hydroxyacetyl)-aminoethanol as a metabolite of Tri in man*

Two volunteers (W.D. and D.H.) were exposed for 6 hr to 200 ppm of Tri by inhalation. Urine was collected as described in Materials and Methods. The fractions obtained after HPLC of the 24 hr urine, presumably containing HAAE, were pooled and the resulting residue was then dissolved in bis-trimethylsilyl acetamide for transformation of HAAE to the volatile bis-trimethylsilyl ether and was analysed by GC/MS, using an OV-1 capillary column for separation. Typical fragments of authentic silylated HAAE (m/z = 248, 173, 160, Fig. 2) were monitored during the gas chromatographic run and their relative intensities in the recorded mass spectra were plotted (Fig. 3).

The plots clearly revealed a high abundance of typical mass fragments with the retention time of authentic HAAE. The mass spectra recorded and processed from these scans were identical to that of the reference. The same procedure was also carried out on a Se-54 capillary column, thus proving the identity of HAAE as a metabolite of Tri in man. GC of the urinary fraction and comparison of the integrated areas permitted calculation of the total amount of HAAE excreted with the 24 hr urine, i.e. 43.6 mg (14.3 mg/l).

DISCUSSION

Species differences in metabolic conversion rates of Tri between rats and mice

The differences between rats and mice in metabolic transformation of a single oral dose of [^{14}C]Tri found in this study are in good agreement with the results obtained by several other authors ([12, 14]; C. R. Elcombe, personal communication) after oral or intraperitoneal administration, or exposure by inhalation to various concentrations of Tri. In mice, the much higher biotransformation rate of Tri, which shows no saturation kinetics up to doses of 2.4 g/kg, and the observed hepatotoxicity after prolonged administration of high Tri doses may contribute to the different response of this species in contrast to rats to a possible carcinogenic potential of the compound [14]. The higher sensitivity of mice to a possible carcinogenic effect of Tri has been claimed to be due to an epigenetic mechanism exerted by Tri or one of its metabolites, trichloroacetic acid (C. R. Elcombe, personal communication; [17]).

Detection of new metabolites of Tri

In addition to 1,1,1-trichloro compounds, which are the main end-products of Tri biotransformation, three other metabolites have been identified, indicating that at least four different pathways are operative in the metabolism of Tri (Fig. 5). The formation of 1,1,1-trichloro compounds may well be explained by a rearrangement of the putative primary oxidative metabolite, 1,1,2-trichlorooxirane (**1**) (see Fig. 4), to chloral (**2**). This reaction fails to occur in thermal rearrangement of synthetic trichlorooxirane *in vitro* in the absence of metals; under such conditions, dichloroacetyl chloride (**6**) is the only rearrangement product [17]. On the other hand, electrophilic compounds (Lewis acids, e.g. $AlCl_3$, $FeCl_3$) may easily

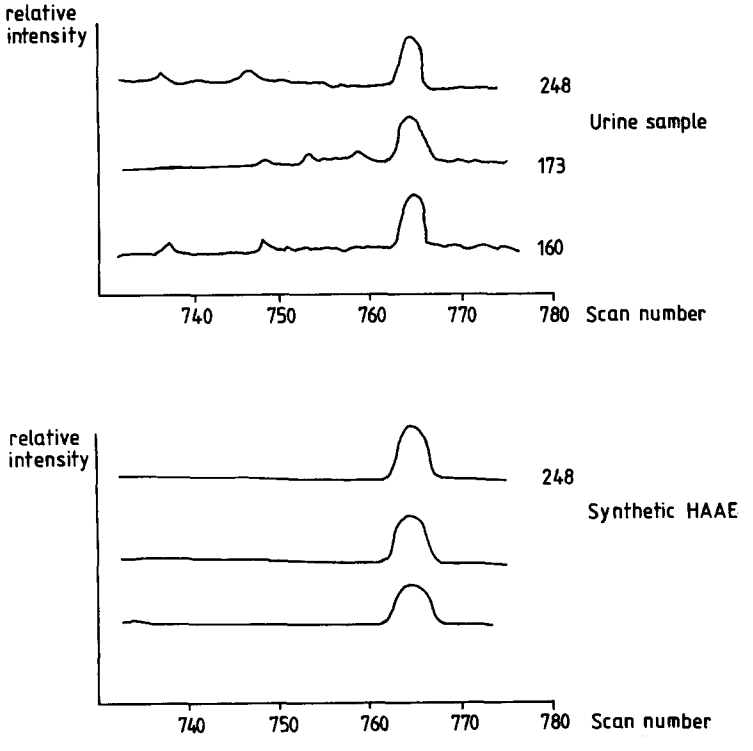

Fig. 3. Intensities of typical fragments of silylated HAAE and silylated human urine fraction under identical chromatographic conditions.

