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Chemical Structure, Reactivity, and Carcinogenicity of Halohydrocarbons

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This review summarizes studies concerning the covalent binding of [^{14}C]TCE to rat liver microsomal protein and exogenous DNA, *in vitro*, the enhancement of this binding by inducers of mixed-function oxidases, and inhibition of binding by inhibitors of these enzymes. Furthermore, recent studies on this type of binding in various strains of mice and rats of both sexes and using microsomal preparations from various organs are briefly reviewed. Other work reviewed here concerns the synthesis of TCE epoxide and its reaction with nucleophiles since it is believed that TCE epoxide is the activated carcinogenic intermediate of TCE. The utility of structural prognostication of carcinogenic activity and the importance of considering possible metabolic pathways for other chlorinated olefins is also discussed.

Introduction

Since the time of the discovery of the carcinogenicity of vinyl chloride (VC) in laboratory animals (*1, 2*) and humans (*3*) there has been an extensive renewed interest in and researches on halogenated hydrocarbons, both unsaturated and saturated (*4–6*).

The carcinogenicity findings with VC led to studies on its metabolism (*7*) and possible modes of action (*5, 8, 9*). Simultaneously studies were undertaken in a number of laboratories on other compounds in this class, some of which are also being examined in our laboratory.

Based on its chemical structure, reactivity and possible metabolic pathways, we predicted that trichloroethylene (TCE) will be carcinogenic, particularly to the liver (*10, 11*). Studies were performed by us on the binding of [^{14}C]TCE to rat liver microsomal proteins *in vitro* (*12, 13*), as well as the synthesis of the epoxide of TCE and its reactions with nucleophilic trapping agents (*14*). More recently, we have also completed studies on the *in vitro* binding of [^{14}C]TCE to microsomal proteins and to DNA in mice and rats of various strains and both sexes and using microsomal preparations from various organs (*15*).

The studies outlined in the previous paragraph, as well as some as yet unpublished work from this laboratory, will be briefly reviewed in this report.

Structural Prognostication of Carcinogenicity

Past studies on structure-activity correlations in this laboratory have dealt with direct-acting carcinogens of the epoxide (*16*) and halo ether (*17, 18*) classes of compounds. Based on such studies it has become possible, within specific classes of compounds, to suggest the likelihood of carcinogenicity. This subject was recently reviewed (*11*). Moreover, this approach has important implications in occupational cancer and this aspect was discussed at a recent symposium of the National Academy of Sciences (*19*).

The same principles of structure-activity relationships apply also to indirect-acting carcinogens, i.e., those which have to be metabolically activated to carcinogenic intermediates. However, for these compounds known or likely metabolic pathways for activation and for detoxification need also to be considered. This is, in fact, of overriding importance and some of these possible metabolic pathways will be discussed in a later section of this report.

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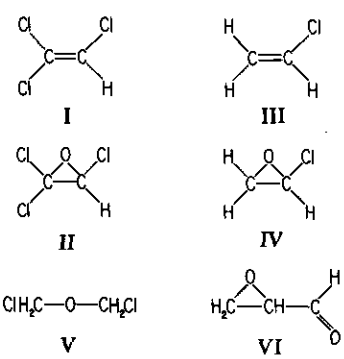
Trichloroethylene

This widely used industrial chemical and anesthetic was chosen for the first phase of our work on halogenated hydrocarbons because of its structural analogy to VC and to some other known indirect-acting carcinogens. TCE was also chosen after consideration of its known and suggested metabolic pathways.

Powell suggested many years ago (*20*) that TCE (I) is probably metabolized to an epoxide, epoxy-1,1,2-trichloroethane (II). This suggestion was based on his extensive metabolic studies on TCE. Subsequently, we suggested that VC (III) exerts its carcinogenicity by metabolism to the activated carcinogenic intermediate (IV) i.e., chloroethylene epoxide (*10*). It was pointed out (*10*) that IV is structurally analogous to the direct-acting carcinogens bis(chloromethyl) ether (V) and glycidaldehyde (VI). The chemistry and carcinogenicity of V and VI were described by us in earlier research reports (*16, 21–23*).

