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COMMENTARY

REACTIVE METABOLITES AND CARCINOGENICITY OF HALOGENATED ETHYLENES

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Oxiranes as biological reactive intermediates of haloethylenes

A field of rapidly expanding knowledge is that of biological reactive intermediates. In this discussion halogenated compounds, e.g. vinyl chloride, play a prominent role [1]. For the chlorinated ethylenes, preliminary considerations connecting structure and activity have been published by Henschler and coworkers [2–5]. They suggested the following: (a) all chlorinated ethylenes are initially biotransformed by microsomal monooxygenase (s) to epoxide (oxirane) intermediates which are subsequently 'detoxified', e.g. by molecular rearrangement (see Fig. 1); (b) reactivities and hence toxicities of individual chlorooxirane intermediates depend on the type of chlorine substitution in that symmetric substitution renders the epoxide relatively stable and not mutagenic whilst asymmetric substitution causes unstable and, therefore, mutagenic epoxides; (c) trichloroethylene is exempt from this general rule because its epoxide 2,2,3-trichlorooxirane, although asymmetrically substituted and reactive, is immediately further transformed at the cytochrome P-450 site to trichloroacetaldehyde (chloral).

The latter is now supported by a recent inhalatory carcinogenicity study in 3 animal species which revealed no indication for carcinogenicity of trichloroethylene [6]. Also, mutagenicity data originally obtained with *E. coli* K 12 [3] were generally confirmed by using *S. typhimurium* strains [7]. This study by Bartsch *et al.* [7] also included one brominated and one fluorinated ethylene, vinyl bromide and vinylidene fluoride: both asymmetrically substituted haloethylenes were bioactivated to mutagenic metabolites, but to much different extents. Vinylidene fluoride showed only 'marginal mutagenicity'.

Covalent binding, mutagenicity and carcinogenicity

During the last few years more data became available on covalent binding of haloethylene metabolites to proteins and to nucleic acids; carcinogenicity bioassays were also performed. Furthermore, the pharmacokinetics of these compounds has been systematically investigated. This now extends the data base for a reasonable comparison.

A compilation of some relevant literature data [8–31] is given in Table 1. Along with the current opinion that epoxides are essential intermediates in metabolism of these compounds, all the haloethylenes in which this effect has been studied are in fact biotransformed to protein alkylating intermediates [2, 8, 9, 15, 16, 21, 22, 25, 26, 29]. However, alkylation of nucleic acids is not a common feature as perchloroethylene [26], and probably trichloroethylene (as discussed in [24]), do not lead to defined alkylation products at nucleic acid bases, much in contrast to compounds like vinyl bromide [30], vinyl chloride [10–12] and vinylidene chloride [17, 32].

Table 1. Covalent macromolecular binding of metabolites, mutagenicity and carcinogenicity of halogenated ethylenes

Haloethylene	Covalent protein binding *in vitro*	Covalent protein binding *in vivo*	Covalent binding to nucleic acids *in vitro**; *in vivo*†	Mutagenicity in bacterial tests after metabolic activation	Carcinogenicity in animal bioassays
Vinyl fluoride	×	×	×	×	×
Vinylidene fluoride	×	×	×	(+) [7]	+‡ [13]
Vinyl chloride	+ [8]	+ [9]	+ [10–12]*,†	+ [3, 7]	+ [14]
Vinylidene chloride	×	+ [15, 16]	+¶ [17]†	+ [3, 7]	+/− [18–20]
1,2-*cis*-Dichloroethylene	×	×	×	− [3]	×
1,2-*trans*-Dichloroethylene	×	×	×	− [3]	×
Trichloroethylene	+ [21]	+ [22]	+/−§ [23, 24]*	(+) [7]	− [6]
Perchloroethylene	+ [25]	+ [2, 26]	− [26]†	− [3, 7]	−‖ [27, 28]
Vinyl bromide	+ [29]	×	+ [30]*,†	+ [7]	+ [31]

+ = positive, − = negative; × = not yet reported; (+) = "marginal mutagenicity" [7]; +/− = positive and negative reported.

‡ Liposarcomas reported in rats after oral administration, the biological significance of which is still not clear.

§ No specific alkylation products identified [23].

‖ Not carcinogenic in rats [27, 28]; hepatomas in B6C3F1 mice after high oral dosage [28], the biological significance of which being subject of present discussion [25, 26].

¶ Low degree of alkylation reported.