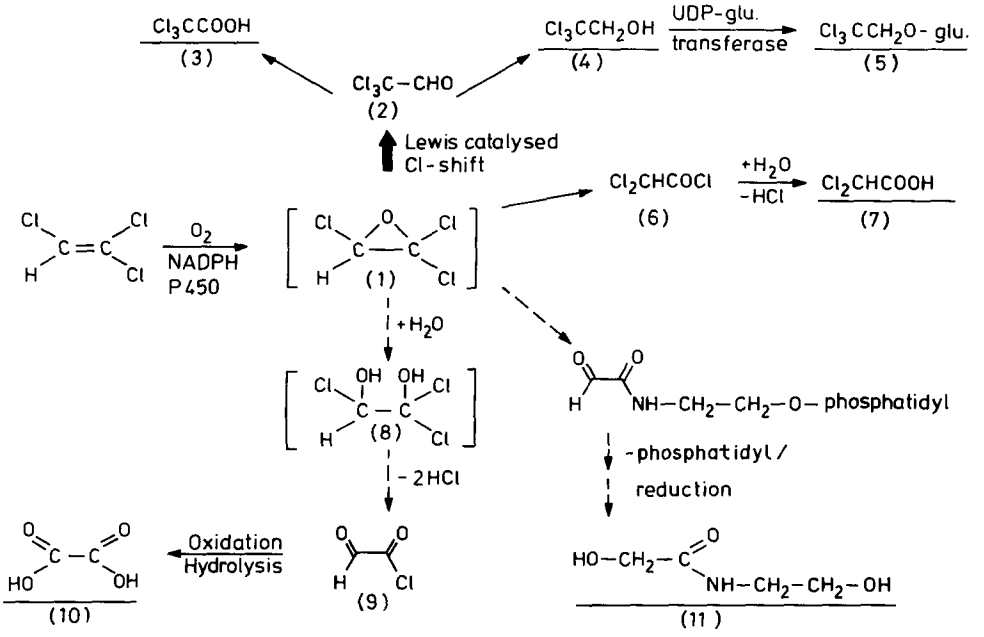

Fig. 4. Metabolic pathways of Tri (identified metabolites are underlined). **1**, Trichlorooxirane; **2**, chloral; **3**, trichloroacetic acid; **4**, trichloroethanol; **5**, trichloroethanol glucuronide; **6**, dichloroacetyl chloride; **7**, dichloroacetic acid; **8**, 1,2,2-trichloro-1,2-dihydroxyethane; **9**, glyoxylic acid chloride; **10**, oxalic acid; **11**, *N*-(hydroxyacetyl)-aminoethanol. Suggested pathways are indicated by dashed lines.

induce a conversion of the epoxide to chloral *in vitro* [17]. To elucidate this marked difference, it has been postulated that under *in vivo* conditions the trivalent iron in cytochrome P-450 may act as a Lewis acid and catalyse the transformation of the epoxide to chloral; this would explain the almost exclusive formation of the final excretion products trichloroacetic acid (**3**), trichloroethanol (**4**) and its glucuronide (**5**).

The very small quantities of trichloroacetic acid found in the experiments with mice may be due to an intense enterohepatic recirculation of trichloroacetic acid as an alkali-labile conjugate in this species. Trichloroacetic acid also forms a coenzyme A conjugate in the mouse, which is slowly excreted with the faeces [18].

Dichloroacetic acid (**7**), which at the given dose was found in substantial amounts only in rats, obviously stems from an overpowering of the deactivation system, which is catalysing the formation of chloral. Rearrangement of the epoxide outside the hydrophobic premise of cytochrome P-450 without interaction of metals is expected to yield dichloroacetyl chloride (**6**). This compound reacts readily with water to form dichloroacetic acid. CO_2, the formation of which [12] is confirmed in this study, may be the product of decarboxylation of di- or trichloroacetic acid [19].

Oxalic acid (**10**) may be formed as the end-product of enzymatic or non-enzymatic cleavage of the epoxide. This hydrolytic ring-opening produces a highly unstable vicinal diol (**8**), which is expected to undergo spontaneous elimination of two molecules of HCl. The glyoxylic acid chloride (**9**) formed may easily react with water and the glyoxylic acid will be further oxidized to oxalic acid.

N-(Hydroxyacetyl)-aminoethanol (**11**), the other chlorine-free metabolite, was identified in three species as a major biotransformation product of Tri. Mechanisms of its formation cannot yet be satisfactorily explained. One hypothesis proposes the reaction of Tri-derived oxidative intermediates either with ethanolamine itself or with phosphatidylethanolamine, a major constituent of membranes, and the ensuing metabolic breakdown of the alkylated lipids. The lipid alkylation could be

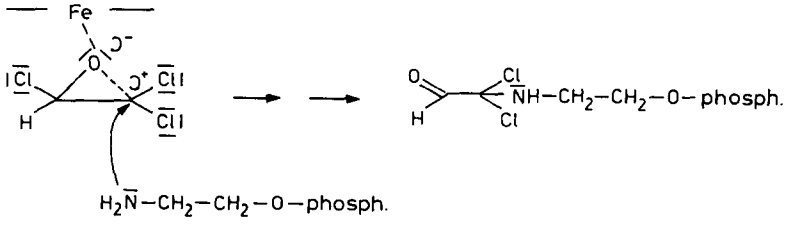

Fig. 5. Suggested mechanism of phospholipid alkylation: enhancement of reactivity at C-1 towards nucleophiles by the interaction of a Lewis acid with the oxidative intermediates of Tri.

favoured by an electron shift induced by a Lewis acid (e.g. cytochrome P-450), forming a species with enhanced reactivity towards nucleophiles, as shown in Fig. 5.