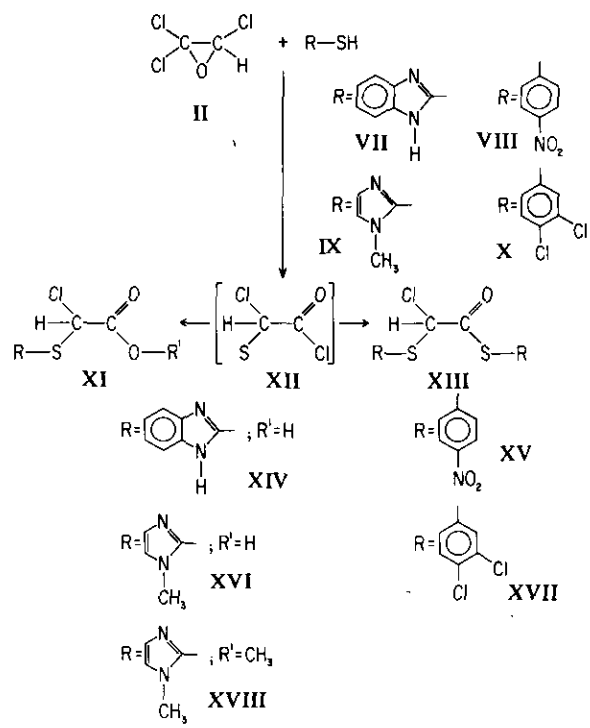


In a recent report the carcinogenicity assays on TCE were described (*24*). The compound is carcinogenic to the liver by feeding to B6C3F1 hybrid mice, but not to Osborne-Mendel rats. It should be pointed out that TCE must be considered a weak carcinogen because of the high dosages used in these feeding experiments.

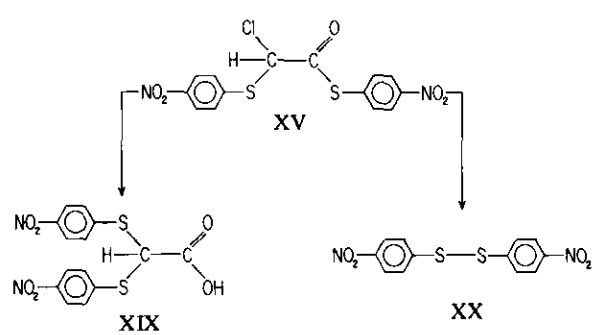
Moreover, there is a concern about the matter of metabolic overload which may inactivate detoxifying enzymes.

Epoxy-1,1,2-trichloroethane (II) was synthesized by the autoxidation of TCE (I) with oxygen and ultraviolet irradiation in the presence of benzoyl peroxide. The product was fully characterized (*14*) and it was found that the compound has a half-life of 1.3 min (pseudo first-order kinetics, rate 0.215 sec^{-1}) at pH 7.4 and 37°C.

In the same report (*14*) we described the reaction of II with a variety of nucleophiles shown in structures VII–XX. The purpose of these organic-chemical studies was to obtain suitable agents for intercepting the epoxide, if it were formed from [^{14}C] TCE, using *in vitro* biochemical experiments with rat liver microsomal preparations. Because of the high reactivity of the epoxide, II, it was important that such intercepting nucleophilic reagents



react rapidly with II, have sufficient water solubility; that the products could be readily extracted by organic solvents from an aqueous system, and that the nucleophiles selected are nontoxic to the microsomal enzymes. All these criteria were met by the nucleophiles and some of their products



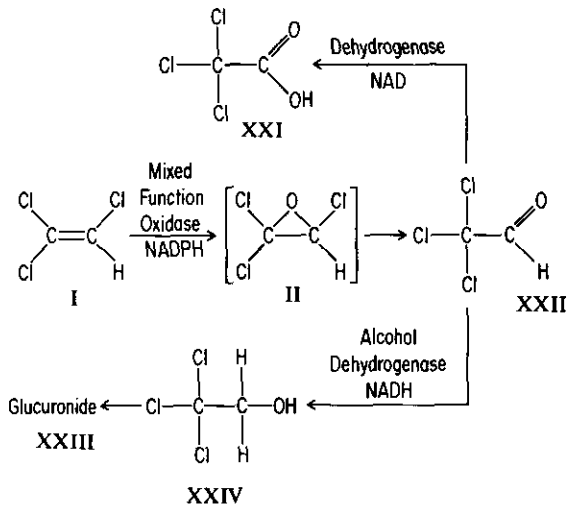
shown in structures VII–XX. These interception experiments are currently underway using [^{14}C]TCE with rat liver microsomal preparations *in vitro*. For the determination of the covalent interaction of TCE with rat liver microsomal proteins, *in vitro*, microsomal preparations were obtained using established procedures (*25*). The [^{14}C]TCE used was of >99% radiochemical purity as determined by gas chromatograph-mass spectrometry and was used as such.

