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Incubation of ^{14}C-Trichloroethylene Vapor with Rat Liver Microsomes: Uptake of Radioactivity and Covalent Protein Binding of Metabolites

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Summary. Microsomal uptake irreversible protein binding of labelled trichloroethylene was measured following incubation with rat liver microsomes in an all-glass vacuum system.

If the cofactor for oxidative metabolism, NADPH, is not added, the gaseous trichloroethylene rapidly equilibrates with the microsomal suspension. Addition of NADPH results in a further uptake of ^{14}C-trichloroethylene from the gas phase, linearly with time, which is due to enzymic metabolism. This part of uptake is inhibited by some arylimidazoles and 1.2.3-benzothiadiazoles. The compounds of greatest inhibitory potency were 6-chloro-1.2.3-benzothiadiazole and 5,6-dimethyl-1.2.3-benzothiadiazole. Part of the metabolites of ^{14}C-trichloroethylene formed by rat liver microsomes were irreversibly bound to microsomal protein, amounting up to 1 nmol per mg microsomal protein per hour. Model experiments on uptake of ^{14}C-trichloroethylene from the gas phase by albumin solutions and liposomal suspensions (from lecithin) showed a rapid equilibration of trichloroethylene also with these systems. Comparison with previous analogous data on vinyl chloride revealed an about 10 times higher affinity of trichloroethylene to albumin and lipid, consistent with the behaviour of both compounds in the rat liver microsomal system.

Key words: Trichloroethylene – Vinyl chloride – Rat liver microsomes – Covalent protein binding of trichloroethylene – Albumin – Lecithin liposomes

Introduction

Trichloroethylene is widely used as degreasing solvent in the industry. In analogy to the pattern of the closely related vinyl chloride it has been argued that this compound possibly might be cancerogenic and/or mutagenic (Van Duuren, 1975). This view is supported by animal experiments (National Cancer Institute, 1976), by mutagenic effects of metabolites of trichloroethylene and of vinyl chloride in bacterial test systems (Greim et al., 1975), and by covalent binding of trichloroethylene (Van Duuren

and Banerjee, 1976) and vinyl chloride (Kappus et al., 1975, 1976) metabolites to microsomal proteins.

As vinyl chloride, trichloroethylene is regarded to be initially metabolized by hepatic mixed function oxidases to a labile epoxide (oxirane) derivative (Bonse et al., 1975) which undergoes secondary conversion. Rat liver microsomes transform trichloroethylene to chloralhydrate (Leibman, 1965; Byington and Leibman, 1965); the latter may be reduced to trichloroethanol or oxidized to trichloroacetic acid. In man, metabolism of trichloroethylene follows the same pathways (Ertle et al., 1972; Müller et al., 1974). After human exposure to trichloroethylene, both trichloroethylene and trichloroethanol are stored in tissues and released slowly (Müller et al., 1974).

Because of the formal chemical analogies between trichloroethylene ($Cl_2C = CHCl$) and vinyl chloride ($CH_2 = CHCl$) a comparison of biological and biochemical effects of both compounds seems desirable. However, this requires identical experimental systems for both compounds. Metabolic surveys on vinyl chloride are difficult because of its gaseous nature. Trichloroethylene, however, is a liquid with a boiling point of 87°. Therefore, especially all in vitro studies on trichloroethylene used liquid trichloroethylene as substrate which had been added to the incubation system. To allow comparison with vinyl chloride, both compounds, vinyl chloride and trichloroethylene, should be applied to an in vitro system in identical manner. At normal temperature (25°) the partial pressure of saturated trichloroethylene vapor is 70 torr.

The aim of the present report is to compare the uptake of gaseous vinyl chloride and of trichloroethylene vapor by rat liver microsomes under identical conditions and to obtain data regarding the comparative covalent binding of metabolites of both compounds to rat liver microsomal protein in vitro. Therefore, we performed similar experiments with ^{14}C-trichloroethylene as we had already done with ^{14}C-vinyl chloride (Kappus et al., 1976) and incubated rat liver microsomes in a closed system in an atmosphere containing different amounts of trichloroethylene vapor. Furthermore, model experiments have been carried out with albumin solutions and liposomal suspensions to study the affinity of trichloroethylene for protein and lipid structures.

