CHEMICAL MANUFACTURERS ASSOCIATION

OCT - 8 1990

October 4, 1990

TO: Vinylidene Chloride Panel

I have enclosed three (3) documents provided to me by Bill Hayes of Dow. These documents include:

(1) A meeting report describing a research proposal on vinylidene chloride. The research will involve a study on the mechanisms of vinylidene chloride nephrotoxicity, which will be sponsored by a group of European companies (BASF, ICI, Solvay, Dow Europe). The study is expected to start in the 4th quarter of 1990; however, a study report may not be available until 1992. The study will be performed at the University of Würzburg.

(2) A study protocol for the mechanism study.

(3) A published article by Trevor Green on the potential mechanisms of carcinogenicity for chloroethylenes. Vinylidene Chloride is discussed.

These documents are simply for your information and no action is required.

If you have any questions, please do not hesitate to contact me (phone: 202/887-1189; fax: 202/887-1237) or my staff assistant, Amy Kosko (phone: 202/887-1345).

Sincerely,

Jonathon T. Busch

Jonathon T. Busch
Manager, Vinylidene Chloride Panel

SL 064650

[illegible] 202-887-1100 Telex 89617 CMA WSH

DOW HEALTH & ENVIRONMENTAL SCIENCES

MEETING REPORT

MEETING WITH: (full name, abbreviation, address)

BASF Ludwigshafen/Solvay/ICI

WRITTEN BY: Jan Wilmer DATE MET: 02/27/90 WRITTEN: 03/05/90

Present:

Gelbke, Hoffmann, Munk, Jaeckh and Druschke (BASF), Christ de Rooy, Solvay and Trevor Green, (ICI), Wilmer (DOW Europe)
Guest: Dr. W. Dekant (University of Wuerzburg)

Purpose:

As a result of a review of the toxicology of VDC by the German BUA, more data are needed to explain the mechanism of the kidney tumor induction in male mice and to explain differences between species. The purpose of the meeting was to discuss a research proposal on VDC submitted by Dr. Dekant.

Main conclusions of the discussions taken place at BASF were:

- the studies should focus on the following aspects:
  a) glutathione/β-lyase pathway: formation of GSH adducts
  b) epoxidation of VDC in the liver followed by transportation of metabolites to the kidney
  c) P-450 mediated metabolic activation in the kidney
  d) studies will be started with Swiss mice. Depending on the results obtained in mice, species differences will be investigated in rats and human tissues (risk assessment).
  e) experiments will be carried out along the most appropriate route of exposure (inhalation).

- the 4 companies expressed their willingness to support this 3-year research proposal (after revision of the present draft)
- Dr. Dekant will submit revised proposal by the end of March which will then be discussed at the June meeting of the PVDC group
- Dr. Hoffmann or Dr. Jaeckh (BASF) will be the monitoring scientist. BASF will help the Univ. of Wuerzburg to introduce Good Laboratory Practice (on a limited scale)
- conditions and finances: standard contract between BASF and Univ. of Wuerzburg. BASF will recharge the other companies
- proposal of BASF to share the costs (200,000 to 240,000 DM) on a equal base was accepted by ICI and Solvay, but not by Dow (proposal: "name plate capacity"). According to BASF's business manager Druschke, this was in contrast to what was agreed within the PVDC group (David Russell, Chris Dyball please clarify)
- Dow USA will be asked to inform about status of VDC in the U.S EPA and ongoing activities within CMA (action: J. Wilmer)

BASF
Abteilung Toxikologi
Department of T xicology

6700 Ludwigshafen
West Germany

hdh-kb: 1024

STUDY PROTOCOL

Studies on the mechanism of the nephrotoxicity of

1,1-Dichloroethene

Testing facility:	Institut für Toxikologie und Pharmakologie der Universität Würzburg
Head of the Institute:	Prof. Dr.med. Henschler
Head of the Research group:	Priv. Doz. Dr. W. Dekant

This protocol consists of 12 pages.

SL 064652

1. GENERAL

1.1. Purpose of the study

The objective of the study is to provide information on the biochemical mechanism of the highly species and sex specific nephrotoxicity and carcinogenicity of 1,1-Dichloroethene (DCE).

Details on the scientific background for these experiments are given in the Appendix.

1.2. Signatures

	Name	Signatur
Study Director:		
Head of Institute:		to be signed after approval of the proj ct
Study assistants:		
Quality Assurance:		
Monitoring Scientists:		

1.3. Time Schedule

Start of study:	
Estimated completion date:	Dates to be set upon approval of the project
Estimated date of Draft Report:	
Final Report:	

1.4. Archiving

During the study all data are recorded and stored in the laboratory.

The signed study protocol, the signed final report and all raw data are archived at BASF Aktiengesellschaft, Ludwigshafen (Dept. of Toxicology).

All samples of biological material generated in the course of this study will be disposed of 4 months from the date of the final report, unless an extended storage period is requested in the protocol or by amendment or further storage in the institute is not appropriate.

1.5. Quality assurance

Performance of study:
The study will be performed in compliance with the principles of Good Laboratory Practice*.

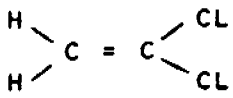
Amendments:
This protocol can be amended at the discretion of the study director. Detailed descriptions of all amendments will be signed by the study director. The amendment will be added to the original signed protocol and will be distributed according to the Distribution Schedul .

---

* Note: The institute is mainly engaged in independent basic research. Procedures according to GLP will be established with the help of BASF toxicology to the extent which is possible in such a facility.

2. *MATERIAL AND METHODS**

2.1. Test Article

Name:	1,1-Dichloroethene (DCE) "Vinylidene Chloride" (VDC)
Chemical Structure:	
Molecular Formula:	$C_2H_2CL_2$
Origin:	
Batch Lot No.:	
Purity:	
Aggregate State:	
Stability:	
Storage:	
Precautions:	
Preparations of test article:	

Note to be continued for other test materials (eg. labelled substances) upon approval of the project.

* Details will be given upon approval of the project.

2.2. Test System

- Rodents

Animals:	1) Rats 2) Mice
Strain:	1) ..... 2) .....
Origin:	
Sex:	
Age:	about ... weeks
Weight:	Rats: about 150 - 200 g; Mice: 20 - 22 g (at start of acclimatization weight will be checked prior to dosing)
Rationale:	Study data have to be set in relation to other rat and mouse toxicity data. Rat and mouse were found to be the most appropriate species for such experiments.

- Husbandry:

Room:	Animals will be held in a climatized area of ....
Caging:	During acclimatization in macrolon cages, type 3 During experiment in a special all-glass closed inhalation system (see figure in Appendix)
Identification:	Individual number (tattooing
Diet:	Pellet standard diet (Altrain, Lage)
Water:	Tap water ad libitum

Analysis of Food:	In view of the aim and duration of this study contaminants occuring in this commercial feed ought not to influence the results. The same applies for the tap water.
Selection of Animals:	Selection will be done randomized from the study pool.

- Cell Systems

Renal tubular epithelial cells:	
Animals:	
Strain:	
Origin:	
Sex:	
Age:	
Cell isolation:	isolation of epithelial cells will be done as described by Vamvakas et al. 1989 [1]
Bacteria for the Ames Test:	
Strains:	
Origin:	

2.3. Study Design

2.3.1. General Comments

Due to the character of the study only a broad outline of experiments can be given in the following sections. Any experimental detail will be recorded in the raw data.

Note: some additional details will be set after approval of the project prior to the start of the experiments in cooperation with the monitoring scientists.

The study is divided in two main sections with several subsections.

- Mechanistic Studies

  In this section experiments will be performed to investigate the extent and rate of DCE metabolism including S-conjugation in mice. If such metabolism is found and elucidated in mice further experiments will be added to investigate sex specificity in mice and possibly its species specificity between mice and rats. If metabolism including S-conjugation is not found to occur to a relevant extent then the formation of reactive metabolites by renal cytochrome P-450 will be investigated in mice.

- Studies on the genotoxicity and cytotoxicity of DCE metabolites

  If in the first section S-conjugated metabolites are found and identified then in this section of the study their possible genotoxicity or cytotoxicity will be investigated.

In the following short descriptions of the above mentioned sections and subsections are given.

2.3.2. Mechanistic studies

- Formation of S-conjugates

a) Are mercapturic acids indicative for the formation of toxic S-conjugates excreted in urine after inhalation of ^{14}C-DCE to mice?

Male Swiss mice are sensitive to DCE-induced renal carcinogenicity after longterm inhalation. In this first series of experiments, Swiss mice will be exposed to DCE by inhalation in a closed inhalation syst m. DCE uptake from the chamber atmosphere will be determined by monitoring DCE-concentrations in the gas phase by GC. The mice will th n be transferred to metabolic cages and urine and feces will be collected for 72 h (Dekant et al., 1986a). Urinary metabolites indicative for the biosynthesis of toxic glutathione conjugates [S-(1-chlorovinyl)-N-acetyl-L-cystein, S-(2-chloroacetyl)-N-acetyl-L-cysteine] will be identified and quantified using GC/MS; structure confirmation will be performed with synthetic reference compounds. The sensitivity and specificity of the analytical methods used will be determined with synthetic S-(1-chlorovinyl)-N-acetyl-L-cysteine and S-(2-chloroacetyl)-N-acetyl-L-cysteine (Vamvakas et al., 1989).

b) Are toxic glutathione conjugates of DCE formed by conjugation of DCE with glutathione in vitro?

To further investigate the conditions for the formation of S-conjugates DCE is incubated in the presence of microsomal and cytosolic enzymes obtained from liver and kidney of male Swiss mice with ^{35}S-labeled glutathione. The incubations mixtures (Dekant et al., 1987) will be processed and separated by high pressure liquid chromatography with diode array detection. Glutathione conjugates formed can be clearly differentiated from ^{35}S-glutathione using these methods; S-(1-chlorovinyl) glutathione can be detected by its specific UV-sp ctra. The conjugates formed will then be isolated and their structure will be determined by mass spectrometry (thermospray and GC/MS), NMR and UV spectrometry (Vamvakas et al., 1989). Reference compounds will be produced by chemical synthesis to confirm th proposed structures. If the experiments conducted demonstrate e biosynthesis of toxic glutathione conjugates of DCE male mice, the following topic will be addressed:

c) **What is the ratio of the metabolic pathway via glutathione conjugation to the total metabolization in vivo? Are there species and sex differences?**

d) **Is the proportion of DCE which is toxified via glutathione conjugation dose-dependent?**

To determine dose, sex and species specific differences in the formation of toxic glutathione conjugates and its competition with P-450 dependent biotransformation, metabolites representative for both pathways will be quantified by GC/MS after inhalation of different doses of DCE in male and female mice and rats.