These *in vitro* binding studies have been described in detail by us (*13*). In brief, the studies showed that TCE binds covalently to microsomal proteins. The binding was decreased by 7,8-benzoflavone. This compound inhibits the binding of 7,12-dimethylbenz(a)anthracene to DNA, RNA, and protein in mouse skin (*26*). The binding of TCE to rat liver microsomal protein was blocked by β-diethylaminoethyl diphenylpropyl acetate (SKF-525A), which is known to inhibit the metabolism of various substrates of cytochrome P-450 (*27*). As expected, phenobarbital enhanced the binding of TCE to microsomal proteins. Finally, the effect of an inhibitor of epoxide hydrase (*28*), 1,2-epoxy-3,3,3-trichloropropane (TCPO) on the same *in vitro* system was examined and found to cause an enhancement of TCE binding to microsomal protein. The addition of competing nucleophiles such as reduced glutathione, 1-methyl-2-mercaptoimidazole, and mercaptoethanol all decreased the binding of TCE to microsomal proteins.

All of these findings are consistent with the suggestion that an epoxide intermediate is formed and that such an epoxide may be the activated carcinogenic intermediate of TCE in liver carcinogenesis.

The likely metabolic pathway of TCE in animals and man is summarized in the scheme involving structures I, II, and XXI–XXIV which is consistent with the metabolic studies and the suggestion of Powell (*20*) concerning an epoxide intermediate.

In recent experiments (*15, 30*), the binding of TCE to liver microsomal proteins of male B6C3F1 hybrid mice which are susceptible to TCE-induced liver tumorigenesis (*24*) was found to be significantly higher than the binding of [^{14}C]TCE to microsomal protein from male Osborne-Mendel rats, which were resistant to TCE-induced hepatocellular carcinoma in the same earlier study (*24*). The *in vitro* binding of [^{14}C]TCE to liver microsomal proteins was higher for male compared with female B6C3F1 mice; females have been reported (*24*) to show a lower incidence of TCE-induced hepatocellular carcinoma compared with males. Microsomal proteins from the lung, stomach, and kidney of



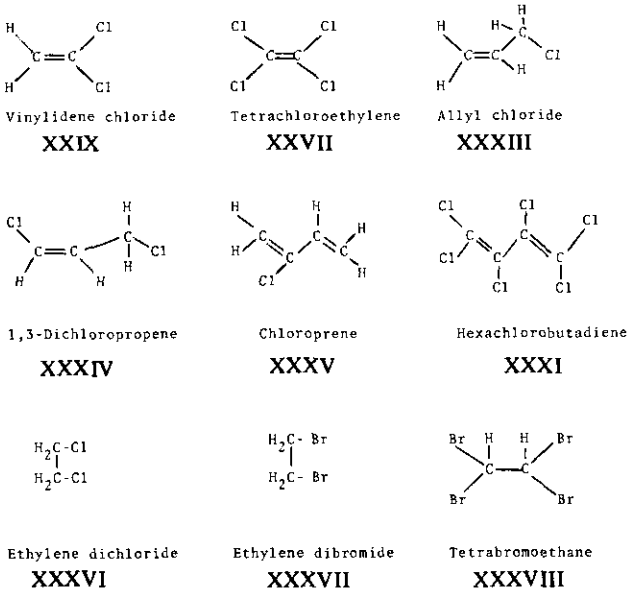
B6C3F1 hybrid mice also metabolized TCE as indicated by the covalent binding of [^{14}C]TCE to microsomal protein from these organs.

Incubation of [^{14}C]TCE with salmon sperm DNA in the presence of microsomal preparations from B6C3F1 hybrid mice resulted in covalent binding of [^{14}C]TCE to DNA. This binding was much higher in the presence of microsomal proteins from male compared with female mice. The binding to DNA and protein was enhanced by *in vivo* phenobarbital administration. These findings are described in detail in a recent report from this laboratory (*15*).