Materials and Methods

[1, 2-^{14}C] Trichloroethylene (spec. activity 1.7 mCi/mmol) was synthesized by New England Nuclear, Boston, Mass. Radiochemical purity, as established by NEN using gas-liquid-chromatography on porapak N, was 99%.

The synthesis of the 1.2.3.-benzothiadiazole derivatives used for inhibiting metabolism of trichloroethylene has been previously described (Gil and Wilkinson, 1976). 1-Naphthyl-4(5)-imidazole and its (4-phenyl)-phenyl analog have been synthesized according to Wilkinson et al. (1974).

Liposomes from egg lecithin were prepared according to Wollenberg et al. (1976).

Male Wistar rats of 180–200 g were used as liver donors. Liver microsomes were prepared according to Remmer et al. (1967).

Rat liver microsomes were incubated with an NADPH-regenerating system at 37° under an atmosphere which contained different amounts of gaseous ^{14}C-trichloroethylene. The concentration of the substrate, trichloroethylene, in the air was expressed as its partial pressure. For these experiments the same apparatus was used

which has been already used for incubation of microsomes with gaseous ^{14}C-vinyl chloride (see Kappus et al., 1976). Determination of uptake of ^{14}C-trichloroethylene radioactivity and of the ^{14}C-trichloroethylene metabolites irreversibly bound to the microsomal protein was exactly as described for ^{14}C-vinyl chloride (Kappus et al., 1976).

Results

Gaseous trichloroethylene rapidly equilibrates with microsomal suspensions. Figure 1 demonstrates that the major portion of ^{14}C-trichloroethylene is taken up within the first three minutes, if the cofactor NADPH (or a regenerating system) is not added. If NADPH is present, a further uptake of radioactivity from the gas phase occurs, linear with time, which should be attributed to metabolism of trichloroethylene to non-volatile, polar, metabolites (Leibman 1965; Byington and Leibman, 1965; Leibman and McAllister, 1967).

Figure 2 shows the influence of substrate concentration (i.e. the partial pressure of ^{14}C-trichloroethylene in the gas phase) on microsomal uptake of trichloroethylene radioactivity, both with and without addition of the NADPH-regenerating system. The enzymic conversion of trichloroethylene which is expressed by the increment of uptake on addition of the cofactor NADPH reaches saturation at comparatively low concentrations of trichloroethylene in the atmosphere.

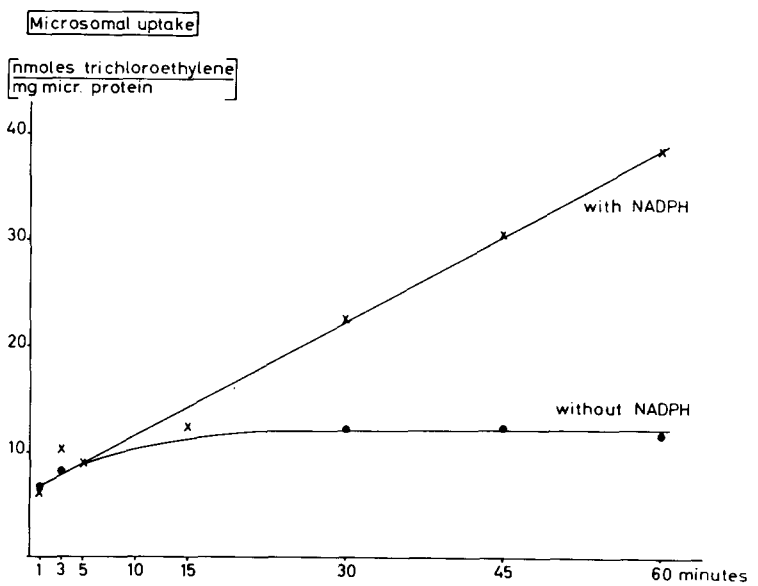

Fig. 1. Microsomal uptake of gaseous ^{14}C-trichloroethylene (partial pressure below 0.01 torr) by rat liver microsomal suspensions ●—●—● incubation without addition of NADPH x——x——x incubation with an NADPH-regenerating system

