Biochemical Pharmacology, Vol. 24, pp. 2013-2017. Pergamon Press, 1975. Printed in Great Britain.

MUTAGENICITY IN VITRO AND POTENTIAL CARCINOGENICITY OF CHLORINATED ETHYLENES AS A FUNCTION OF METABOLIC OXIRANE FORMATION

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(Received 10 July 1975; accepted 19 August 1975)

All chlorinated ethylenes undergo biotransformation in mammalian organisms, the main pathway being an oxidation to oxiranes, as the first step. The stability of these oxiranes varies widely and depends on the number of chlorine substitutions and on the relative position of the substituents in the molecule. Symmetrically substituted oxiranes from the tetrachloro- and the isomeric 1,2-dichloroethylenes seem to be rather stable [1),2)]. In the case of vinyl chloride and trichloroethylene the polarity of the unsymmetrical oxiranes exerts lesser stability and induces intramolecular rearrangement[3),4)]. Attempts to prepare the oxirane with the highest polarity from 1.1-dichloroethylene (1.1-DCE) remained unsuccessful till now. In the reaction of 1.1-DCE with m-chloroperoxybenzoic acid,the corresponding oxirane could not be detected; instead, the chloroacetyl chloride was isolated as the product of oxirane-rearrangement[5)].

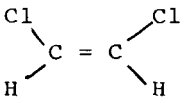
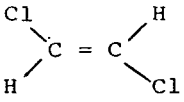
However, no direct relationship exists between chemical reactivity and biological effects because the thermal rearrangement of the oxiranes leads to different chemical species: either acyl chlorides or aldehydes[4)] which are suspect of causing quite different biological effects. Besides rearrangement, oxiranes may react directly with biologic nucleophiles. Thus, the mutagenic

activity of vinyl chloride (VCM) has been claimed to be exerted by a direct alkylating action of the oxirane[6),7)].

VCM has been demonstrated to be carcinogenic in animals[8),9)] and in man (for references, see[10)]). Quite recently, a carcinogenic activity of trichloroethylene (TRI) has been reported in mice after long-term administration of rather high daily oral doses (0.5 or 1.0 g/kg). This report[11)] in connection with the above considerations prompted us to determine the mutagenicity of the whole series of chlorinated ethylenes with a metabolic activating microsomal enzyme system in vitro.

Materials and Methods. Tetrachloroethylene, trichloroethylene, cis- and trans-1,2-dichloroethylene, 1,1-dichloroethylene were obtained from Merck & Co., Darmstadt, as a.g. reagents; vinylchloride as a purified gas (> 99,9%) from BASF, Ludwigshafen. Mutagenic activity of the derivatives formed during microsomal activation was tested in a metabolizing in vitro system[12)] with E coli K 12. One can use this bioauxotrophic strain in four mutation systems to test mutagenic agents: In the three back mutation systems gal^+, arg^+, and nad^+, and in the MTR system, where forward mutation leads to resistance to 5-methyl-DL-tryptophane[13)]. For the experiments 6 to 9 x 10^8 cells of an overnight culture were suspended in 1.5 ml incubate containing 5 mg microsomal protein, isolated from mouse livers, and the NADPH generating system 5 mM $MgCl_2$, 16 mM DL-isocitrate-Na_3, 0.66 mM NADP-Na_3, 20 µl isocitrate-dehydrogenase (20 milliunits/µl) in 0.1 M phosphate buffer pH 7.4, as well as different concentrations of the test compounds. These concentrations were selected from preliminary experiments so that they did not reduce cell survival by more than 20 per cent (Table 1). After 2 hours of incubation in a shaking water bath at 37°, the reaction was terminated in ice. The incubate was diluted in saline and plated on appropriate selective media as described previously[14)]. Survival of the E coli K 12 strain was determined by plating on the complete medium. Mutagenicity is expressed as colony-forming units (cfu) that were counted on the appropriate selective media per cfu counted on the complete medium. Liver microsomes were isolated from male mice pretreated for 10 days with 0.1 per cent phenobarbital in the drinking water to increase microsomal enzyme activity[12)].