- Formation of reactive intermediates by renal cytochrome P-450 in mice

In case that no indications for the biosynthesis of toxic glutathione conjugates will be obtained, the program will be modified to include experiments to address the problem of P-450 bioactivation in the kidney selectively in male mice. For this purpose, the covalent binding of ^{14}C-DCE derived radioactivity to renal macromolecules will be determined in vivo after inhalation of ^{14}C-DCE and the sex and species dependent extent of binding will be determined. Urinary metabolites will also be identified by GC/MS (Dekant et al., 1986b; Dekant et al., 1986c). To ascertain the involvement of P-450 dependent bioactivation reactions in the covalent binding of DCE-derived radioactivity in vivo, amino acid adducts derived from the reactive metabolite formed, chloroacetyl chloride, will be attempted to identify using GC/MS and HPLC-methods after hydrolysis of the isolated proteins obtain d from mice kidney. Reference compound can be synthesized by the chemical reaction of chloroacetyl chloride with selected amino acids containing nucleophilic sites not participating in the peptide bond wich are likely reaction sites of the electrophile in proteins (histidine, lysine, serine, cysteine).

To determine sex and species differences in the extent of renal cytochrome P-450 dependent bioactivation of DCE, the extent of metabolism of ^{14}C-DCE by P-450 will be quantified in kidney microsomes obtained from male and female rats and mice. Products possibly formed (chloroacetic acid, dichloroacetaldehyde, phospholipid-adducts, heme alkylation; Dekant et al., 1987) will be quantified by gas chromatography, covalent binding of ^{14}C-DCE derived reactive intermediates will be quantified by liquid szintillation counting (D kant et al., 1986c).

2.3.3. Studies on genotoxicity of metabolites formed

In case that potentially toxic glutathione conjugates are formed in mice, the following questions will also be addressed:

Are the glutathione and cysteine conjugates that ar formed genotoxic and cytotoxic?

On the basis of the results of the studies mentioned before glutathione and cysteine S-conjugates of DCE produced synthetically will be investigated in the Ames-Test (Dekant et al., 1986b) and in LLC-PK1 cells (Vamvakas et al., 1989), a cell line from pig kidneys, for mutagenicity or for the induction of unscheduled DNA synthesis as a measure of genotoxic effects. The cytotoxicity of these compounds will be determined in freshly isolated renal tubular epithelial cells (Vamvakas et al., 1989).

3. REPORTING

Results will be reported and presented in a study report including a short summary, a description of the used methods and a short evaluation of the results.

If appropriate data will be presented in tables and graphs. Appropriate statistical methods will be used.

4. LITERATURE

Costa, A.K. and Ivanetich, K.M. (1982):

Vinylidene chlorides: its metabolism by hepatic microsomal cytochrome P-450 in vitro.
Biochem. Pharmacol., 31, 2083 - 2092

Dekant, W., Metzler, M. and Henschler, D. (1986a):

Identification of S-1,2,2-trichlorovinyl-N-acetylcysteine as a urinary metabolite of tetrachloroethylene: Bioactivation through glutathione conjugation as a possible explanation of its nephrocarcinogenicity.
J. Biochem. Toxicol., 1, 57 - 72.

Dekant, W., Vamvakas, S., Berthold, K., Schmidt, S., Wild, D. and Henschler, D. (1986b):

Bacterial ß-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene.
Chem.-Biol. Interactions, 60, 31 - 45

Dekant, W., Schulz, A., Metzler, M. and Henschler, D. (1986c):

Absorption, elimination and metabolism of trichloroethylene: a quantitative comparison between rats and mice.
Xenobiotica, 16, 143 - 152

Dekant, W., Martens, G., Vamvakas, S., Metzler, M. and Henschler, D. (1987):

Bioactivation of tetrachloroethylene - Role of glutathione S-transferase-catalyzed conjugation versus cytochrome P-450-dependent phospholipid alkylation.
Drug Metab. Dispos., 15, 702 - 709.

Dekant, W., Vamvakas, S. and Anders, M.W. (1989):

Bioactivation of nephrotoxic haloalkenes by glutathione conjugation: Formation of toxic and mutagenic intermediates by cysteine conjugate ß-lyase.
Drug Metab. Rev., 20, 43 - 83

Liebler, D.C. and Guengerich, F.P. (1983):

Olefin oxidation by cytochrome P-450: evidence for group migration in catalytic intermediates formed with vinylidene chloride and trans-1-phenyl-1-butene.
Biochemistry, 22, 5482 - 5489

Liebler, D.C., Meredith, M.J. and Guengerich, F.P. (1985):

Formation of glutathione conjugates by reactive metabolites of vinylidene chloride in microsomes and isolated hepatocytes.
Cancer Research, 45, 186 - 193

Liebler, D.C., Latwesen, D.G. and Reeder, T.C. (1988):

S-(2-chloroacetyl)glutathione, a reactive glutathione thiol ester and a putative metabolite of 1,1-dichloroethylene.
Biochemistry, 27, 3652 - 3657

Okine, L.K. and Gram, T.E. (1986):

In vitro studies on the metabolism and covalent binding of [14C]1,1-dichloroethylene by mouse liver, kidney and lung.
Biochem. Pharmacol., 35, 2789 - 2795

Reichert, D., Werner, H.W., Metzler, M. and Henschler, D. (1979):

Molecular mechanisms of 1,1-dichloroethylene toxicity: excreted metabolites reveal different pathways of reactive intermediates.
Arch. Toxicol., 42, 159

Vamvakas, S., Dekant, W. and Henschler, D. (1989):

Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes.
Mut. Res., 222, 329 - 335

Vamvakas, S., Kremling, E. and Dekant W. (1989):

Metabolic activation of the nephrotoxic haloalkene 1,1,2-trichloro-3,3,3-trifluoro-1-propene by glutathione conjugation.
Biochem. Pharmacol., 38, 2297 - 2304

BASF
Abteilung Toxikologie
Department of Toxicology

APPENDIX

State of Research:
Scientific background for this study

1,1-Dichloroethene (DCE) is an important reactive intermediate in industrial syntheses and is also often used as a copolymer in th production of plastics. DCE is the compound with the severest toxic effects among the chlorinated ethenes, and even low doses result in liver and kidney lesions after repeated administration of DCE. The carcinogenicity of DCE is still open to debate. DCE is mutagenic in the Ames test, it caused tumors in the liver in son long term studies; in one study, it selectively induced renal tumors in male mice.

Some of the bioactivation mechanisms which lead to hepatotoxic effects have been elucidated. The bioactivation mechanism presented in Figure 1 to explain the hepatotoxic effects has be n supported by the experimental results (Costa and Ivanetich, 1982; Liebler and Guengerich, 1983; Liebler et al., 1985)*.

---

* References are given in Section 4 of the study protocol

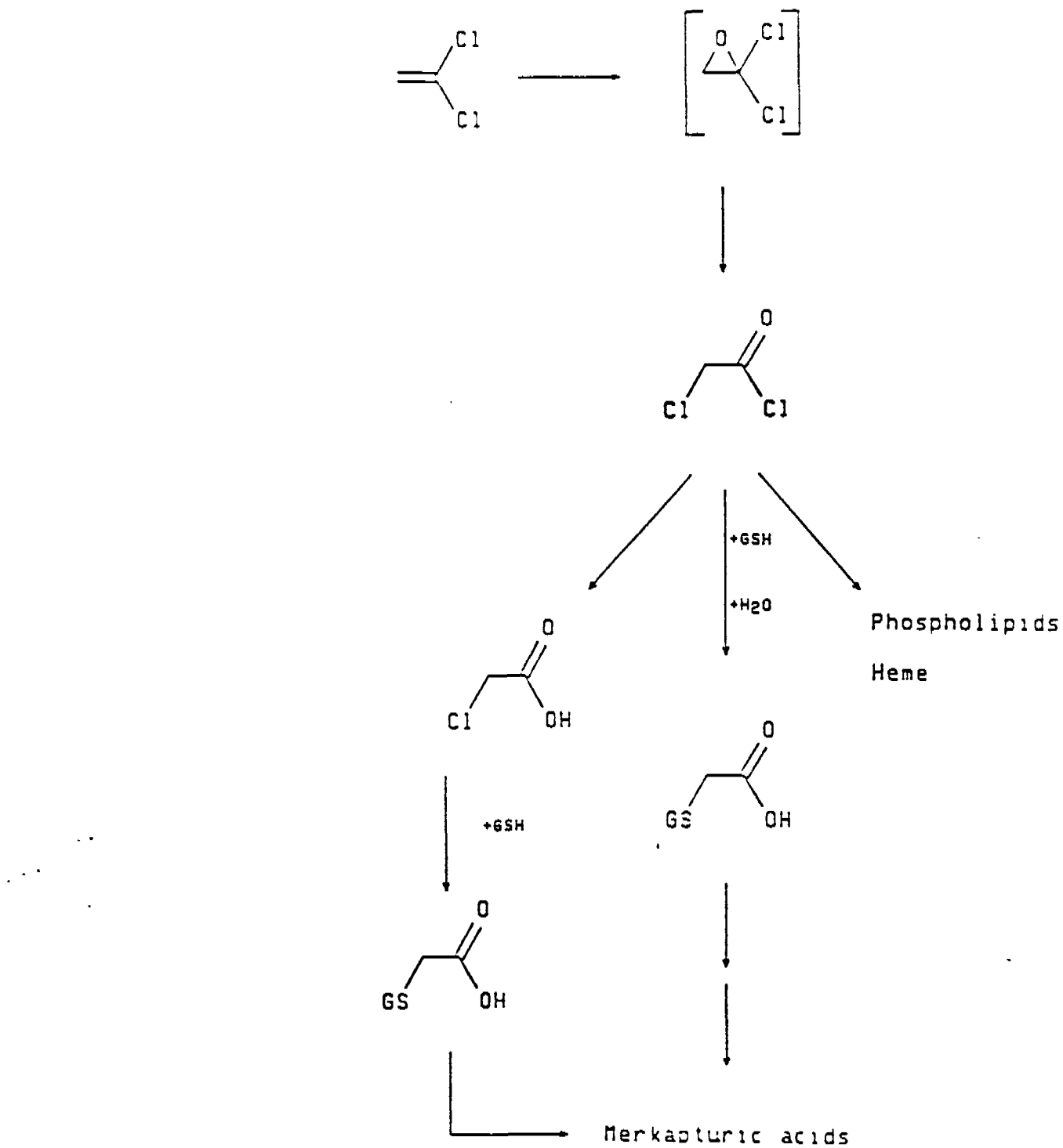


Fig. 1: Metabolism of 1,1-dichloroethene by cytochrome P-450

SL 064666

DCE is a substrate for cytochrome P-450 dependent monooxigenases. Chloroacetyl chloride is formed as a product of this reaction possibly via an epoxide-intermediate. Chloroacetyl chloride is a highly reactive acylation agent and reacts with cellular macromolecules such as proteins and phospholipids; toxic effects may be induced bythis covalent binding. All other metabolites which have been identified up to now can also be attributed to reactions of chloroacetyl chloride with water, glutathione and other nucleophiles. Indications of a reaction of chloroacetyl chloride with phospholipids were obtained by the identification of an etanolamine conjugate (Reichert et al, 1979) as a DCE metabolite in vivo.