This is consistent with Henschler's structural theory, and it lends biochemical support to the outcome of both mutagenicity [3, 7] and carcinogenicity [6, 13, 14, 18–20, 27, 28, 31] tests. Hence, there are different lines of support (metabolism and covalent binding, mutagenicity, carcinogenicity) to the view [33] that epoxides of halogenated ethylenes in fact must possess a certain degree of instability to effect a genotoxic action.

On the other hand, it seems feasible that an epoxide, being too unstable to reach the DNA target in significant amounts, could even be less active than a more stable one. Quantitative data interpretable in this direction have now been accumulated.

Metabolic rates and formation of preneoplastic hepatocellular foci

Recent publications have impressively demonstrated that metabolic and pharmacokinetic data must be incorporated into the interpretation of toxicological data, especially when inhalation experiments are concerned [34, 35]. Therefore, in view of the very dissimilar metabolic rates of haloethylenes, it is mandatory to base a mechanistic and quantitative comparison of oncogenic effects on metabolic data, i.e. on the rates or amounts of intermediary epoxide produced by the initial metabolic step (Fig. 1). There is sufficient pharmacokinetic data [36, 37] for such calculations.

Oncogenic effects of different haloethylenes can be quantitated on the base of histochemical examination of preneoplastic nucleoside-5'-triphosphatase ("ATPase") deficient foci in rats exposed to these chemicals from the time of birth on; a detailed discussion of this methodology has been published [38, 39].

Table 2 contains metabolic data [36, 37] and data on formation of hepatic preneoplastic foci [25, 38–43] after exposure of young Wistar rats to haloethylenes. A suitable endpoint for such a comparison [39] is the percentage of ATPase deficient hepatocytes after an exposure period of 10 weeks. The exposure concentrations of 2000 ppm ensured a saturation of metabolizing enzymes (V_{max} conditions) [36]; only vinylidene chloride, because of its high acute toxicity, was applied at a concentration that produced a metabolic rate of about $\frac{1}{2}$ V_{max} [37]. In accordance with Henschler's general rules (*vide supra*) trichloroethylene [38] as well as perchloroethylene [25, 39] do not induce preneoplastic foci. The other compounds show a wide range of oncogenic activities, extended over 3 orders of magnitude with the extremes of vinyl chloride [38] and vinylidene fluoride [41]. This huge range is much reduced when the observed preneoplastic effect is related to the amount of haloethylene metabolites produced under the experimental exposure conditions (400 hr exposure; last column of Table 2). Such an estimation is realistic because newborn rats develop their full ability to metabolize vinyl chloride within their first week of life [44].

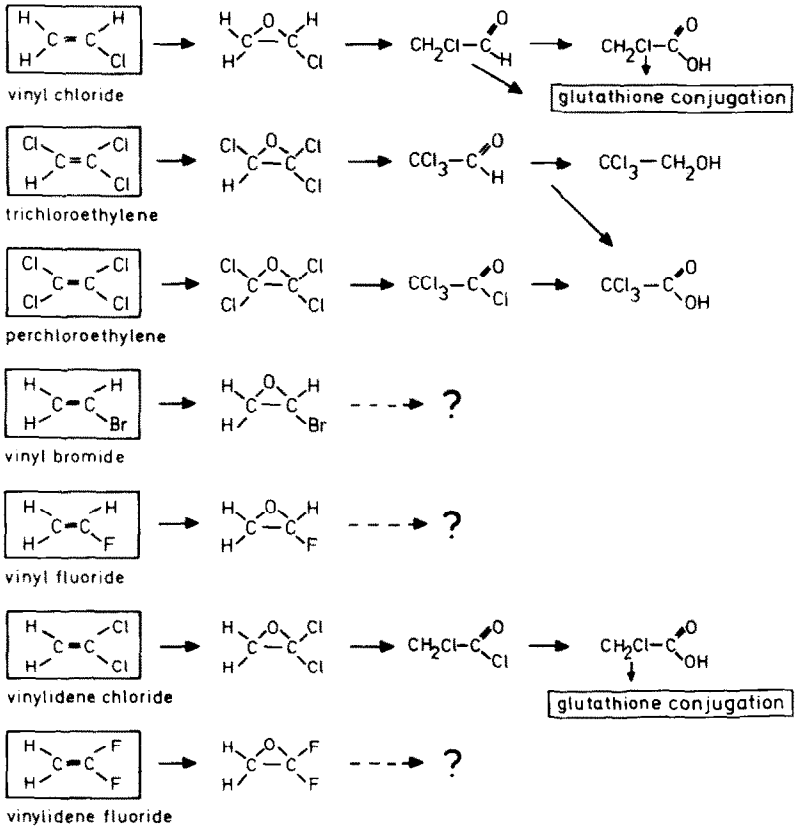

Fig. 1. Initial steps of metabolism of halogenated ethylenes [2–5, 7, 11].