It should be noted that since the announcement of the carcinogenicity of TCE by the National Cancer Institute (*29*), a number of substitutes for TCE have been introduced in industry. Some of these compounds are listed in Table 1. These compounds have not undergone chronic bioassay for carcinogenicity. Hence their use as substitutes is regrettable since all four are expected to cause liver damage, and compounds XXVI and XXVII are in all likelihood also liver carcinogens.

Table 1. Substitutes for trichloroethylene in degreasing, manufacture of decaffeinated coffee, and other uses.

Structure		Name
$Cl_3C—CH_3$	XXV	1,1,1-Trichloroethane
CH_2Cl_2	XXVI	Dichloromethane
$Cl_2C=CCl_2$	XXVII	Tetrachloroethylene
$Cl_3C—CCl_3$	XXVIII	Hexachloroethane



Carcinogenicity Assays of Vinyl Bromide and Poly(vinyl Bromide)

Poly(vinyl bromide) (PVBr) and copolymers containing the vinyl bromide (VBr) moiety have been under investigation for possible use as flame retardants.

Because of our continuing interest in flame retardants (*31*) and because of the structural closeness of vinyl chloride and VBr, the latter compound and its polymer were tested for carcinogenicity in mice. (Both materials were kindly provided by Ethyl Corporation, Detroit, Mich.) The results of these experiments are given in detail in Tables 2 and 3, since they have not been published elsewhere.

Skin application of VBr in acetone solution and PVBr in commercial aqueous latex suspension using female ICR/Ha Swiss mice, 30 per group, did not result in any skin tumors in either group. When tested as initiators with phorbol myristate acetate (PMA) as promoter (two-stage carcinogenesis), the test group (VBr followed by PMA) did not show any significant increase in tumors induced compared with the control group (PMA alone). The positive control group showed the expected high tumor incidence (*32*). Although VBr has a low boiling point (15.8°C/760 mm), it is expected that the high dose used in the skin application experiments, 15 mg/application in 0.1 ml acetone, would result in absorption into the skin of a substantial amount of the material before evaporation.

The two materials were also tested by subcutaneous injection in mice (Table 3); VBr was inactive. PVBr, on the other hand, resulted in a high incidence of liposarcomas. However, the material was injected as an aqueous suspension and hence this interesting finding probably is due to physical carcinogenesis and thus does not bear on the matter of VBr and/or PVBr as chemical carcinogens. To our knowledge inhalation studies on VBr have not, as yet, been reported.

Table 2. Carcinogenicity of vinyl bromide and poly(vinyl bromide) tested as initiators and whole carcinogens on mouse skin.[a,b]

Initiator and dose, mg/0.1 ml acetone (except where noted)	Days to first tumor	Number of mice with papillomas[c] / Total number of papillomas
Vinyl bromide, 15.0	412	1/1[d]
Polyvinyl bromide (0.1 ml commercial suspension)	175	1/1
DMBA, 0.02 (positive control)	62	30/320 (19)
PMA (control)	44	1 (1)
No treatment (160 mice)	—	0

[a]Thirty female ICR/Ha Swiss mice per group; duration 420 days; PMA 2.5 μg, three times/week.
[b]Both compounds were completely inactive when tested as whole carcinogens on mouse skin by repeated application, 3 × wkly at the same doses and group sizes given above, also for 420 days.
[c]Number of mice with squamous carcinoma in parentheses.
[d]One additional animal bore a subcutaneous fibrosarcoma.

Table 3. Carcinogenicity of vinyl bromide and poly(vinyl bromide): subcutaneous injection in mice.[a]

Compound and dose (once per week)	Number of mice with local tumors
Vinyl bromide, 25 mg/0.05 ml trioctanoin	0
Polyvinyl bromide, 0.05 ml commercial suspension	19 liposarcomas[b]
β-Propiolactone, 0.3 mg/0.05 ml trioctanoin (positive control)	18 sarcomas 3 squamous carcinomas
Trioctanoin only, 0.05 ml	0
No treatment (60 mice)	0

[a]Thirty female ICR/Ha Swiss mice per group; duration 420 days.
[b]Discontinued treatment after 48 treatments.

Both materials were also tested by feeding in rats. The results of these tests are incomplete and will be published elsewhere when the histopathology is complete.