Bioactivation mechanisms which could explain the selectiv nephrotoxicity and carcinogenicity of DCE in mice have, however, not yet been defined. The administration of DCE results in lesions at the proximal tubules; tubular adenocarcinomas were induced by long-term administration to mice. This observation may provide one way of elucidating the mechanisms responsible for the organ specificity. Identical toxic effects on the kidney with regard to both acute toxicity and carcinogenicity were found for several structurally related halogenated alkenes, such as hexachlorobutadiene, dichlorodifluoroethene, tetrachloroethene, trichloroethene, tetrafluoroethene, trichlorotrifluoropropene and dichloroacetylene (overview in Dekant et al., 1989). The mechanisms of organotropic carcinogenicity and toxicity for these compounds have already been elucidated well, including substantial contributions from our own group. The following hypothesis which could explain the organ specificity of these compounds (Fig. 2):

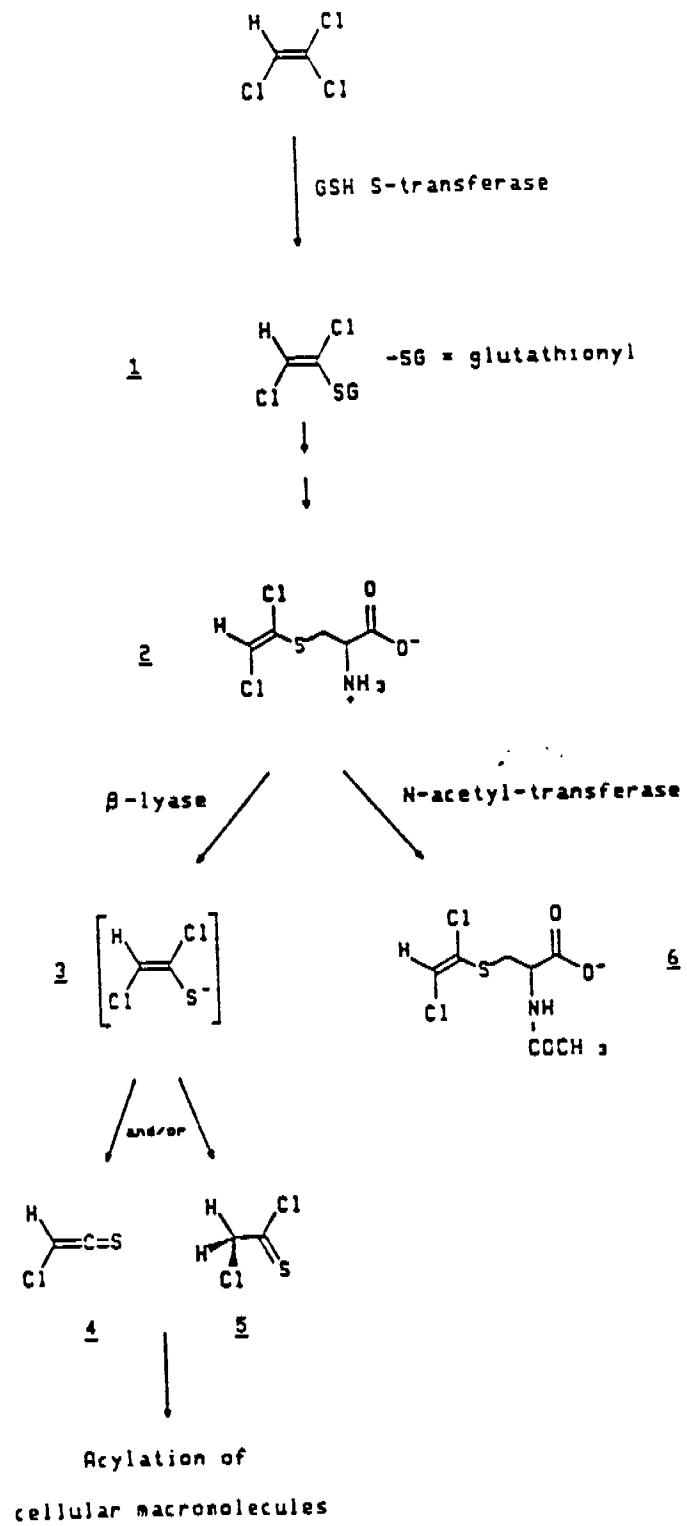


Fig. 2: Mechanism of haloalkene bioactivation by the glutathione conjugate/cysteine conjugate β-lyase pathway

In the liver, trichloroethene and many other substances from this group which are nephrotoxic and/or nephrocarcinogenic are conjugated by glutathione S-transferases with glutathione. The glutathione conjugates formed are transported, either intact of after enzymatic degradation by γ-glutamyl transpeptidase and dipeptidases, to the kidney and concentrated there. The cysteine S-conjugates that are formed after degradation are converted further into mercapturic acids by N-acetyl transferases. These are excreted in the urine. However, cysteine S-conjugates of halogenated alkenes are also substrates for enzymes from the group of the cysteine conjugate β-lyases which cleave these S-conjugates with the formation of pyruvate, ammonium ions and reactive intermediates, thioacyl fluorides and thioketenes. These electrophilic and highly toxic intermediates cause the primary lesion at DNA which may lead to tumor formation and they cause acute toxic effects by the reaction with other macromolecules. β-lyases and the enzymes of the mercapturic acid biosynthesis which are responsible for the degradation of glutathione conjugates are present in the proximal tubules of the kidney in high concentrations. This topographical distribution and the ability of the kidney to concentrat amino acids and their derivatives may explain the organ-specific ffect. Most of these steps have been validated (Dekant et al., 1989).

Toxic glutathione conjugates may also be formed within the metabolism of DCE. Investigations in which the influence of various pretreatments on nephrotoxicity and the covalent binding of ^{14}C-DCE metabolites in the kidney was determined indicate that precursors of the toxic metabolites are formed in the liver rather than in the kidney and reach the kidney via transport mechanisms (Okine and Gram, 1986). These observations agree with the concept of the formation of toxic glutathione conjugates in the metabolism of DCE.

Besides the metabolism via the glutathione conjugate/cysteine conjugate ß-lyase pathway, the formation of direct reactive glutathione conjugates is also conceivable with DCE. Chloroacetyl glutathione, a reactive thiol ester and an alkylating agent with a half-life of 4 hours under physiological conditions has been postulated as a metabolite of DCE (Liebler et al., 1988); this compound is possibly formed by the reaction of chloroacetyl chloride with one molecule of glutathione and may, especially after the depletion of glutathione by high concentrations of electrophilic DCE metabolites, excreted from the liver, reach the kidney and induce toxic effects there by reacting with macromolecules (Fig. 3). At higher concentrations of glutathione, a detoxification of chloroacetyl glutathione with a further molecule of glutathione is, however, likely in liver.

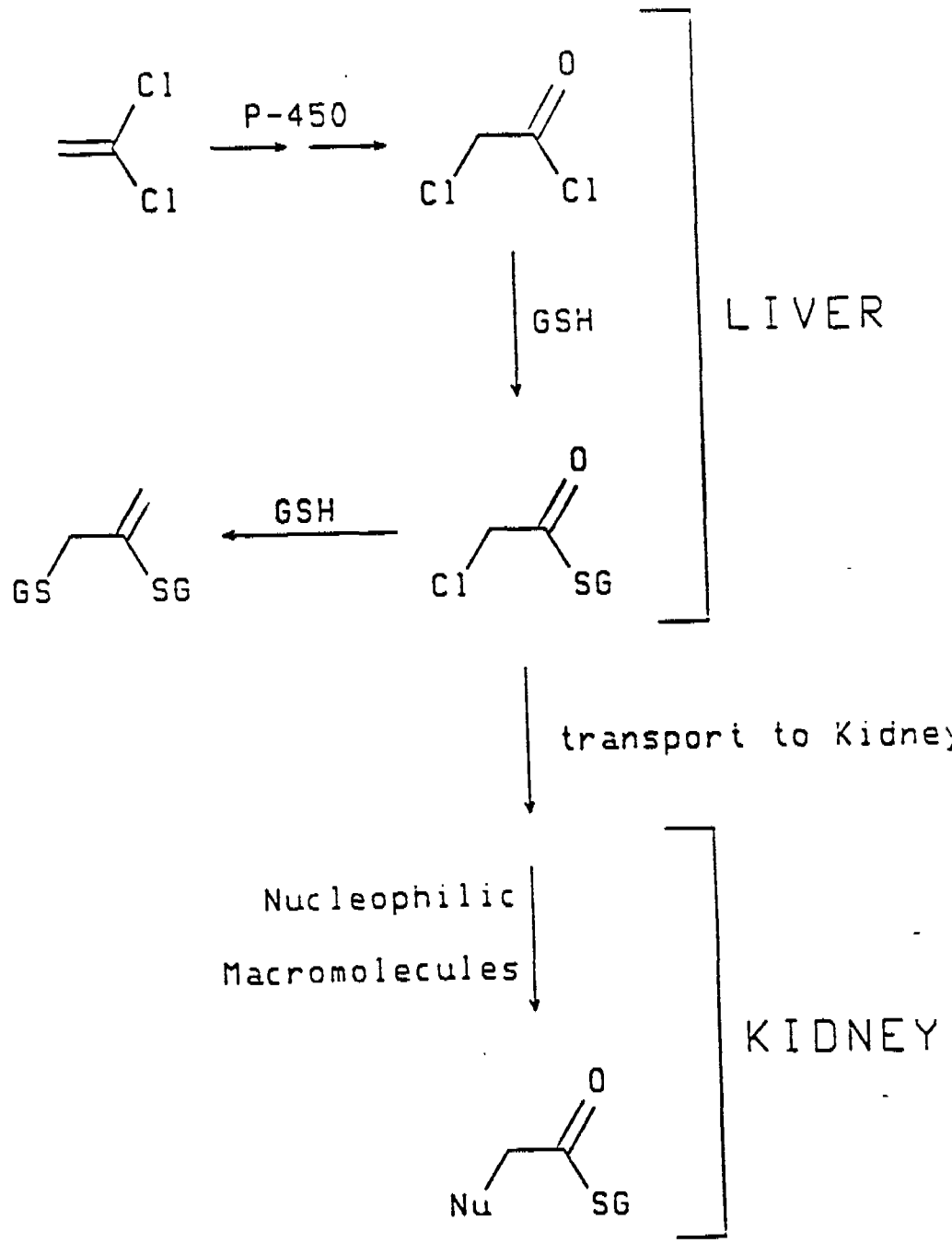


Fig. 3: Possible formation of the alkylating agent S-(2-chloroacetyl)glutathione in the metabolism of 1,1-dichloroethen (Liebler et al., 1988)

Cytochrome P-450 dependent bioactivation might also account for species and sex specific nephrotoxicity. P-450 isozymes catalyzing the activation of DCE to electrophiles (1,1-dichlorooxirane and chloroacetyl chloride) may be expressed specifically in the kidney of male mice thus causing organ specific toxicity and carcinogenicity by P-450 activation reactions of DCE in situ.

