STUDIES ON LIVER MICROSOMAL METABOLISM AND INTERACTION OF VINYL CHLORIDE AND RELATED COMPOUNDS IN RELATION TO POSSIBLE CARCINOGENICITY

H. M. BOLT[1], J. G. FILSER[1], M. WIEGAND[1], A. BUCHTER[2]
and W. BOLT[2]

Institute of Toxicology, University of Tübingen[1] and Institute for Occupational and Social Medicine, University of Cologne[2], F.R. Germany

ABSTRACT

Vinyl chloride and its analogues interact with hepatic microsomal cytochrome P-450. A consequence of this interaction is the oxygenation at the ethylene double bond to epoxide derivatives. In some instances these are regarded as possible »ultimate« mutagens and carcinogens. On the other hand, interaction with cytochrome P-450 may lead to inhibition of the metabolism of drugs and other xenobiotics. Inhibition is maximal by compounds which are only slowly metabolized, e.g. *trans*-1,2-dichloroethylene, perchloroethylene, and vinylidene fluoride. Vinyl chloride which is rapidly metabolized by cytochrome P-450 exerts a weak inhibitory effect only. The effects were examined *in vitro* with rat liver microsomes and different concentrations of halogenated hydrocarbons in the gas phase. p-Hydroxylation of aniline and demethylation of aminopyrine were used as indicator reactions.

The proven carcinogenic action of vinyl chloride and questions of possible carcinogenicity of related compounds are of major concern in occupational medicine. During the last few years it has been recognized that vinyl chloride does not represent a primary carcinogen *per se*, but that it is »activated« by the microsomal monooxygenase system of the liver to at least one alkylating and toxic metabolite, possibly the epoxide derivative, chloroethylene oxide[3].

Comparative studies on the metabolism of chlorinated ethylenes have suggested that all the compounds of this chemical class share a common primary metabolic epoxidation[6]; mutagenicity and carcinogenicity of either of these compounds probably depend on the stability and relative reactivity of the appropriate epoxide[7]. Hence, the carcinogenicity of vinyl chloride and possible carcinogenicity of its congeners rely on interaction of these compounds with the hepatic microsomal monooxygenase, especially with cytochrome P-450 which plays a key role in the formation of epoxides.

Vinyl chloride binds to hepatic cytochrome P-450 as a typical "type-I--compound"[13,27]. Unknown metabolites of vinyl chloride cause a destruction of hepatic microsomal heme and hence of cytochrome P-450[12,13,26], but on the other hand a long-term exposure to elevated vinyl chloride levels may result in induction of the cytochrome P-450 system[23].

It is well established that some halogenated hydrocarbons may inhibit oxidative drug metabolism by binding to cytochrome P-450[8]. The toxicological impact of this type of interaction of occupationally used compounds with hepatic microsomal enzymes has recently been stressed[10].

This investigation uses the rat liver microsomal system *in vitro* and compares the metabolic "activation" of vinyl chloride and of some related compounds (vinyl bromide, trichloroethylene) with the inhibitory effect of halogenated ethylenes towards microsomal reactions of oxidative drug metabolism. Some compounds that are very slowly metabolized but bind to cytochrome P-450 may markedly inhibit oxidative drug metabolism.

MATERIALS AND METHODS

Materials

Vinyl chloride, vinyl fluoride and vinylidene fluoride were donated by Dynamit Nobel AG, Troisdorf/Germany. Vinylidene chloride, vinyl bromide, 1,1,1-trichloroethane (methyl chloroform), perchloroethylene and *trans*-1,1--dichloroethylene were purchased from Aldrich Europe, Brussels/Belgium. *Cis*--1,1-dichloroethylene was a product of Ferak, Berlin/Germany. Trichloroethylene was from Merck AG, Darmstadt/Germany.

Microsomal incubations

Male Wistar rats (200−250 g, Ivanovas, Kisslegg/Germany) were pretreated with phenobarbital to increase hepatic monooxygenase levels. This was done by a single i.p. dose of 80 mg/kg, followed by five days of phenobarbital administration, 0.1%, in drinking water. Liver microsomes were prepared in the usual manner[25]. Microsomal incubations were carried out under air, containing definite partial pressures of halogenated compounds in the gas phase. For this purpose an all-glass incubation system was used which has been described in detail elsewhere[16]. Different concentrations of halogenated volatile compounds in the gas phase were adjusted to inhibit microsomal demethylation of aminopyrine and p-hydroxylation of aniline. p-Hydroxylation of aniline was assayed as described by Schenkman and co-workers[28].