Similar ethanolamine adducts have been identified as end-products of biotransformation of 1,1-dichloroethylene [20] and halothane [21]. In halothane metabolism, trifluoroacetylated microsomal lipids have also been identified in *in vivo* and *in vitro* experiments [22]. Another possibility could be nucleophilic attack of an endocyclic amino group of the haem moiety in cytochrome P-450, resulting in a 'suicidal' inactivation of the microsomal monooxygenase system right after the accomplished oxidation of the Tri molecule. Compounds containing double and triple carbon–carbon bonds are known to destroy the porphyrin ring system of cytochrome P-450 during oxidative metabolism, as demonstrated for vinyl chloride [23] and a variety of olefinic and acetylenic compounds [24]. Further metabolic breakdown of the alkylated haems could also result in the ethanolamine adduct in urine.

Further investigations to confirm or reject these hypotheses are under way in these laboratories.

The results of this study show that inactivation of Tri or its electrophilic metabolic intermediates, respectively, occurs *in vivo* along at least four different pathways. The identified metabolites, the mechanisms of their formation and their proportions of total urinary activity reveal that only a very small percentage if any at all, of the reactive intermediates formed during biotransformation of Tri may reach the critical target molecules in the organism. These results may be an explanation for the questionable carcinogenic and mutagenic properties exhibited by trichloroethylene *in vivo* and *in vitro* and do not favour the concept of a direct genotoxic effect of this chlorinated ethylene.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft, Bonn, and the Doktor-Robert-Pfleger-Stiftung, Bamberg. The skilful technical assistance provided by Ms. E. Kretzschmar and Ms. J. Colberg (recording of mass spectra) is gratefully acknowledged.

REFERENCES

1. National Cancer Institute, Carcinogenesis Bioassay of Trichloroethylene, U.S. Department of Health, Education and Welfare, Public Health Service, CAS No. 79-01-6, February 1976.
2. D. Henschler, E. Eder, T. Neudecker and M. Metzler, *Archs Toxic.* **37**, 233 (1977).
3. D. Henschler, W. Romen, H. M. Elsässer, D. Reichert, E. Eder and Z. Radwan, *Archs Toxic.* **43**, 237 (1980).
4. *IARC Monogr.*, Suppl. 4, 247 (1982).
5. H. Greim, G. Bonse, Z. Radwan, D. Reichert and D. Henschler, *Biochem. Pharmac.* **24**, 2013 (1975).
6. D. Henschler, *Environ. Hlth. Perspect.* **21**, 61 (1977).
7. E. M. Waters, H. B. Gerstner and J. E. Juff, *J. Toxic. environ. Hlth* **2**, 671 (1977).
8. D. E. Hathaway, *Cancer Lett.* **8**, 263 (1977).
9. B. L. van Duuren and S. Banerjee, *Cancer Res.* **36**, 2419 (1976).
10. H. M. Bolt and J. G. Filser, *Environ. Hlth Perspect.* **21**, 107 (1977).
11. M. L. Cunningham, A. J. Gandolfi, K. Brendel and I. G. Sipes, *Life Sci.* **29**, 1207 (1981).
12. L. G. Parchman and P. N. Magee, *J. Toxic. environ. Hlth* **9**, 797 (1982).
13. K. Bergman, *Archs Toxic.* **54**, 181 (1983).
14. W. T. Stott, W. F. Quast and P. G. Watanabe, *Toxic. appl. Pharmac.* **62**, 137 (1982).
15. B. L. Brian, *J. Chromatogr.* **66**, 138 (1972).
16. T. Ertle, D. Henschler, G. Müller and M. Spassowski, *Archs Toxic.* **29**, 177 (1972).
17. G. Bonse and D. Henschler, *CRC Crit. Rev. Toxic.* **4**, 395 (1976).
18. T. Green, M. S. Pront and W. N. Prawan, *Abstr. 8th Eur. Workshop on Drug Metabolism*, p. 130, Liège (1982).
19. D. Henschler and R. Hoos, in *Biological Reactive Intermediates*—II, Part A, pp. 659–666. Plenum Press, New York (1982).
20. D. Reichert, H. Werner, M. Metzler and D. Henschler, *Archs Toxic.* **42**, 159 (1979).
21. E. N. Cohen, J. R. Trudell, H. N. Edmunds and E. Watson, *Anesthesiology* **43**, 392 (1975).
22. R. Müller and A. Stier, *Naunyn-Schmiedeberg's Archs Pharmac.* **21**, 234 (1982).
23. F. P. Guengerich and T. W. Strickland, *Molec. Pharmacol.* **13**, 993 (1977).
24. R. R. Ortiz de Montellano, H. S. Beilan, K. L. Kunze and B. R. Mico, *J. biol. Chem.* **256**, 4395 (1981).