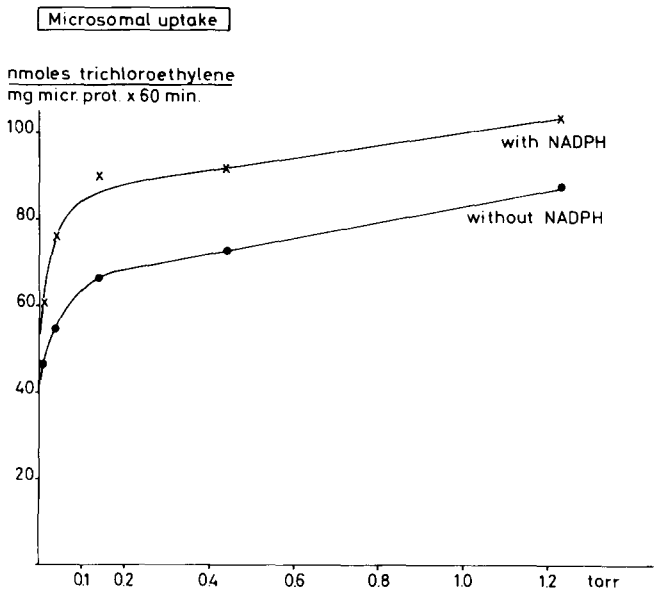

Fig. 2. Dependence of microsomal uptake of gaseous ^{14}C-trichloroethylene by rat liver microsomes on concentration of substrate. Substrate concentration is given as the partial pressure of ^{14}C-trichloroethylene in the atmosphere (torr). •—•—• uptake in absence of NADPH x——x——x uptake in presence of an NADPH-regenerating system. Incubation time was 60 minutes at 37°. torr = partial pressure of TRI.

Previous experiments (Bolt et al., 1976) have shown that hepatic metabolism of vinyl chloride can effectively be inhibited by some compounds which interact with cytochrome P-450 and which originally have been designed as insecticide synergists (Wilkinson, 1971; Wilkison and Brattsten, 1972). These include derivatives of imidazole and of 1.2.3-benzothiadiazole. By means of these compounds some cytochrome P-450 dependent oxygenations may be differentially inhibited (Bolt and Kassel, 1976). Table 1 compares the inhibitory potency of some 1.2.3-benzothiadiazoles with that of two arylimidazoles at the same concentration (10^{-4} M inhibitor). At this low concentration SKF 525 A (obtained from Smith, Kline and French, Philadelphia, Pa.) shows no inhibitory effect. This only becomes evident at concentrations above 10^{-3} M (Van Duuren and Banerjee, 1976). In general, all the 1.2.3-benzothiadiazoles tested inhibit metabolism of trichloroethylene more than the two imidazoles (i.e., 1-naphthyl--4(5)-imidazole and (4-phenyl)-phenyl-4(5)-imidazole).

To answer the question as to whether the NADPH-independent part of uptake of trichloroethylene by microsomes ought to be due to dissolution of this compound in the lipid or protein moiety of microsomal membranes, further experiments examined solubility of trichloroethylene in albumin solutions and in liposomal suspensions. Table 2 shows that both, albumin and liposomes are capable of taking up tricholoroethylene from the gas phase. Capacity of liposomes for binding trichloroethylene is slightly higher than that of albumin. From a quantitative point of view, liposomes and albumin take up about one-fourth of trichloroethylene compared to the amount taken up by microsomes in absence of NADPH. This relation agrees with that which has been calculated for vinyl chloride (Kappus et al., 1976)

Table 1. Inhibition of microsomal metabolism of trichloroethylene. Microsomal metabolism of trichloroethylene has been determined as the increase of uptake of gaseous ^{14}C-trichloroethylene on addition of NADPH. Each value represents the mean of double determinations. Concentration of inhibitors was 10^{-4} M. Incubation time was 60 min at 37°.