Other Chlorinated Hydrocarbons

Because of the extensive use of chlorinated hydrocarbons in chemical industry, some of them are becoming ubiquitous environmental contaminants and possibly environmental hazards. They may occur in films used as food wrappings, latex paint, rubber, etc. (*4–6*) and some of them occur in drinking water. A few of the compounds of concern to us that have been shown to occur in drinking water (*33*) are listed in Table 4. The presence of these compounds in drinking water must be ascribed to contamination of water supplies by effluents from chemical industry. The carcinogen chloroform (*34, 35*) also occurs in drinking water. However, this compound is also produced from naturally occurring humic materials in the disinfection of water supplies by chlorine (*36, 37*). The latter source must account, at least in part, for its presence in drinking water.

Table 4. Some chloroolefins in drinking water.[a]

Name		Structure
Vinyl chloride	III	$CH_2=CHCl$
Vinylidene chloride	XXIX	$CH_2=CCl_2$
1,2-Dichloroethylene (*cis* and *trans*)	XXX	$ClCH=CHCl$
1,1,2-Trichloroethylene	I	$Cl_2C=CHCl$
Tetrachloroethylene	XXVII	$Cl_2C=CCl_2$
Hexachlorobutadiene	XXXI	$Cl_2C=C(Cl)-C(Cl)=CCl_2$
1-Chloropropene-1	XXXII	$H-C(Cl)=CH-CH_3$

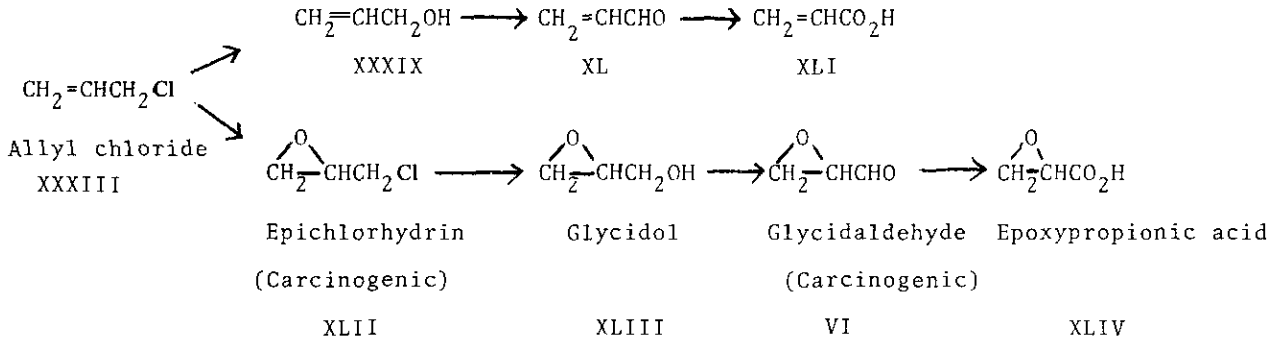
[a]EPA data (*33*).

The manufacture, chemistry and toxicology of these compounds have been reviewed recently (*38*). The purpose of this section of the present review is to discuss some compounds which are of current interest and under investigation in many laboratories, including our own.

The compounds of major interest are shown in structures XXVII, XXIX, XXXI, and XXXIII–XXXVIII. These compounds merit special attention for several reasons: (a) the structural analogy of some of these compounds (the chlorinated olefins) to vinyl chloride and TCE and hence possible carcinogenicity; (b) their large-scale production and use in chemical industry leading to environmental pollution by these agents; (c) some of these compounds are known carcinogens in rodents, e.g. ethylene dibromide (*39*), hexachlorobutadiene (*40*), and vinylidene chloride (*41*). Hexachlorobutadiene was recently shown to cause kidney tumors in rats by ingestion, but details of these experiments are not, as yet, available. Ethylene dichloride is discussed elsewhere in this volume (*42*).

Studies in this laboratory on structures XXVII, XXIX, XXXI and XXIII–XXXVIII are concerned with their chemistry, metabolism, carcinogenicity and determination of the nature of the activated carcinogenic intermediates for those compounds which are known carcinogens. These studies are carried out by using the same approaches described earlier in this review for trichloroethylene, and also including bioassays.