The subject of the investigations is to elucidate the mechanisms of the selective nephrotoxicity of DCE in male mice focusing on the formation of toxic glutathione conjugates and the marginal conditions of their formation, especially the dependenc on species, sex and dose. On the basis of the detection of such reactions in the metabolism of DCE, a molecular mechanism is to be established to explain the specific nephrotoxicity and nephrocarcinogenesis. In case toxic glutathione S-conjugates will not b identified, the experiments will concentrate on determining the contribution of P-450 dependent bioactivation reactions to DCE nephrotoxicity in mice. The results obtained are to contribute to an improved assessment of the hazard for man by exposur to DCE.

Working Hypothesis

Based on theoretical considerations, the nephrotoxicity of DCE in male mice may be the consequence of a selective bioactivation by one of the following reaction sequences:

- Metabolism of DCE by glutathione S-transferase to S-(1-chlorovinyl)glutathione, processing of this glutathione S-conjugate to S-(1-chlorovinyl)-L-cysteine which is cleaved in part by cysteine conjugate β-lyase in kidney to reactive intermediates. Acetylation of S-(1-chlorovinyl)-L-cysteine results in the urinary excretion of S-(1-chlorovinyl)-N-acetyl-L-cysteine.

- Formation of (2-chloroacetyl)glutathione by formation of chloroacetyl chloride, reaction of this acetyl chloride to the electrophilic thioester (2-chloroacetyl)glutathione which is selectively concentrated in the kidney.

- Cytochrome P-450 dependent bioactivation of DCE selectively in the kidney of male Swiss mice resulting in high concentration of reactive intermediates in the target organ.

The aim of the study is to elucidate the molecular mechanisms of the nephrotoxicity of DCE.

Annu Rev Pharmacol Toxicol 1990. 30:73–89

CHLOROETHYLENES: A Mechanistic Approach to Human Risk Evaluation

Trevor Green

ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, United Kingdom

KEY WORDS: carcinogenicity, species differences, peroxisomes, hyaline droplets, epoxides

INTRODUCTION

The four chlorinated ethylenes, vinyl chloride (chloroethylene), vinylidene chloride (1,1-dichloroethylene), 1,1,2-trichloroethylene and 1,1,2,2-tetrachloroethylene form a group of structurally related chemicals with properties invaluable to modern industrial society. Polymers and copolymers of vinyl chloride and vinylidene chloride have become the most important synthetic resins in use today, with worldwide production of PVC exceeding 18 million tons in 1988. The solvent properties of trichloroethylene and tetrachloroethylene have resulted in their widespread use in metal degreasing, dry cleaning, and a wide variety of industrial applications. These chemicals have now been in common use for more than 50 years. During this time workers have been exposed to a wide range of concentrations, in some cases for periods of 20 years or more, which has allowed a significant data base to be compiled about the short- and long-term effects of these chemicals on human health. In the last 20 years this information has been supplemented by numerous studies in laboratory animals.

The chloroethylenes are either gases or volatile liquids and the principal route of exposure is by inhalation. Exposure to high concentrations results in CNS depression and narcosis (1–4); trichloroethylene, for example, has been used as an anesthetic for many short-duration surgical procedures (5). Other effects have been reported, ranging from acro-osteolysis, part of a phenomenon known as vinyl chloride disease (6), to liver, kidney, and lung changes

0362-1642/90/0415-0073$02.00

(7, 8). Attempts to reproduce acro-osteolysis in laboratory animals led to the discovery of the carcinogenicity of vinyl chloride. In a study exposing rats to vinyl chloride by inhalation, Viola (9) and Viola et al (10) reported an increased incidence of skin, lung, and bone tumors. Maltoni et al (11) confirmed these findings and also reported an increased incidence of angiosarcoma of the liver. In the same year, 1974, Creech & Johnson (12) reported three deaths from angiosarcoma of the liver among workers employed on a vinyl chloride plant in the USA. The rarity of this type of tumor and the results of Maltoni's animal studies immediately established a connection between exposure to vinyl chloride and human liver cancer. This led to one of the most extensive epidemiological and toxicological studies of any industrial chemical.

One consequence of the work on vinyl chloride was the subsequent testing of the other chloroethylenes for the ability to cause cancer. That the structurally similar chloroethylenes would have properties similar to vinyl chloride appeared to be confirmed when each was shown to cause cancer in laboratory animals. There were, however, significant differences. Whereas vinyl chloride was established as a human carcinogen and was found to be mutagenic and carcinogenic in all tests used to measure these responses, a marked lack of mutagenicity and species-, strain-, and sex-dependent responses in two-year cancer studies characterized the evaluation of the other chloroethylenes. Furthermore, there was no evidence from occupationally exposed populations of any increase in any type of human cancer. Consequently, the risks to man from exposure to these chloroethylenes were much less well defined than for vinyl chloride.

The variable results in the cancer bioassays and the lack of activity in many mutagenicity tests spawned a series of mechanistic studies on the differences between vinyl chloride and the other three chemicals in this group. According to these studies, at least two, if not all three, polychlorinated ethylenes act in an entirely different manner from vinyl chloride and involve mechanisms that were unknown in 1974 when the carcinogenicity of vinyl chloride was first discovered. Consequently, the risks to man are better understood and a scientific basis now exists for the control and use of these chemicals. This paper reviews the mechanisms of action of each of the chlorinated ethylenes and considers the relevance of the animal studies to people exposed to these chemicals. The cancer studies and mutagenicity assays have been reviewed by others and are only considered if relevant to the mechanisms involved in the development of cancer.

VINYL CHLORIDE

By 1986, twelve epidemiology studies had identified 120 cases of vinyl chloride-induced angiosarcoma of the liver in populations employed in

the manufacture of vinyl chloride (13), compared to an incidence in the general population of 0.10 per million. Increases in other tumors including liver, brain, lung, thyroid, lymphatic tissue, and skin were noted but a relationship could not be established between them and vinyl chloride exposure.

Hemangiosarcoma of the liver was also induced in rats, mice, and hamsters exposed to vinyl chloride (11). As in man, such tumors are extremely rare in these species. Other tumors associated with vinyl chloride exposure included zymbal gland tumors in rats and hamsters, nephroblastomas in rats, pulmonary and mammary gland tumors in mice, and forestomach papillomas and melanomas in hamsters. The same range of tumors was seen in rats following either oral administration or inhalation exposure. Tumors were observed over a wide range of doses ($\times 10^3$) from as low as 10ppm and, in some studies, after short exposures (14). The wide range of effects in several species was considered characteristic of a genotoxic carcinogen. This view was supported by the short-term mutagenicity tests. Vinyl chloride was mutagenic and clastogenic in vivo, and in vitro when in the presence of an appropriate metabolic activating system (8). Clastogenicity has also been reported in workers exposed to high concentrations of vinyl chloride (15).

There seems little doubt that vinyl chloride is mutagenic and carcinogenic as a result of its metabolism by microsomal mixed function oxidases (cytochromes P-450) to chloro-oxirane (chloroethylene oxide) (16, 17). This highly electrophilic epoxide is a potent mutagen when tested directly or when generated from vinyl chloride in the presence of an appropriate metabolizing system (18, 19). DNA-alkylation products that lead to mispairing, depurination, and fragmentation have been isolated from animals exposed to vinyl chloride and from a variety of in vitro systems (20, 21). These products are consistent with chloro-oxirane being the ultimate carcinogenic form of vinyl chloride. The other known mutagenic metabolite of vinyl chloride is chloroacetaldehyde, the rearrangement product of chloro-oxirane (19). Although less mutagenic than the epoxide, chloroacetaldehyde is known to react with DNA to give the same alkylation products and could therefore have a role in the carcinogenicity of vinyl chloride (20).

The major detoxification pathway for these two mutagenic metabolites is by conjugation with glutathione leading to the excretion of S-(2-hydroxyethyl) cysteine and thiodiglycolic acid in urine (Figure 1) (22, 23). The epoxide also appears to be a substrate for epoxide hydratase and gives an unstable diol that is further metabolized to become incorporated into the citric acid cycle and yield carbon dioxide in vivo. That vinyl chloride metabolism is a saturable process (24) is reflected by the incidences of angiosarcoma and the reduction of the slope of the dose-response curve to zero at higher dose levels (14). There are no known major species differences in the metabolism of vinyl chloride.

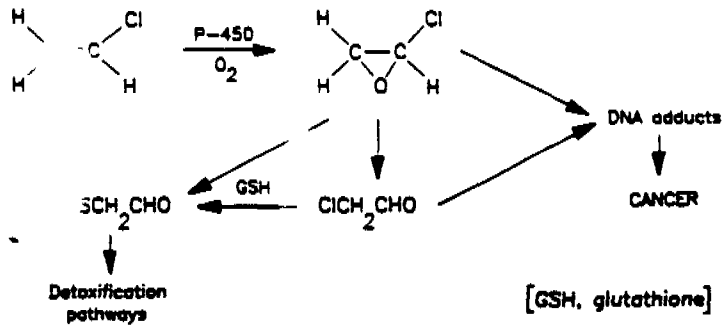


Figure 1 The metabolic activation of vinyl chloride.