inhibitor	% inhibition
SKF 525 A	0
(4-phenyl)-phenyl-4(5)-imidazole	15.2
1-naphthyl-4(5)-imidazole	43.8
5-nitro-1.2.3-benzothiadiazole	64.5
6-methoxy-1.2.3-benzothiadiazole	71.1
5-chloro-1.2.3-benzothiadiazole	80.8
6-bromo-1.2.3-benzothiadiazole	81.9
6-ethoxy-1.2.3-benzothiadiazole	89.6
6-chloro-1.2.3-benzothiadiazole	100.0
5,6-dimethyl-1.2.3-benzothiadiazole	100.0

Table 2 also compares the reversible binding of ^{14}C-trichoroethylene by albumin and liposomes with the amount of ^{14}C-trichloroethylene metabolites which is irreversibly (covalently) bound by rat liver microsomal protein. The time course of covalent binding of trichloroethylene metabolites by microsomal protein (Figure 3) is linear over a period of at least 60 minutes which also agrees with the previous findings on vinyl chloride (Kappus et al., 1976).

Table 2. Reversible binding of ^{14}C-trichloroethylene to albumin and liposomes and irreversible (covalent) binding of ^{14}C-trichloroethylene metabolites to rat liver microsomal protein: a) Dependence of uptake of radioactivity by albumin solutions and liposomal suspensions on partial pressure of gaseous ^{14}C -trichloroethylene. b) Dependence of covalent binding to microsomal protein on partial pressure of ^{14}C-trichloroethylene.

partial pressure of trichloroethylene (torr) in the gas phase	a) reversible binding: nmol trichloroethylene per mg albumin	liposomes	b) irreversible binding to protein: (nmol trichloroethylene per mg microsomal protein)
0.0136	9	13	0.53
0.044	10	14	0.57
0.140	11	16	0.71
0.442	15	18	0.74
1.225	15	18	1.02

Incubation time was 60 min at 37°C. Figures show the mean of double experiments.

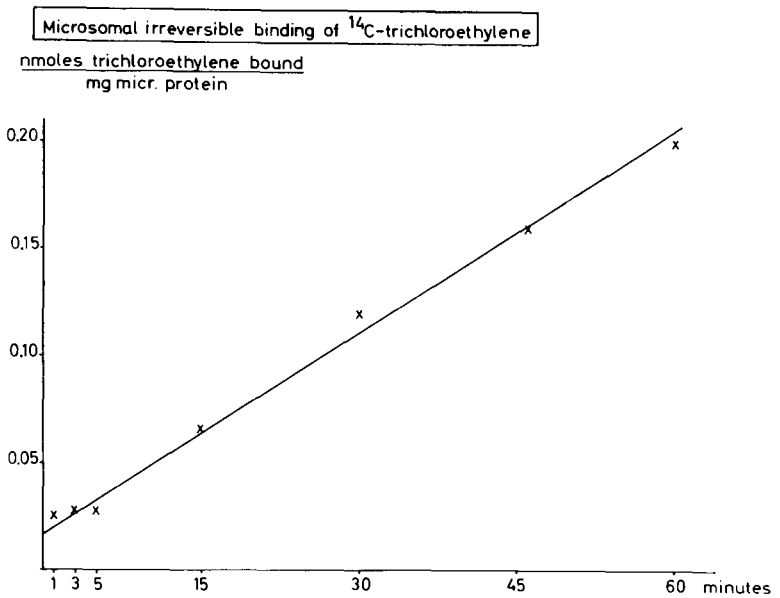

Fig. 3. Timal course of covalent binding of ^{14}C-trichloroethylene metabolites to microsomal protein on exposure of a microsomal suspension, containing NADPH-regenerating system, to gaseous ^{14}C-trichloroethylene (partial pressure below 0.01 torr)