Demethylation of aminopyrine was followed by determination of the generated formaldehyde[22]. Substrate solution (0.1 ml 0.16 M aminopyrine), 0.1 ml 5 mM $MgCl_2$, 0.1 ml 80 mM DL-Na-isocitrate, 0.3 ml Tris-HCl buffer pH 7.5 (0.1 M), 0.3 ml microsomal suspension (5 mg microsomal protein/ml) and 5 µl isocitric dehydrogenase solution (Boehringer Mannheim) were placed in reaction vessels[16] and the mixture was chilled on ice. After addition of 0.1 ml 10 mM NADP, connection of the vessels to the incubation apparatus[16], adjustment of

the appropriate concentration of the halogenated compound in the gas phase and five minutes of equilibration the reaction was started, the ice bath was replaced by a 37 °C water bath. Samples were drawn at different time intervals from 5 through 30 minutes after the start of the reaction. To every 0.3 ml of the reaction mixtures, 0.3 ml 7.5% trichloroacetic acid was added to stop the reaction. After centrifugation of the proteins the supernatant served for the determination of formaldehyde which had been formed during the enzymic reaction. Determination followed the method of Nash[22].

RESULTS

Some aliphatic halogenated hydrocarbons may inhibit hepatic drug metabolism while others do not exhibit this effect[8]. This statement also pertains to the chemical class of halogenated ethylenes. Of the two oxidative rat liver microsomal reactions tested, aminopyrine demethylation is much more sensitive to inhibition than p-hydroxylation of aniline (Table 1). Aniline hydroxylation is

TABLE 1

Effects of volatile halogenated ethylenes and of methyl chloroform present in the gas phase on aniline hydroxylation and on aminopyrine demethylation by rat hepatic microsomes *in vitro*. 100 % = total inhibition of reaction.

Compound	Partial pressure in gas phase		% inhibition of	
	torr	kPa	aniline hydroxylation	aminopyrine demethylation
Vinyl chloride	55	7.3	5	6
Vinyl fluoride	55	7.3	3	6
Vinylidene chloride	51	6.8	7	12
Vinylidene fluoride	52	7.0	6	25
Vinyl bromide	54	7.2	12	17
trans-DCE	50	6.7	12	40
cis-DCE	47	6.3	n.d.	12
Trichloroethylene	15	2.0	0	0
Perchloroethylene	6.3	0.8	0	9
1,1,1-Trichloroethane (methyl chloroform)	22.4	3.0	0	23

DCE = 1,2-dichloroethylene
n.d. = not determined
Control values (without inhibitor): p-hydroxylation of aniline: 1.35 ± 0.07 (S.D.; n = 3) nmol/mg microsomal protein per min; demethylation of aminopyrine: 11.8 ± 2.4 (S.D.; n = 12) nmol/mg microsomal protein per min.

only slightly inhibited by *trans*-1,2-dichloroethylene and by vinyl bromide. Inhibition of aminopyrine demethylation by those compounds is more pronounced; it can be elicited especially by *trans*-1,2-dichloroethylene, to a lesser degree by vinylidene fluoride and by vinyl bromide. *Cis*-1,2-dichloroethylene is