In conclusion, only one additional point will be made, and that concerns the matter of structural prognostication of carcinogenicity already alluded to in an earlier section of this review. For compounds suspected to be carcinogenic, it is of importance to examine the known, as well as postulated metabolic pathways if it appears likely that the parent compound is an indirect-acting carcinogen. As



an example, the possible intermediates that can be visualized for the metabolism of allyl chloride, XXXIII are shown in structures VI and XXXIX–XLIV. This example is particularly interesting, since two of the possible intermediates, epichlorhydrin (XLII) and glycidaldehyde (VI) are known direct-acting carcinogens (*17, 43*).

The author is indebted to his collaborators whose work is summarized as part of this review and referred to in the references. They are: S. Banerjee, B. M. Goldschmidt, S. A. Kline, G. Loewengart, S. Melchionne and A. Smith.

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REFERENCES

1. Viola, P. L., Bigotti, A., and Caputo, A. Oncogenic response of rat skin, lungs, and bones to vinyl chloride. Cancer Res. 31: 516 (1971).
2. Maltoni, C., and Lefemine, G. Carcinogenicity bioassays of vinyl chloride: Current results. Ann. N. Y. Acad. Sci. 246: 195 (1975).
3. Creech, J. L., Jr., and Johnson, M. N. Angiosarcoma of the liver in the manufacture of polyvinyl chloride. J. Occup. Med. 16: 150 (1974).
4. Selikoff, I. J., and Hammond, E. C., Eds. Toxicity of vinyl chloride-polyvinyl chloride. Ann. N. Y. Acad. Sci. 246: (1975).
5. Saffiotti, U., and Wagoner, J. K., Eds. Occupational carcinogenesis. Ann. N. Y. Acad. Sci. 271: (1976).
6. WHO-NIEHS Conference on potential environmental health hazards from technological developments in rubber and plastic industries. Environ. Health Perspect. 17: (1976).
7. Antweiler, H. Studies on the metabolism of vinyl chloride. Environ. Health Perspect. 17: 217 (1976).
8. Bartsch, H., et al. Mutagenicity and metabolism of vinyl chloride and related compounds. Environ. Health Perspect. 17: 193 (1976).
9. Osterman-Golkar, S., et al. Alkylation of DNA and proteins in mice exposed to vinyl chloride. Biochem. Biophys. Res. Commun. 76: 259 (1977).
10. Van Duuren, B. L. On the possible mechanism of carcinogenic action of vinyl chloride. Ann. N. Y. Acad. Sci. 246: 258 (1975).
11. Van Duuren, B. L. Structural prognostication of carcinogenicity and tumor-enhancing activity in various chemicals. Third International Symposium on Detection and Prevention of Cancer. Marcel Dekker, New York, in press.
12. Banerjee, S., and Van Duuren, B. L. Interaction between trichloroethylene and hepatic endoplasmic reticulum (abstract). Proceedings Third International Symposium on Detection and Prevention of Cancer. International Study Group for Detection and Prevention of Cancer, New York, 1976, p. 4.
13. Van Duuren, B. L., and Banerjee, S. Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res. 36: 2419 (1976).
14. Kline, S. A., and Van Duuren, B. L. Reactions of epoxy-1,1,2-trichloroethane with nucleophiles. J. Heterocycl. Chem. 14: 455 (1977).
15. Banerjee, S., and Van Duuren, B. L. Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA *in vitro*. Cancer Res., in press. tion.
16. Van Duuren, B. L. Carcinogenic epoxides, lactones, and halo-ethers and their mode of action. Ann. N. Y. Acad. Sci. 163: 633 (1969); and references cited therein.
17. Van Duuren, B. L., et al. The carcinogenic activity of alkylating agents. J. Nat. Cancer Inst. 53: 695 (1974).
18. Van Duuren, B. L., Goldschmidt, B. M., and Seidman, I. Carcinogenic activity of di- and trifunctional α-chloro ethers and of 1,4-dichlorobutene-2 in ICR/ha Swiss mice. Cancer Res. 35: 2553 (1975); and references cited therein.
19. Van Duuren, B. L. Carcinogens in the workplace. In: Proceedings of Symposium on Public Information in the Prevention of Occupational Cancer. 1976. National Academy of Sciences and National Research Council, Washington, D. C., 1977, p. 23.