The vast amount of information available about vinyl chloride, which is very briefly summarized here, corroborates the mutagenicity and carcinogenicity of this chemical. This conclusion is consistent with its metabolism to a mutagenic epoxide and the development of cancer by a mechanism involving alkylation of DNA. Vinyl chloride is therefore a classical genotoxin causing cancer by somatic mutation.

THE MUTAGENICITY AND CARCINOGENICITY OF VINYLIDENE CHLORIDE, TRI- AND TETRA-CHLOROETHYLENE

Vinylidene chloride, trichloroethylene, and tetrachloroethylene are all metabolized by cytochrome P-450 to products consistent with the formation of an epoxide intermediate (25, 26) and are identical in this respect to vinyl chloride. Although the stability and reactivity of the epoxides have been intensively investigated (27–30), there is no direct evidence that they exist as free chemical species in biological systems. It has been suggested that these epoxides never leave the enzyme site as free species (31), unlike vinyl chloride epoxide, which has been detected spectroscopically and trapped with a variety of nucleophiles (32, 33). A further indirect measure of the formation and reactivity of these epoxides is the mutagenicity of the parent chemical in vivo, and in vitro in the presence of suitable metabolic activation. Again, these results provide little evidence that these epoxides exist in mammalian systems. Weak responses have been seen for vinylidene chloride in prokaryotic and some eukaryotic systems (34, 35). The responses are highly dependent upon species or the source of the metabolic activation used in the in vitro tests, with mouse tissues the most active (36, 37). A similar pattern is seen for trichloroethylene where the responses are perhaps even weaker, e.g. no evidence of point mutations in bacteria (38, 39), some in yeast (40, 41), but chromosomal effects in the mouse micronucleus assay (42). Tetrachloroethylene appears not to be mutagenic or clastogenic either in vivo or in vitro

in tests where pure material was used (39, 43). DNA-binding studies have been reported for each of the chloroethylenes, although there is little evidence of binding except with vinyl chloride where specific adducts have been identified (44–47). There is therefore a clear gradation from a potent mutagen and clastogen, vinyl chloride, through to tetrachloroethylene, which appears to have no genotoxic properties in short-term tests. This conclusion is based on an overview of a complex area that in detail is beyond the scope of this review. Many of the mutagenicity tests reported in the literature are confounded by the use of impure material and the presence of mutagenic stabilizers that have been used in some commercial grades of these chemicals. Nonetheless, the conclusion is valid when properly conducted tests of pure material are considered.

The pattern of inconsistent responses seen in the mutagenicity tests is repeated in the two-year cancer studies. The carcinogenicity of vinylidene chloride, the closest analog of vinyl chloride and therefore the one that might be expected to most resemble vinyl chloride in its properties, has never been fully resolved. Of eighteen chronic studies only one reported a carcinogenic effect (48). Male Swiss mice exposed to 25ppm by inhalation had a low incidence of renal adenocarcinoma. The effect was not seen at 10ppm, nor in female mice, in other species at higher dose levels, or in mice of other strains. In light of these negative results, this apparently real but unique response fails to resolve the issue of whether or not vinylidene chloride is a carcinogenic hazard to man. A further bioassay is unlikely to resolve this issue.

Trichloroethylene and tetrachloroethylene have also shown marked species-, strain- and sex-specific responses in two-year studies (43, 49–54). Both chemicals are clearly carcinogenic in the liver of B6C3F1 mice in several studies by either inhalation or ingestion (32, 49–51). However, trichloroethylene did not induce tumors in Swiss mice (52) nor did either chemical cause liver tumors in rats (43, 49–51). A low incidence of kidney tumors was reported in male rats exposed to tetrachloroethylene in two studies (43, 53), and lung tumors have been observed in CD-1 mice exposed to trichloroethylene in another study (54).

Thus, in contrast to vinyl chloride where there are consistent responses in the cancer bioassays and a clear genotoxic mechanism, the other chloroethylenes fail to give a clear indication of their genotoxic potential in short-term tests and the cancer studies fail to give a clear indication of the hazard to man. Many of these issues have largely been resolved by mechanistic studies that explained the species and sex differences, and linked the relevance of the animal studies to exposed human populations.

Vinylidene Chloride-Induced Mouse Kidney Tumors

Perhaps the least satisfactory explanation of a species-specific response among the chloroethylenes is found for vinylidene chloride. This may reflect

the degree of uncertainty over its carcinogenicity. Kidney tumors were found in only one study that used Swiss mice and then in only two out of eighteen surviving males (48). Severe nephrotoxicity was a feature of the study at the dose level wh tumors were seen but not at lower dose levels where no tumors occurr Of the species and strains used in the eighteen cancer studies, the male Swiss mouse was more susceptible than rats, hamsters, or other strains of mice to the nephrotoxic effects of vinylidene chloride. Thus, it is tempting to make a connection between the kidney damage and the low incidence of tumors seen in these mice.

Other possible mechanisms have been considered. Vinylidene chloride is metabolized by a saturable pathway to a putative epoxide intermediate that rearranges to chloroacetyl chloride (25, 55). The major detoxification pathways for these intermediates are hydrolysis and conjugation with glutathione, resulting in a variety of metabolites and the depletion of hepatic glutathione levels. Studies have investigated the metabolism and pharmacokinetics of vinylidene chloride in different species and organs, seeking an explanation for the response seen exclusively in the mouse kidney. Alkylation of macromolecules is highest in the mouse kidney (56–58) and is dependent on cellular glutathione levels (59). Oesch et al (36) compared the effe s of vinylidene chloride on hepatic and renal enzymes and reported a red tion in epoxide hydratase and glutathione-S-transferase activity in male mouse kidney that was not seen in female mice or in rats of either sex. Although a reduction in these detoxification enzymes is consistent with the outcome of the cancer studies, one enzyme, epoxide hydratase, apparently plays a very minor role in the metabolism of vinylidene chloride.

In conclusion, higher metabolic rates, higher levels of covalent binding, and possible reductions in the levels of detoxifying enzymes are all consistent with the male Swiss mouse's susceptibility to kidney tumors. Implicit is the belief that vinylidene chloride is carcinogenic as a result of its metabolism to a reactive electrophile such as the epoxide. However, the crucial factor in determining the species and strain differences may be the susceptibility of the Swiss mouse to the cytotoxic effects of vinylidene chloride. There is some evidence that tumors are not seen in the absence of toxicity; this suggests that the weak alkylating effects of vinylidene chloride metabolites may not be sufficient to cause cancer. Kidney damage and the resulting cell division may facilitate the expression of the weak genotoxic potential of the metabolites or, alternatively, kidney damage may alone lead to cancer by a nongenotoxic mechanism. Whichever mechanism applies, the effects seem to be unique to the male Swiss mouse and to have little relevance to other species, including man.

Tri- and Tetrachloroethylene Induced Mouse Liver Tumors

Both trichloroethylene and tetrachlorethylene cause a very similar increase in the incidence of hepatocellular carcinoma in B6C3F1 mice following either

oral administration or inhalation exposure for two years (43, 49–51). Short-term exposure to these chemicals causes liver growth and marked peroxisome proliferation in mice but not rats (60–62). The link between peroxisome proliferation and cancer in rodents strongly suggests that this response is the basis of the species difference in carcinogenicity (63–65). The identification and subsequent cancer bioassay of the metabolite responsible for the peroxisome proliferation confirmed this hypothesis. Both chemicals are metabolized to trichloroacetic acid (Figure 2), which causes peroxisome proliferation in rats and mice in vivo and in rat and mouse hepatocytes in vitro (60). A two-year study in which trichloroacetic acid was administered to B6C3F1 mice in drinking water found a 32% incidence of hepatocellular carcinoma within 61 weeks of the start of the study (66). Tumors were not found in the livers of control animals at that time. There can be little doubt therefore that trichloroacetic acid is the metabolite of trichloroethylene and tetrachloroethylene responsible for the liver tumors seen in mice. These studies also demonstrate that the epoxide intermediates proposed in the metabolism of both chemicals have no role in the initiation or development of these tumors.

Trichloroacetic acid is a common metabolite of trichloroethylene and tetrachloroethylene in most animal species including rats, mice, and man (26, 67–70). When trichloroacetic acid was administered to rats and mice hepatic peroxisome proliferation was seen in both species (60). However, this response and liver cancer were seen only in mice and not rats when the parent chemicals were administered in the two-year cancer studies. The reason for this apparent anomaly is pharmacokinetic; trichloroacetic acid-induced peroxisome proliferation is a threshold phenomenon (60). In the mouse, the metabolism of trichloroethylene and tetrachloroethylene is linear over a wide range of dose levels and the threshold is easily exceeded at the high dose

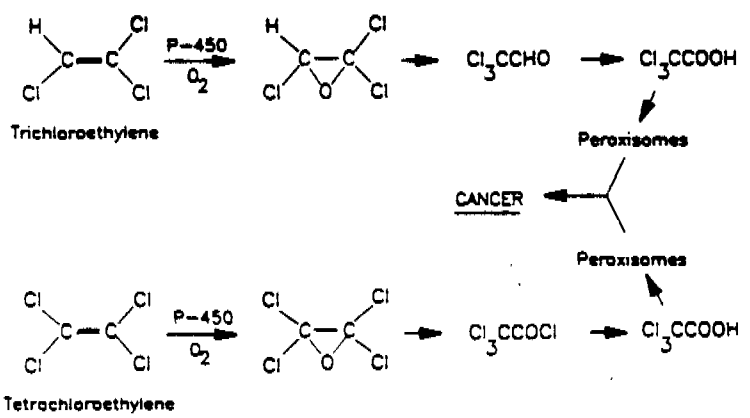


Figure 2 The metabolism of tri- and tetrachloroethylene in relation to the development of liver tumours in $B_6C_3F_1$ mice.

levels used in the cancer studies. In the rat, metabolism becomes saturated at relative low dose levels and the threshold concentration of trichloroacetic acid required to induce peroxisome proliferation is not exceeded at any dose of the parent chemical (26, 60, 71). The effects of this species difference in metabolism is clearly seen in the blood levels of trichloroacetic acid in the two species. For example, the peak in blood levels was seven times higher in the mouse than the rat when both species were exposed to 400ppm tetrachloroethylene for six hours (72).

These experiments produced an entirely consistent explanation for the mechanism of action of these two chemicals and for the basis of the rodent species differences. The mechanism involves peroxisome proliferation mediated by a common metabolite, trichloroacetic acid. The finding that trichloroacetic acid is a complete carcinogen appears to rule out any role for electrophilic epoxide metabolites in the hepatocarcinogenicity of these chemicals, which is consistent with their lack of mutagenicity. Also consistent with a nongenotoxic mechanism is the nature of the threshold response for peroxisome proliferation, which is also the basis of the species difference between rats and mice and is derived from simple pharmacokinetic differences between the two species. Thus, mice are susceptible to liver cancer as a direct result of the high metabolic rates frequently found in this species and rats are protected by lower rates and saturation of the metabolic pathway.