much less active in our system than *trans*-1,2-dichloroethylene. Slight inhibitions are observed with vinyl chloride and vinyl fluoride. A typical inhibition experiment with *trans*-1,2-dichloroethylene is shown in Figure 1.
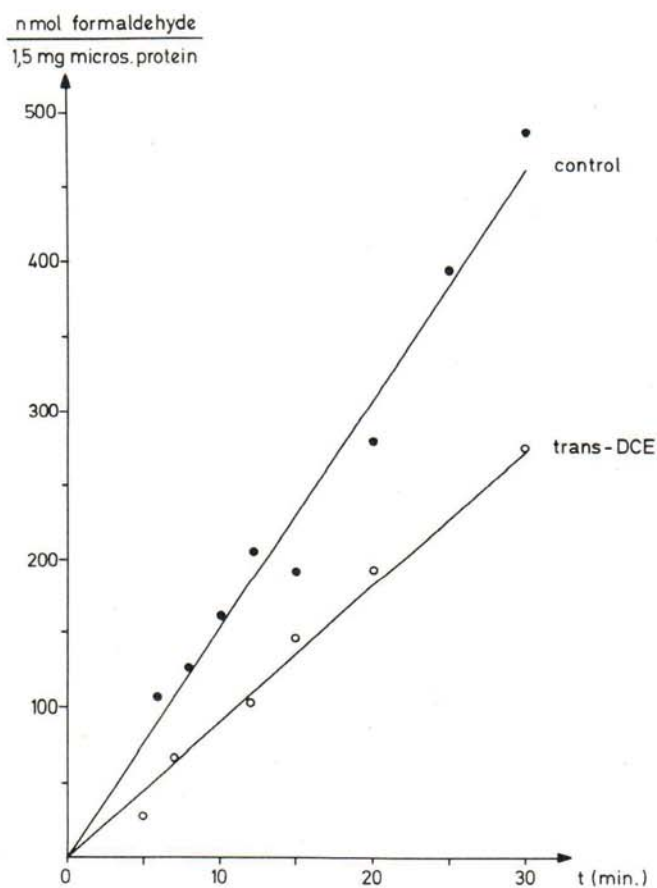

FIG. 1 — Demethylation of aminopyrine by rat liver microsomes *in vitro* and inhibition by *trans*-1, 2-dichloroethylene. Solid circles: control, not inhibited; open circles: in presence of *trans*-1,2- -dichloroethylene in atmosphere, partial pressure = 7 kPa (53 torr).

Experiments with different concentrations of *trans*-1,2-dichloroethylene and vinylidene fluoride in the gas phase, as compared to methyl chloroform (Figure 2) suggest different mechanisms of inhibition of aminopyrine demethylation by *trans*-1,2-dichloroethylene and by vinylidene fluoride: vinylidene fluoride shows progressive inhibition of aminopyrine demethylation with higher concentrations.
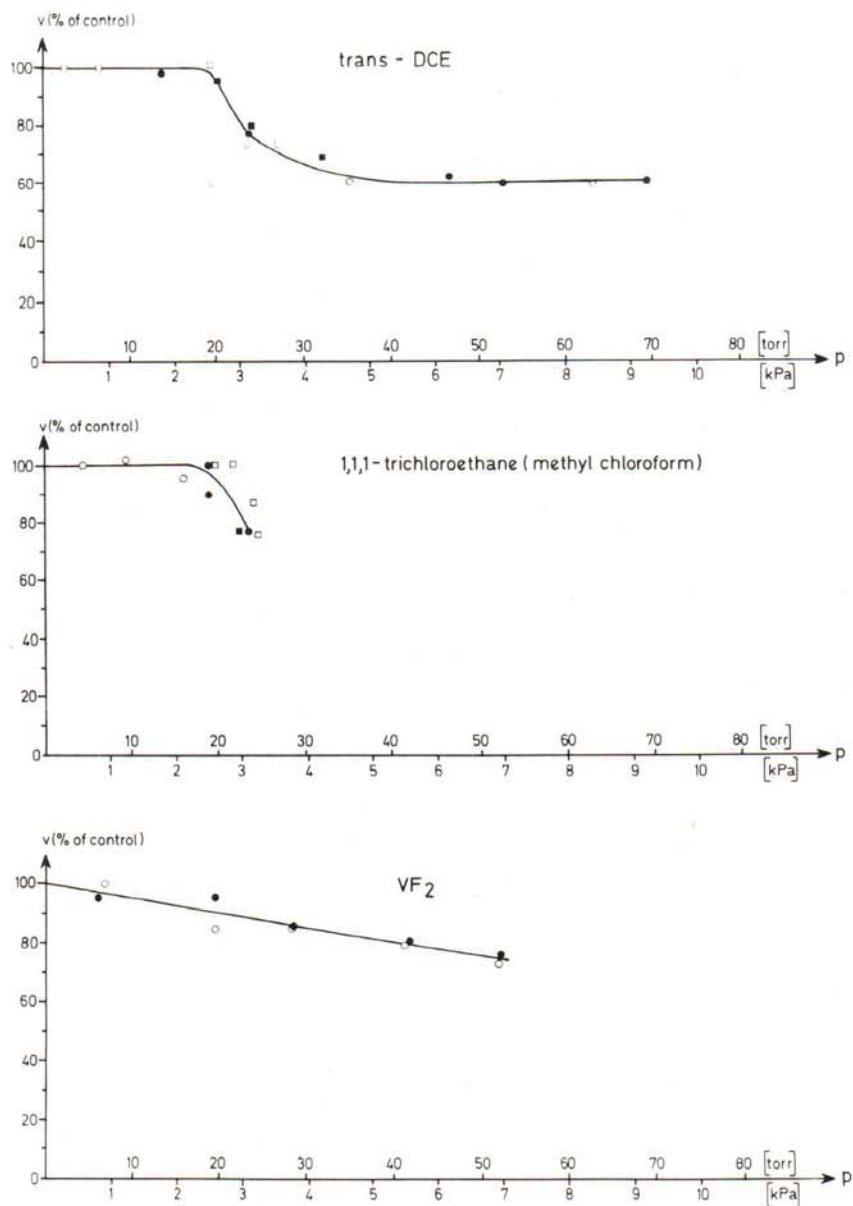
FIG. 2 – Inhibition of liver microsomal demethylation of aminopyrine *in vitro* by different amounts of *trans*-1,2-dichloroethylene (*trans*-DCE), 1,1,1-trichloroethane (methyl chloroform) and vinylidene fluoride (VF_2) in the atmosphere. Partial atmospheric pressures of the halogenated hydrocarbons are given in torr and in kPa. Different symbols represent different sets of experiments.