Tab.1: Mutagenicity of chlorinated ethylenes after incubation in a metabolic activating microsomal system

	concentration in the medium[x] at 37° C [mM]	%survival of bacteria	% of spontaneous mutation rate in different operons of E coli K_{12}			
			gal+	arg+	MTR	nad+
$Cl_2C = CCl_2$ Tetrachloroethylene	0.9	99±1	100	100	100	100
$Cl_2C = CHCl$ Trichloroethylene	3.3	76±4	123±23	232±36	114±18	100
$Cl_2C = CH_2$ 1.1-Dichloroethylene	2.5	74±7	120±14	229±26	100	100
 cis-1.2-Dichloroethylene	2.9	88±5	100	100	100	100
 trans-1.2-Dichloroethylene	2.3	90±3	100	100	100	100
$ClCH = CH_2$ Vinyl chloride	10.6	72±3	231±20	663±141	172±35	148±24

x) determined by GC analyses after injection of 5 µl of the liquid compounds; except vinyl chloride where the gas was introduced by bubbling through the liquid at 15° C.

Results and Discussion. The results of the experiments are listed in Table 1. Cytotoxicity of the chlorinated ethylenes varies widely. To obtain 80-100 per cent survival of the tester strain, only 1 mM of trichloroethylene could be used but 10 mM of vinyl chloride.

No mutagenic activity of the chlorinated ethylenes was detected in the test system without microsomal enzyme activity. When the complete incubate with metabolically active microsomes was used, conversion of VCM, 1.1-DCE as well as TRI induced mutations, the latter compounds being less mutagenic than VCM. The highest mutation rates were detected in the arginine genes, whereas reversibili-

ty in the gal^+ and nad^+ systems and the forward mutation to MTR resistance were less sensitive to the mutagenic effects of the metabolites. Tetrachloroethylene and the cis- and trans-isomers of dichloroethylene were not metabolized to mutagens at all.

Direct comparison of the mutagenic activity of the chlorinated ethylenes is not possible since different substrate concentrations had to be used to minimize cell death of the tester strain. However it is evident that mutagenicity of VCM is several times higher than that of 1.1-DCE and TRI.

Our results are indicative of a conspicuous correlation between the stability of the oxiranes, as outlined earlier, and the mutagenicity of all six chlorinated ethylenes: those forming very unstable oxiranes (VCM, 1.1-DCE,TRI) induce mutations in the test system, whereas the others (Per, cis- and trans-1.2-DCE) forming much mor stable oxiranes, do not.

The mutagenicity of trichloroethylene, though only slight in extent in the gal^+ system, which is known as very sensitive, has not been anticipated. Trichloroethylene is metabolized in vitro and in vivo to the scarcely reactive chloral hydrate and furtheron to trichloroethanol and trichloracetic acid[15),16)] The latter compounds are not known to induce cytotoxic or genetic effects. Conversion of TRI-oxirane to chloral in vivo is a quite unexpected reaction because thermal rearrangement in vitro entirely forms dichloroacetyl chloride[17)]. This different behaviour should be further investigated. The results of such experiments may contribute to the better understanding of the mutagenic and potentially carcinogenic properties of trichloroethylene.

Acknowledgement: The skilful technical assistence of Mrs. Hesse and Steinhilber is gratefully acknowledged.

REFERENCES

1. D.M.Frankel, C.E.Johnson, H.M.Pitt, J.org.Chem. 22, 1119 (1957)
2. K.Griesbaum, R.Kibar, B.Pfeffer, Liebigs Ann.Chem. 1975, 214
3. H.Gross, J.Freiberg, Journ.f.Prakt.Chem. 311, 506 (1969)
4. G.Bonse, Th.Urban, D.Reichert, D.Henschler, Biochem.Pharmacol.(1975) in press
5. G.Bonse, D.Henschler, unpublished results
6. U.Rannug, A.Johansson, C.Ramel, C.A.Wachtmeister, Ambio 3, 194 (1974)
7. H.Bartsch, C.Malaveille, R.Montesano, Int.J.Cancer 15, 429 (1975)
8. P.L.Viola, A.Bigotti, A.Caputo, Cancer Res. 31, 516 (1971)
9. C.Maltoni, G.Lefemine, Environm.Res. 7, 387 (1974)
10. J.W.Lloyd, J.occup.Med. 16, 809 (1974); 17, 333 (1975)
11. Memorandum, Dept.of Health, Education & Welfare, Washington, 20.3.1975
12. P.Czygan, H.Greim, AJ Garro, F.Hutterer, F.Schaffner, H.Popper, O.Rosenthal, DY Cooper: Cancer Res. 33, 2983 (1973)
13. J.Ellenberger, G.Mohn, Arch.Toxicol. 33, 225 (1975)
14. G.Mohn, J.Ellenberger, D.McGregor, Mutation Res. 25, 187 (1974)
15. K.C.Leibman, Mol.Pharmacol. 1, 247 (1965)
16. J.W.Daniel, Biochem.Pharmacol. 12, 795 (1963)
17. J.Derkosch, personal communication (1974)