The above experiments and bioassays all used the B6C3F1 strain of mouse, and for the trichloroethylene studies, oral gavage as the route of administration. The Swiss mouse exposed by inhalation is apparently not susceptible to trichloroethylene-induced liver cancer in the same way (52), although the reasons for this strain difference are unknown. Swiss mice metabolize trichloroethylene at a similar rate and to a similar extent and are also susceptible to peroxisome proliferation (60, 71). Thus, other than differences in the dose and route of administration in the two cancer bioassays, and the known sensitivity of the B6C3F1 mouse to liver carcinogens, there is no clear explanation for this strain difference in carcinogenicity.

The relevance of the mechanism identified in B6C3F1 mice for humans exposed to tri- and tetrachloroethylene has been assessed in a number of studies comparing metabolic rates in mice, rats, and man, and the response of human liver tissue to the key metabolite, trichloroacetic acid (60). The metabolism of both trichloroethylene and tetrachloroethylene is limited in man by saturation of the metabolic pathway at relatively low exposure levels (67, 73, 74). In this respect, man resembles the rat and may not be able to produce sufficient trichloroacetic acid to stimulate peroxisome proliferation. There is, however, an even more significant difference between mice, rats, and man in their response to trichloroacetic acid. The peroxisome proliferation seen in vivo in rats and mice treated with trichloroacetic acid has been reproduced in vitro using mouse and rat hepatocytes (60). Under the same

conditions, trichloroacetic acid failed to induce peroxisome proliferation in human hepatocytes. Consequently, the mechanism believed to operate in the mouse appears to be unique to that species. Only the mouse has the combination of high metabolic rates and the ability to respond to trichloroacetic acid as a peroxisome proliferator that results in cancer. The rat is limited by metabolic saturation; man is limited by both metabolic saturation and a lack of trichloroacetic acid-stimulated peroxisome proliferation. These conclusions are perhaps not too surprising. The mouse as the smallest of the species of interest would be expected to have the highest metabolic rates. It is also well known that peroxisome proliferation differs according to species and is much more common in rodents than in any other species.

Tetrachloroethylene Induced Rat Kidney Tumors

A low incidence of renal tubular cell adenomas and adenocarcinomas was found in male F344 rats exposed to tetrachloroethylene by inhalation for a lifetime (43). The incidence was not statistically significant, but because adenomas are rare in control F344 rats and malignant kidney tumors are not normally observed at all, several studies have sought a mechanistic explanation for their origin.

Previous lifetime studies of tetrachloroethylene in rats have all been affected by poor survival within the dosed groups (51, 76). In each case there was evidence that survival was reduced as a result of chemically induced nephropathy distinguishable from the age-related nephropathy normally seen in this strain of rat. Of the species tested, the male rat was the most sensitive to these effects. Mortality was slight in the NTP inhalation study (43), but again there was evidence of kidney damage characterized by tubular enlargement and hyperplasia. There are therefore striking similarities between tetrachloroethylene and vinylidene chloride in that the species most sensitive to the nephrotoxic effects of the chemical has a low incidence of kidney tumors after two years of exposure. It is possible that a similar mechanism involving sustained cytotoxicity and cell division may apply.

Other well-defined mechanisms have been proposed to account for these tumors. High oral doses of tetrachloroethylene (1–1.5g/kg/day) cause an accumulation of the protein alpha-2u-globulin (hyaline droplets) in renal proximal tubular cells of male rats (75, 77–79). Tubular casts were formed and focal areas of proximal tubular regeneration were apparent in these studies. The effects were specific to the male rat and were not seen in females or in mice of either sex. Hyaline droplet formation or protein droplet nephropathy has been linked with a significant number of male rat-specific kidney carcinogens and is believed to be part of a cycle of necrosis and cellular regeneration that results in cancer (77, 80–83). Such a mechanism would explain the specificity of the tumors and would not be dissimilar in effect to the cytotoxicity and hyperplasia reported from the two-year studies. Howev-

er, although these responses were observed after high oral doses of tetrachloroethylene, they were not seen after inhalation exposure for 28 days (75) at the dose levels (up to 400ppm) used in the NTP inhalation study (43). Apparently, hyaline droplet formation, like peroxisome proliferation and other nongenotoxic responses, is a threshold phenomenon and the highest dose used in the NTP study is below the threshold. Further short-term studies using higher doses (1000ppm) by inhalation did induce this response in male rats (75). Thus, although a well-established link exists between hyaline droplet formation and renal cancer in male rats, the lack of effect at the top dose level used in the two-year inhalation study (43) questions the significance of this phenomena in the development of tumors in that study. A contribution from hyaline droplet formation to the development of these tumors cannot entirely be ruled out based on the results of a 28-day study. The potential for causing this effect has clearly been established and hyaline droplet formation may still occur during a lifetime study, even at the lower dose levels used in the NTP study (43).

Yet another mechanism has been proposed to explain the low incidence of male rat kidney tumors seen after exposure to tetrachloroethylene. Unlike the previous mechanisms, this one involves the formation of a mutagenic metabolite in the kidney. Tetrachloroethylene is metabolized by a second minor pathway involving hepatic glutathione conjugation (75, 84) (Figure 3). The conjugate is metabolized by the mercapturic acid pathway and excreted in urine as the N-acetyl cysteine derivative. The precursor of this metabolite, the cysteine conjugate of tetrachloroethylene, is a substrate for the renal enzyme β-lyase and is mutagenic in the Ames bacterial mutation assay when activated by rat kidney fractions (75, 85, 86). The lack of response of tetrachloroethylene itself in this assay is probably due to a failure of the standard test to

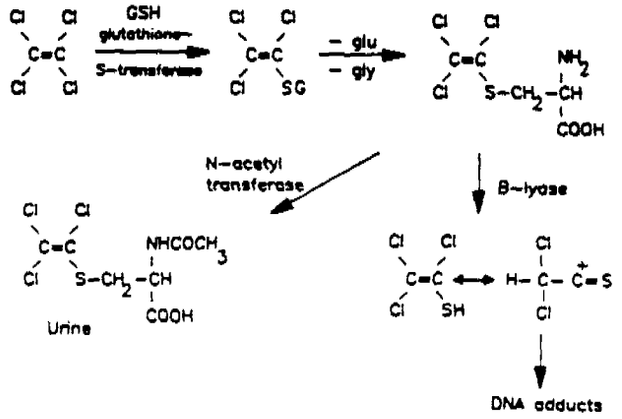


Figure 3 The metabolism of tetrachloroethylene by glutathione conjugation and activation by renal β-lyase in the rat.

replicate the number of steps involved in the activation of the chemical by this pathway. These studies have been taken as evidence for the involvement of genotoxicity in the development of these tumors. Activity by this pathway is consistent with the male rat as the susceptible species, since glutathione conjugation and B-lyase activity is greater in male than female rats or mice of either sex (75). The pathway is minor, even in the male rat, and only increases significantly when the major cytochrome P-450 pathway has saturated (75).

A surfeit of explanations exists for what is actually a very low incidence of tumors in one sex of one species of laboratory animal. This may equally be true with most mechanisms of carcinogenesis in that many factors influence the initiation and development of tumors. In this case it is entirely possible, if not probable, that the overall mechanism involves a low level of genotoxicity derived from glutathione conjugation and activation by β-lyase, in conjunction with cell division stimulated by cytotoxicity or hyaline droplet formation. Each apparently separate mechanism may in combination provide the initiation and promotion steps believed to be essential for chemical carcinogenesis.

Most aspects of the mechanisms believed to be responsible for the kidney tumors seen in male rats are in fact unique to the male rat. Hyaline droplet formation and the protein alpha-2u-globulin are found only in the male rat and not in other laboratory animals or in man. Even in the male rat the effect is highly dose-dependent. Cytotoxicity and kidney damage are features of high continuous exposures and are unlikely to occur during occupational use of tetrachloroethylene. The other mechanism involving glutathione conjugation has been investigated in a series of studies comparing the key metabolic steps in rat, mouse, and human tissues (75). Human kidney possesses the enzyme β-lyase with activity towards the cysteine conjugate of tetrachloroethylene similar to that in the mouse and approximately 30-fold lower than that in male rat kidney. Experiments using human liver fractions failed to detect glutathione conjugation of tetrachloroethylene and concluded, based on the limit of detection of the assay, that the difference in rate between the rat and man was at least an order of magnitude.

In conclusion, three possible mechanisms have been reported to explain the male rat kidney tumors; each is very much dependent upon dose, two exhibit genuine thresholds, and the third is based on a pathway that is minor at low dose levels. It is unlikely that these mechanisms will occur in man; glutathione conjugation has not been detected in human liver, hyaline droplet formation is exclusive to the male rat and chronic toxicity is only likely to occur after exposure to high concentrations over a prolonged period.

OTHER TUMORS

In addition to the multiplicity of tumor types reported following vinyl chloride exposure, other tumors have also been reported for trichloroethylene and

tetrachloroethylene. An increased incidence of lung adenomas and adenocarcinomas has been reported in mice exposed to trichloroethylene by inhalation (54) and an increase in mononuclear cell leukemia was seen in F344 rats exposed to tetrachloroethylene (43). A recent NTP study (87) exposing four strains of rat to trichloroethylene also reported a low incidence of kidney tumors.

No satisfactory explanations for the mechanisms involved in the development of these tumors are presently available, although two of the results may be features of the strain of animal used and the conduct of the study rather than a true carcinogenic response to the chemical. The incidence of mononuclear cell leukemia in control F344 rats in the study (43) was 56% (males) and although this was statistically increased by exposure to tetrachloroethylene, its relevance for man is questionable. This type of tumor was not seen in other strains of rat, is known to be of high and variable incidence in the F344 rat, and is not found in man.

The low incidence of kidney tumors reported in rats exposed to trichloroethylene appears to be largely a result of chronic toxicity rather than from a more specific mechanism. Although reported, this study (87) was considered inadequate for assessing carcinogenicity because of poor survival within the dosed groups. Nephrotoxicity was again a feature of the study and the lack of dose-, sex-, or strain-dependence on the distribution of the kidney tumors suggests that toxicity may well be the cause of the low incidence observed. Other mechanisms, detected with tetrachloroethylene, apparently are either absent or present at much lower levels.