By means of a Dixon-plot (Figure 3) a theoretical 50% inhibition was calculated to occur at a partial pressure of 21 kPa (160 torr) in the gas phase of the microsomal incubation system.

Trans-1,2-dichloroethylene, however, behaves differently in that it is ineffective at atmospheric partial pressures below 2.5 kPa, and a maximal effect occurs at 5 kPa which cannot be further increased. This might be due to the solubility properties of *trans*-1,2-dichloroethylene in the microsomal lipid phase. Furthermore, it is interesting to note that, with respect to aminopyrine demethylation, methyl chloroform (1,1,1-trichloroethane) behaves very similarly to *trans*-1,2-dichloroethylene (Figure 2). However, experiments with higher concentrations of the compound in the gas phase are not possible because of the higher boiling point and lower volatility of methyl chloroform, than of the other compounds investigated.

Trans-1,2-dichloroethylene, vinylidene fluoride and methyl chloroform share the common property that they are all extremely slowly metabolized by rats[9].
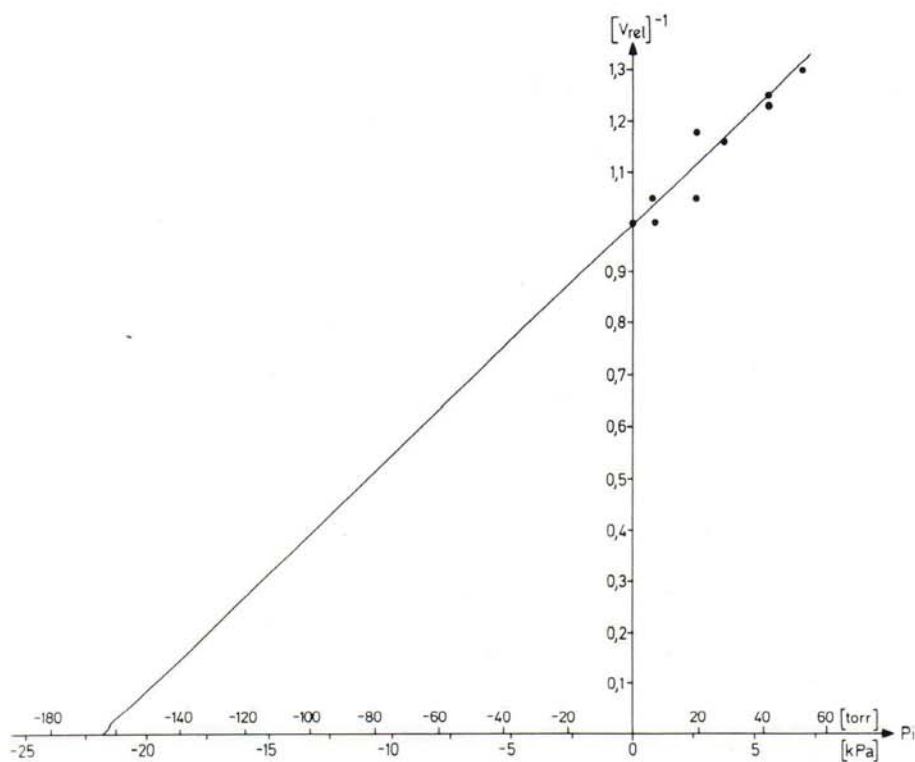