20. Powell, J. F. Trichloroethylene: Absorption, elimination and metabolism. Brit. J. Ind. Med. 2: 142 (1945).
21. Goldschmidt, B. M., Blazej, T. P., and Van Duuren, B. L. The reaction of guanosine and deoxyguanosine with glycidaldehyde. Tetrahedron Letters 13: 1583 (1968).
22. Goldschmidt, B. M., Van Duuren, B. L., and Goldstein, R. C. The reaction of p-nitrobenzylpyridine with electrophiles. J. Heterocycl. Chem. 13: 517 (1976).
23. Van Duuren, B. L., and Loewengart, G. Reaction of DNA with glycidaldehyde. Isolation and identification of a deoxyguanosine reaction product. J. Biol. Chem. 252: 5370 (1977).
24. U. S. Dept. Health, Education and Welfare, National Cancer Institute. Carcinogenesis bioassay of trichloroethylene. Carcinogenesis Technical Report Series, No. 2. U. S. GPO, Washington, D. C., 1976.
25. Levin, W., et al. Partial purification and properties of cytochromes P-450 and P-448 from rat liver microsomes. Arch. Biochem. Biophys. 153: 543 (1972).
26. Kinoshita, N., and Gelboin, H. V. Aryl hydrocarbon hydroxylase and polycyclic hydrocarbon tumorigenesis: Effect of the enzyme inhibitor 7,8-benzoflavone on tumorigenesis and macromolecule binding. Proc. Nat. Acad. Sci. (U. S.) 69: 824 (1972).
27. Cooper, J. R., Axelrod, J., and Brodie, B. B. Inhibitory effects of β-dimethylaminoethyl diphenylpropylacetate on a variety of drug metabolic pathways *in vitro*. J. Pharmacol. Exp. Ther. 112: 55 (1954).
28. Oesch, F. Mammalian epoxide hydrases: Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica 3: 305 (1973).
29. U. S. Public Health Service, National Cancer Institute, Memorandum of Alert Carcinogenesis bioassay of trichloroethylene. Washington, D. C., 1975.
30. Banerjee, S., Van Duuren, B. L., and Goldschmidt, B. M. Microsome-dependent covalent binding of the carcinogen, trichloroethylene, to cellular macromolecules. Proc. 68th Ann. Meeting Amer. Assoc. Cancer Res. 18: 34 (1977).
31. Loewengart, G., and Van Duuren, B. L. Evaluation of chemical flame retardants for carcinogenic potential. J. Toxicol. Environ. Health. 2: 539 (1977).
32. Van Duuren, B. L., et al. Dose-response studies with a pure tumor-promoting agent, phorbol myristate acetate. Cancer Res. 33: 2166 (1973).
33. Environmental Protection Agency. Science Advisory Board. A report: assessment of health risk from organics in drinking water. Office of Research and Development. Washington, D. C., April 30, 1975.
34. Eschenbrenner, A. B., and Miller, E. Induction of hepatomas in mice by repeated oral administration of chloroform, with observations on sex differences. J. Nat. Cancer Inst. 5: 251 (1945).
35. Powers, M. B., and Voelker, R. W. Evaluation of the oncogenic potential of chloroform by long term oral administration in rodents. Proceedings 15th Annual Meeting Society of Toxicology, Atlanta, Ga., March, 1976.
36. Rook, J. J. Formation of haloforms during chlorination of

natural waters. Water Treatment Exam. 23: 234 (1974).
37. Morris, J. C. Formation of halogenated organics by chlorination of water supplies. EPA Report 600/1-75-002, National Technical Information Service, Springfield, Va., March 1975.
38. Fishbein, L. Industrial mutagens and potential mutagens. I. Halogenated aliphatic derivatives. Mutation Res. 32: 267 (1976).
39. Powers, M. B., et al. Carcinogenicity of ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) after oral administration in rats and mice. Toxicol. Appl. Pharmacol. 33: 171 (1975).
40. Anonymous. A toxicological study of hexachlorobutadiene (HCBD) shows that exposure effects are related directly to dosage. Chem. Eng. News 54: 18 (Aug. 30, 1976).
41. Maltoni, C. Recent findings on the carcinogenicity of chlorinated olefins. Environ. Health Perspect. 21: 1 (1978).
42. Weisburger, E. K. Carcinogenicity studies of halogenated hydrocarbons. Environ. Health Perspect. 21: 7 (1978).
43. Van Duuren, B. L., Orris, L., and Nelson, N. Carcinogenicity of epoxides, lactones, and peroxy compounds. Part II. J. Nat. Cancer Inst. 35: 707 (1965).