CONCLUSIONS

The discovery of the carcinogenicity of vinyl chloride led to the assumption that the other chloroethylenes would be similarly carcinogenic. This belief was well founded in that each is carcinogenic in at least one animal species. At this point the similarities between vinyl chloride and the other chloroethylenes end. The expectation that they act in the same way as vinyl chloride through mutagenic epoxide metabolites has not been fullfilled. New mechanisms have been discovered involving peroxisomes, hyaline droplets, and new metabolic pathways that were unknown in the 1970s when vinyl chloride was first investigated. Many of the new mechanisms are believed to be nongenotoxic; they exhibit thresholds and in many cases show marked species-specificity. Chronic toxicity features in all studies where kidney tumors have been observed. That a variety of mechanisms can operate within a close structural group of chemicals is perhaps the most significant conclusion from these studies. Within this finding the species-, sex- and target-organ specificity of these chemicals has been explained and the need clearly illus-

trated for this level of understanding to adequately evaluate the risks to man. The known mechanisms for the chloroethylenes are summarized in Table 1.

The question remains of the use of this type of work in human-risk assessment. The chloroethylenes illustrate clearly the pitfalls of assuming that structurally similar chemicals act by the same mechanism and can therefore be grouped together for risk assessment. Studies comparing mechanisms and species suggest that the risks to man from exposure to vinylidene chloride, trichloroethylene and tetrachloroethylene are considerably less than those associated with vinyl chloride. In some cases there are qualitative, and in others marked quantitative, differences between laboratory animals and man. Quantitative risk assessment is inappropriate where qualitative differences occur between species in response to peroxisome proliferators such as the metabolite trichloroacetic acid, or differences in tetrachloroethylene-induced hyaline-droplet formation. Where quantitative differences in metabolism and pharmacokinetics do exist, such procedures may be appropriate if they take into account those differences in the calculation of the risk to man. Even if the more generic weight-of-evidence approach is taken, the species differences in the cancer studies of vinylidene chloride, trichloroethylene and tetrachloroethylene, their lack of mutagenicity, and the mechanisms involved in the development of the tumors, all suggest the risks to man are minimal.

In-depth mechanistic investigations and species comparisons are still in their infancy and have yet to play a major role in human risk assessment. Clearly, however, such an approach is important and can assist in separating the high-risk chemicals such as vinyl chloride from species-specific carcinogens that may pose little or no threat to man at occupational or environmen-

Table 1 Known mechanisms for the carcinogenicity of the chloroethylenes

Chemicals	Carcinogenic Metabolite	Mechanism	Tumors
Vinyl chloride	Epoxide/chloroacetaldehyde	DNA alkylation	Multiple All species
Vinylidene chloride	Epoxide/acid chloride?	DNA?	Male mouse kidney
	Unknown	Chronic toxicity	Male mouse kidney
Trichloroethylene	Trichloroacetic acid	Peroxisome proliferation	Mouse liver
Tetrachloroethylene	Trichloroacetic acid	Peroxisomes	Mouse liver
	Cysteine conjugate	DNA adducts	Rat kidney
	Unknown	Hyaline droplets	Rat kidney
	Unknown	Chronic toxicity	Rat kidney

tal exposure levels. As this area develops and our understanding of these mechanisms improves, the challenge for regulatory authorities will be to incorporate such data into the risk-assessment process, rather than rely solely on inconsistent animal bioassays and short-term tests.

Literature Cited

1. Selikoff, I. J., Hammond, E. C., eds. 1975. Toxicity of vinyl chloride-polyvinyl chloride. *Ann. NY Acad. Sci.* 246
2. Torkelson, T. R., Rowe, V. K. 1982. Vinylidene chloride. In *Patty's Industrial Hygiene and Toxicology*, ed. G. Clayton, F. Clayton, p. 3545. New York: McGraw-Hill
3. Browning, E., 1965. Trichloroethylene. In *Toxicity and Metabolism of Industrial Solvents*, pp. 189–212. Amsterdam: Elsevier
4. Rowe, V. K., McCollister, D. D., Spencer, H. C., Adams, E. M., Irish, D. D. 1952. Vapour toxicity of tetrachloroethylene for laboratory animals and human subjects. *Am. Med. Assoc. Arch. Ind. Health* 5:566–79
5. Hunter, A. R. 1962. Inhalation anaesthetic agents. *Br. J. Anaethesia* 34: 224–28
6. Cordier, J. M., Fievres, C., Lefeve, M. J., Sevrin, A. 1966. Acro-ostéolyse et lesions cutanées associées chez deux ouvriers affectés au nettoyage d'autoclaves. *Cah. Med. Trav.* 4:3–39
7. IARC. 1989. IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans. Vol. 19. Some monomers, plastics and synthetic elastomers and acrotein. Lyon, France: IARC
8. IARC. 1979. IARC Monographs on the evaluation of carcinogenic risks of chemicals to humans. Vol. 20. Some halogenated hydrocarbons. Lyon, France: IARC
9. Viola, P. L. 1970. Pathology of vinyl chloride. *Med. Lav.* 61:174–80
10. Viola, P. L., Bigotti, A., Caputo, A. 1971. Oncogenic response of rat skin, lungs and bones to vinyl chloride. *Cancer Res* 31:516–22
11. Maltoni, C., Lefemine, G., Chieco, P., Carretti, D. 1974. Vinyl chloride carcinogenesis: current results and perspectives. *Med. Lav.* 65, 421–44
12. Creech, J. L., Johnson, M. N. 1974. Angiosarcoma of the liver in the manufacture of PVC. *J. Occup. Med.* 16:150–51
13. Forman, D., Bennett, B., Stafford, J., Doll, R. 1985. Exposure to vinyl chloride and angiosarcoma of the liver: a report of the register of cases. *Br. J. Indust. Med.* 42:750–53
14. Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., Carretti, D. 1984. Experimental research on vinyl chloride carcinogenesis. In *Archives of Research on Industrial Carcinogenesis* ed. C. Maltoni, M. A. Mehlman. Vol. 2. Princeton: Princeton Sci.
15. Purchase, I. F. H., Richardson, C. R., Anderson, D., Paddle, G. M., Adams, W. G. F. 1978. Chromosomal analysis in vinyl chloride exposed workers. *Mutat. Res.* 57:325–34
16. Barbin, A., Bresil, H., Croisy, A., Jacquignon, P., Malaveille, C., et al. 1975. Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* 67:596–603
17. Ivanetich, K. M., Aronson, E., Katz, E. D. 1977. Interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 *in vitro*. *Biochem. Biophys. Res. Commun.* 74:1411–18
18. Bartsch, H., Malaveille, C., Barbin, A., Bresil, H., Tomatis, L., Montesano, R. 1976. Mutagenicity and metabolism of vinyl chloride and related compounds. *Environ. Health Perspect.* 17:193–98
19. Malaveille, H., Bartsch, H., Barbin, A., Camus, R., Montesano, R. et al. 1975. Mutagenicity of vinyl chloride, chloroethylene oxide, chloro-acetaldehyde and chloro-ethanol. *Biochem. Biophys. Res. Commun* 63:363–70
20. Green, T., Hathaway, D. E. 1978. Interactions of vinyl chloride with rat-liver DNA in vivo. *Chem. Biol. Interact.* 22:211–24
21. Laib, R. J., Gwinner, L. M., Bolt, H. 1981. DNA alkylation by vinyl chloride metabolites. *Chem Biol. Interact.* 37: 219–31
22. Green, T., Hathway, D. E. 1975. Biological fate of vinyl chloride in relation to its oncogenicity. *Chem. Biol. Interact* 11:545–62
23. Watanabe, P. G., McGowan, G. R., Gehring, P. J. 1976. Fate of ^{14}C vinyl chloride after single oral administration

in rats. *Toxicol. Appl. Pharmacol.* 36:339–52
24. Watanabe, P. G., Gehring, P. J. 1976. Dose-dependent fate of vinyl chloride and its possible relationship to oncogenicity in rats. *Environ. Health Perspect.* 17:145–52
25. Jones, B. K., Hathway, D. E. 1978. The biological fate of vinylidene chloride in rats. *Chem. Biol. Interact.* 20:27–41
26. Daniel, J. W. 1963. The metabolism of ^{36}Cl-labelled trichloroethylene and tetrachloroethylene in the rat. *Biochem. Pharmacol.* 12:705–802
27. Henschler, D. 1977. Metabolism and mutagenicity of halogenated olefins—A comparison of structure and activity. *Environ. Health Perspect.* 21:61–64
28. Greim, H., Bonse, G., Radwan, Z., Reichert, D., Henschler, D. 1975. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* 24: 2013–17
29. Bonse, G., Urban, T., Reichert, D., Henschler, D. 1975. Chemical reactivity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. *Biochem. Pharmacol.* 24:1829–34
30. Jones, R. B., Mackrodt, W. C. 1982. Structure-mutagenicity relationships for chlorinated ethylenes: a model based on the stability of the metabolically derived epoxides. *Biochem. Pharmacol.* 31: 3710–13
31. Miller, R. E., Guengerich, F. P. 1982. Oxidation of trichloroethylene by rat liver microsomal cytochrome P450: Evidence for chloride migration in a transition state not involving trichloroethylene oxide. *Biochemistry* 21:1090–97
32. Gothe, R., Calleman, C. J., Ehrenberg, L., Wachtmeister, C. A. 1974. Trapping with 3,4-dichlorobenzenethiol of reactive metabolites formed in vitro from the carcinogen vinyl chloride. *Ambio* 3:234–36
33. Laib, R. J., Bolt, H. M. 1977. Alkylation of RNA by vinyl chloride metabolites in vitro and in vivo: formation of 1-N^6-etheno-adenosine. *Toxicology* 8: 185–95
34. Jones, B. K., Hathway, D. E. 1978. Tissue-mediated mutagenicity of vinylidene chloride in *Salmonella typhimurium* TA1535. *Cancer Lett.* 5:1–6
35. Bronzetti, G., Bauer, C., Corsi, C., Leponni, C., Nieri, R., del Carratore, R. 1981. Genetic activity of vinylidene chloride in yeast. *Mutat. Res.* 89:179–85
36. Oesch, F., Protic-Sabljic, M., Friedberg, T., Klimisch, H. J., Glatt, H. R. 1983. Vinylidene chloride: changes in drug-metabolizing enzymes, mutagenicity and relation to its targets for carcinogenesis. *Carcinogenesis* 4(8):1031–38
37. Bartsch, H., Malaveille, C., Montesano, R., Tomatis, L. 1975. Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. *Nature* 255:641–43
38. Baden, J. M., Kelley, M., Mazze, R. I., Simmons, V. F. 1979. Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane. *Br. J. Anaesthiol.* 51:417–21
39. Bartsch, H., Malaveille, C., Barbin, A., Planche, G. 1979. Mutagenic alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450-linked microsomal mono-oxygenases. *Arch. Toxicol.* 41:249–77
40. Bronzetti, G., Zeiger, E., Frezza, D. 1978. Genetic activity of trichloroethylene in yeast. *J. Environ. Pathol. Toxicol.* 1:411–18
41. Callen, D. F., Wolf, C. R., Philpot, R. M. 1980. Cytochrome-P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. *Mutat. Res.* 77:55–63
42. Duprat, P., Gradiski, D. 1980. Cytogenetic effect of trichloroethylene in the mouse as evaluated by the micronucleus test. *IRCS Med. Sci.* 8:182
43. Mennear, J. H. 1985. NTP Technical report on the toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) in F344/N rats and B6C3F$_1$ mice (Inhalation Studies). *DHSS—NIH Rep. 85-2657*
44. Bergmann, K. 1982. Reactions of vinyl chloride with RNA and DNA of various mouse tissues *in vivo*. *Arch. Toxicol.* 49:117–29
45. Reitz, R. H., Watanabe, P. G., McKenna, M. J., Quast, J. F., Gehring, P. J. 1980. Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: a comparative study with dimethylnitrosamine. *Toxicol. Appl. Pharmacol.* 52:357–70
46. Schumann, A. M., Quast, J. F., Watanabe, P. G. 1980. The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity. *Toxicol. Appl. Pharmacol.* 55:207–19