FIG. 3 – "Dixon-plot" of inhibition of liver microsomal aminopyrine demethylation *in vitro* by atmospheric vinylidene fluoride. Vinylidene fluoride concentrations are given as partial pressures of the compound in the gas phase.

TABLE 2

Metabolism and irreversible protein binding of vinyl chloride and analogues in the rat liver microsomal system *in vitro* (nmol/mg microsomal protein per hour).

Compound	Overall metabolism	Irreversibly protein bound	Reference
Vinyl chloride	39	0.45	16
Vinyl bromide	14	0.34	5
Trichloroethylene	25	1.02	4

The present data, along with those from the literature[11], indicate that halogenated ethylenes might interact with the hepatic drug metabolizing monooxygenase system.

DISCUSSION

The present data show that halogenated ethylenes interact with the hepatic microsomal monooxygenase. This interaction may lead to oxygenation of the substrate, resulting in the formation of an epoxide intermediate with possibly toxic properties[6]. On the other hand, compounds that are metabolized to a minimal extent only, like *trans*-1,2-dichloroethylene, vinylidene fluoride, or even perchloroethylene, may persist at the enzymic site and thus exhibit inhibition of oxygenation of other drugs or xenobiotics that are also biotransformed by the hepatic microsomal monooxygenase system.

The present data obtained *in vitro* can be related to observations *in vivo*. Freundt and Macholz[11] have recently reported that *trans*-1,2-dichloroethylene and *cis*-1,2-dichloroethylene, if acting on rats at an atmospheric concentration of 200 ppm only which corresponds to the current MAC/TLV value, markedly inhibit aminopyrine metabolism.

Vinyl chloride is more rapidly metabolized[3,9], and shows only a weak inhibitory effect on the metabolism of aminopyrine. However, it has been claimed that interference in metabolic "activation" may occur between vinyl chloride and vinylidene chloride at the microsomal cytochrome P-450 level[14], in that vinyl chloride reduces the hepatotoxic effectiveness of vinylidene chloride. Another microsomal effect of vinyl chloride and related compounds which is presently under investigation is induction of lipid peroxidation. The available data [15,17] suggest that the metabolism of these substances may also involve the formation of free radicals. Lipid peroxidation may be one molecular mechanism to understand some pathogenic effects of halogenated hydrocarbons.

Rapid metabolism has been observed to occur with vinyl chloride, vinyl bromide and trichloroethylene. Vinyl chloride[21] and vinyl bromide[1] are carcinogenic whereas carcinogenicity of trichloroethylene is very questionable[20]. Table 2 summarizes the velocities of hepatic microsomal overall metabolism *in vitro* and the amounts of reactive metabolites which are irreversibly bound *in vitro* to microsomal protein. All these compounds are "activated" to

protein alkylating metabolites. This, along with the alkylation of nucleic acids[18,19,20,24] points to a possible carcinogenicity.

The question of carcinogenicity of trichloroethylene is presently under discussion[2,6,20,29,30]. Vinyl bromide which is used for the manufacture of flame--resistant polymers should be handled with similar caution as vinyl chloride[1]. Further studies will evaluate other compounds which show a chemical similarity to vinyl chloride.

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