Table 2. Metabolic rates of haloethylenes and ATPase deficient preneoplastic foci observed after 10 weeks of exposure (8 hr/day; 5 days/week) in newborn female Wistar rats

Haloethylene	Exposure concentration (ppm)	Metabolic rate $\left(\frac{\mu mole}{hr \cdot kg}\right)$	Estimated amount metabolized during exposure (mmole/kg)	Preneoplastic foci after 10 weeks (% of liver area)	% Foci theoretically produced by 1 mole metabolites per kg b.wt
Vinyl fluoride	2000	7 [36, 37]	2.8	0.04 [40]	14.3
Vinylidene fluoride	2000	1.1 [36, 37]	0.44	0.0008 [41]	1.81
Vinyl chloride	2000	110 [36, 37]	44	0.8 [38]	18.2
Vinylidene chloride	100	50 [37]	20	0.003 [42]	0.15
Trichloroethylene	2000	210 [36, 37]	84	0 [38]	0
Perchloroethylene	2000	7 [36]	2.8	0 [25, 39]	0
Vinyl bromide	2000	40 [36, 37]	16	0.07 [43]	4.38

Under the assumption [2–5] of Fig. 1 that the essential initial metabolic step is transformation to halooxiranes, the metabolic rates of haloethylenes determined by pharmacokinetic means must be equivalent to the amounts of epoxide produced. That the epoxide is in fact the ultimately reactive (and carcinogenic) principle has recently been validated for vinyl chloride [45]. Hence, comparison of the data in the last column of Table 2 should give some idea of the comparative oncogenic effects of the different epoxides under realistic conditions *in vivo*. From this comparison, two features may be deduced:

(A) The oncogenic effects of monohaloethylene metabolites decrease in the order: vinyl chloride > vinyl fluoride > vinyl bromide. No details are available on the metabolic pathways of vinyl bromide subsequent to epoxidation and on physico-chemical characteristics of its epoxide, although the implication of this epoxide in metabolism of vinyl bromide has been reasonably suggested [7]. But comparative molecular orbital studies have been published on fluorooxirane and chlorooxirane [46]; these show that the 3-membered ring of fluorooxirane has more tension and is less stable than that of chlorooxirane.

(B) When the 1,1-dihaloethylenes and the monohaloethylenes (i.e., vinylidene fluoride vs vinyl fluoride and vinylidene chloride vs vinyl chloride) are compared (Table 2), it appears that the reactive intermediates of 1,1-dihaloethylenes exert a distinctly lower oncogenic effect than those of the monohaloethylenes. Whilst no experimental reports are available on a hypothetical [7] epoxide of vinylidene fluoride, it is known [5] that 2,2-dichlorooxirane (the epoxide of vinylidene chloride) is considerably more unstable than monochlorooxirane; hence, the former, by contrast to the latter, "resists all attempts of synthesis by conventional methods" [5].

These two points, at a first glance, seem to contradict Henschler's structural rule [2–5] which says that instability of chlorooxirans (and possibly halooxiranes in general) is associated with mutagenicity and carcinogenicity of the parent halogenated ethylenes. Without any doubt, epoxides need to be reactive to successfully attack the DNA target. Also, structurally closely related epoxides can exhibit a wide range of genotoxic activities [47]. For the halogenated ethylenes, however, it appears that the epoxide of vinyl chloride, monochlorooxirane, in quantitative terms might represent an optimum between stability and reactivity to both reach the DNA target and react with it, after being formed at the monooxygenase site. A further decrease in stability could possibly render the oxirane too short-lived to reach the target.

Future experimental and theoretical work should be directed to these questions. The presently available data demonstrate that the haloethylenes represent a family of compounds of very dissimilar biological activities although their simplicity in chemical structure implies a certain uniformity as to the possible routes of metabolic transformation. Hence, ideas to broaden the molecular and theoretical basis of understanding the differences in biological effects must be encouraged.

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