47. Stott, W. T., Quast, J. F., Watanabe, P. G. 1982. The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. *Toxicol. Appl. Pharmacol.* 62:137–51
48. Maltoni, C., Cotti, G., Chieco, P. 1984. Chronic toxicity and carcinogenicity bioassays of vinyl chloride. *Acta Oncol.* 5(2):91
49 National Cancer Inst. 1976. Carcinogenesis bioassay of trichloroethylene. Natl. Cancer Inst. Carcinogenesis Tech. Rep. Ser. 2. Bethesda: US DHEW Publ. No. (NIH) 76-802
50. National Toxicology Programme. 1982. Carcinogenesis bioassay of trichloroethylene. Bethesda: US Dept. Health Hum. Serv., NIH. No. 82-1799
51. National Cancer Inst. 1977. Bioassay of tetrachloroethylene for possible carcinogenicity. Tech. Rep. Ser. 13. Bethesda: US DHEW Publ. No. (NIH) 77-813 PB 272940
52. Henschler, D., Elasser, H. M., Ronen, W., Eder, E. 1984. Carcinogenicity study of trichloroethylene with and without epoxide stabilisers in mice. *J. Cancer Res. Clin. Oncol.* 104:149–56
53. Maltoni, C., Cotti, G. 1986. Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion. *Acta Onocol.* 1:11–26
54. Fukuda, K., Takemoto, K., Tswata, H. 1983. Inhalation carcinogenicity of trichloroethylene in mice and rats. *Ind. Health* 21:243–54
55. Reichert, D. W., Werner, H. W., Metzler, M., Henschler, D. 1979. Molecular mechanism of 1,1-dichloroethylene toxicity. Excreted metabolites reveal different pathways of reactive intermediates. *Arch. Toxicol.* 42:159–69
56. Okine, L. K., Goochee, J. M., Gram, T. E. 1985. Studies on the distribution and covalent binding of 1,1-dichloroethylene in the mouse. *Biochem. Pharmacol.* 34:4051–57
57 Short, R. D., Winston, J. M., Minor, J. L., Seifter, J., Lee, C. C. 1977. Effect of various treatments on toxicity of inhaled vinylidene chloride. *Environ. Health Perspect.* 21:125–29
58 Short, R. D., Winston, J. M., Minor, J. L., Hong, C. B., Seifter, J., Lee, C. 1977. Toxicity of vinylidene chloride in mice and rats and its alteration by various treatments. *J. Toxicol. Environ. Health* 3:913–21
59 Reichert, D., Werner, H. W., Henschler, D. 1978. Role of liver glutathione in 1,1-dichloroethylene metabolism and hepatotoxicity in intact rats and isolated perfused rat liver. *Arch. Toxicol.* 41(3): 169
60. Elcombe, C. R. 1985. Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: A biochemical human hazard assessment. *Arch. Toxicol. Suppl.* 8:6–17
61. Elcombe, C. R., Pratt, I. S., Green, T. 1982. The rate of trichloroacetic acid formation determines the species difference in hepatic peroxisome proliferation due to trichloroethylene. *Pharmacologist* 24:173. Abstr. 432
62. Goldsworthy, T. L., Popp, J. A. 1987. Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. *Toxicol. Appl. Pharmacol.* 88:225–33
63. Reddy, J. K., Lalwani, N. D. 1983. Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* 12:1–58
64. Reddy, J. K., Azarnoff, D. L., Hignite, C. E. 1980. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* 283: 397–98
65. Rao, M. S., Reddy, J. K. 198[illegible] roxisome proliferation and hep[illegible] carcinogenesis. *Carcinogenesis* 8:6[illegible]36
66. Herren-Freund, S. L., Pereira, M. A., Olson, G. 1987. The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid, in mouse liver. *Toxicol. Appl. Pharmacol.* 90:183–89
67. Monster, A., Regouin-Peeters, W., van Schijndel, A., van der Tuin, I. 1983. Biological monitoring of occupational exposure to tetrachloroethylene. *Scand. J. Work Environ. Health* 9:273–81
68. Dekant, W., Haug, R., Henschler, D. 1985. Absorption, elimination and metabolism of tetrachloroethylene. *Arch. Pharmacol.* 329(Suppl. R24)
69. Green, T., Prout, M. S. 1985. Species differences in response to trichloroethylene II. Biotransformation in rats and mice. *Toxicol. Appl. Pharmacol.* 79:401–11
70. Soucek, B., Vlachova, D 1960. Excretion of trichloroethylene metabolites in human urine. *Br. J. Ind. Med.* 17:60–64
71 Prout, M. S., Provan, W. M., Green, T. 1985 Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice *Toxicol. Appl. Pharmacol.* 79:389–400
72. Odum, J., Green, T., Foster, J. R., Hext, P. M 1988. The role of

trichloroacetic acid and peroxisome proliferation in the differences in carcinogenicity of perchloroethylene in the mouse and rat. *Toxicol. Appl. Pharmacol.* 92:103–12

73. Ikeda, M., Ohtsuji, H., Imamura, T., Komoike, Y. 1972. Urinary excretion of total trichloro-compounds, trichloroethanol and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. *Br. J. Ind. Med.* 29:328–33
74. Ohtsuki, T., Sato, K., Koizumi, A., Kumai, M., Ikeda, M. 1983. Limited capacity of humans to metabolize tetrachloroethylene. *Int. Arch. Occup. Environ. Health* 51:381–90
75. Green, T., Odum, J., Nash, J. A., Foster, J. R. 1990. Perchloroethylene induced rat kidney tumour: An investigation of the mechanisms involved and their relevance to man. *Toxicol. Appl. Pharmacol.* In press
76. Rampy, L. W., Quast, J. F., Leong, B. K. J., Gehring, P. J. 1970. Results of long-term inhalation studies on rats of 1,1,1-trichloroethane and tetrachloroethylene formulations. In *Proc. 1st Int. Congr. Toxicol.*, ed. G. L. Plaa, W. A. M. Duncan, p. 562. New York: Academic
77. Goldsworthy, T. L., Lyght, O., Martin, J. T., Popp, J. A. 1986. Chlorinated hydrocarbon nephrotoxicity and cell proliferation. *Pharmacologist* 28(3):479
78. Goldsworthy, T. L., Lyght, O., Burnett, V. L., Popp, J. A. 1988. Potential role of α-2u-globulin, protein droplet accumulation and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene and pentachloroethane. *Toxicol. Appl. Pharmacol.* 96:367–79
79. Green, T., Odum, J., Foster, J. R., Hext, P. M. 1986. Perchloroethylene induced hepatic peroxisome proliferation in mice and renal hyaline droplet formation in rats. *Toxicologist* 6(1):1262
80. Bruner, R. H. 1984. Pathological findings in laboratory animals exposed to hydrocarbon fuels of military interest. In *Renal Effects of Petroleum Hydrocarbons*, ed. M. A. Mehlman, G. P. Hemstreet, J. J. Thorpe, N. K. Weaver, pp. 133–40 Princeton, NJ: Princeton
81. Charbonneau, M., Strasser, J., Lock, E. A., Turner, M. J., Swenberg, J. A. 1988. 1,4,-Dichlorobenzene induced nephrotoxicity: Similarity with unleaded gasoline induced effects. In *Nephrotoxicity Extrapolation from in vitro to in vivo and from Animal to Man*, ed. P. A. Bach, E. A. Lock, ed. New York: Plenum
82. Strasser, J. R., Charbonneau, M., Borghoff, S. J., Turner, M. J., Swenberg, J. A. 1988. Renal protein droplet formation in male Fischer 344 rats after isophorone treatment. *Toxicologist* 8: 136
83. Kanerva, R. L., Ridder, G. M., Lefever, F. R., Alden, C. L. 1987. Comparison of short term renal effects due to oral administration of decalin or d-limonene in young adult male Fischer-344 rats. *Food Chem. Toxicol.* 25:345–53
84. Dekant, W., Metzler, M., Henschler, D. 1986. Identification of S-1,2,2-trichlorovinyl-N-acetylcysteine as a urinary metabolite of tetrachloroethylene: Bioactivation through glutathione conjugation as a possible explanation of its nephrocarcinogenicity. *J. Biochem. Toxicol.* 1:57–72
85. Dekant, W., Berthold, K., Vamvakas, S., Henschler, D., Anders, M. W. 1988. Thioacylating intermediates as metabolites of S-(1,2-dichlorovinyl)-L-cysteine and S-(1,2,2-trichlorovinyl)-L-cysteine formed by cysteine conjugate β-Lyase. *Chem. Res. Toxicol.* 1:175–78
86. Vamvakas, S., Elfarra, A. A., Dekant, W., Henschler, D., Anders, M. W. 1988. Mutagenicity of amino acid and glutathione S-conjugates in the Ames test. *Mutat. Res.* 206:83–90
87. National Toxicology Program. 1988. Toxicology and carcinogenesis studies of trichloroethylene in four strains of rats. (Gavage Studies). Bethesda: US Dept. Health Serv. (NIH) No. 79-01-6