Discussion

Rat liver microsomes rapidly take up trichloroethylene, if this compound is present in gaseous form in the atmosphere. This is in agreement with analogous data on vinyl chloride (Kappus et al., 1976). However, quantitatively trichloroethylene and vinyl chloride behave different: whereas, without addition of NADPH to the microsomal system, under saturation conditions only 4 nmol vinyl chloride are taken up per mg microsomal protein (Kappus et al., 1976), twenty times more trichloroethylene (i.e., about 80 nmol trichloroethylene/mg microsomal protein) is dissolved by the same preparation (see Figure 2). Also, in the case of vinyl chloride (Kappus et al., 1976) there is a plateau if NADPH is added; the apparent high affinity of microsomes for trichloroethylene leads to a further uptake of non-metabolized trichloroethylene at high trichloroethylene levels (Figure 2). These findings are supported by the model experiments with albumin solutions and liposomal suspensions. One milligram albumin reversibly binds only 1.1 nmol vinyl chloride, but 15 nmol trichloroethylene, and 1 mg lecithin binds 1.7 nmol vinyl chloride, but 18 nmol trichloroethylene (see Kappus et al., 1976, and Table 2).

The reason for this behaviour should be a different lipophilicity of trichloroethylene than of vinyl chloride which is especially evidenced by the solubility in liposomal suspensions. Therefore, much more trichloroethylene than vinyl chloride should bind to membrane structures. In the microsomal system, this leads to higher concentrations of trichloroethylene than of vinyl chloride at the site of metabolism. This is also observed in differences in saturation of albumin and liposomes by the two different

substrates: whereas a partial pressure of 2 torr vinyl chloride in the gas phase is necessary to saturate albumin or liposomes (Kappus et al., 1976), 0.5 torr of trichloroethylene in the gas phase has the same effect (see Table 2).

The higher affinity of trichloroethylene for microsomal structures may be expressed by the enzymic turnover: at a partial pressure of 1.225 torr trichloroethylene (see Table 2) 1 mg microsomal protein covalently binds 1.02 nmol trichloroethylene metabolites within 60 min, the corresponding figures for vinyl chloride amounting to 0.5 nmol per mg microsomal protein in 60 min (unpubl.).

That covalent binding data of both compounds, trichloroethylene and vinyl chloride, range in the same order of magnitude, may be consistent with the theory of Van Duuren (1975) that trichloroethylene and vinyl chloride should possibly exert similar effects regarding to chemical induction of tumours. But further studies are necessary which should deal with covalent binding of trichloroethylene metabolites to nucleic acids, especially under in vivo conditions. For vinyl chloride, it could be demonstrated (Laib and Bolt, 1977) that, after exposure of rats to vinyl chloride, adenine moieties of nucleic acids of the liver are chemically modified to 1-N^6-etheno-adenine, a mechanism which has been postulated by Barbin et al. (1975) to be involved in chemical cancerogenicity of vinyl chloride.

The view that trichloroethylene must be metabolized, probably to the intermediary epoxide, to gain mutagenic (Greim et al., 1975) or cancerogenic (Van Duuren, 1975; Van Duuren and Banerjee, 1976) activity stimulates experimental research for compounds that effectively can block metabolism of trichloroethylene. SKF 525 A which has been used to inhibit microsomal metabolism of trichloroethylene (Leibman, 1965; Van Duuren and Banerjee, 1976) is not effective when applied in low concentrations. However, the present data show that substituted benzothiadiazoles, especially 6-chloro-1.2.3-benzothiadiazole and 5,6-dimethyl-1.2.3-benzothiadiazole, very effectively inhibit microsomal metabolism of trichloroethylene (see Table 1).

The high affinity for trichloroethylene of protein and lipid membrane structures which results from the present investigation is also of considerable impact for interaction of trichloroethylene with the living organism. For example, the constant of equilibrium for vinyl chloride (v.c.),

$$K = \frac{\text{concentration of v.c. in rat tissues}}{\text{concentration of v.c. in the atmosphere}}$$

which can be determined if metabolism is completely blocked by a potent inhibitor, amounts to 2.86 (Bolt et al., 1977). The corresponding value for trichloroethylene, however, amounts to about 13 (unpubl.). This means that atmospheric trichloroethylene is more than four times more concentrated by the rat organism than vinyl chloride is. This pattern of trichloroethylene has some pharmacokinetic consequences which will be discussed in detail elsewhere.

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