ARMSTRONG LABORATORY

AL-TR-1992-0116

AD-A262 588

# STRUCTURE-ACTIVITY COMPARISON OF HYDRAZINE TO OTHER NASOTOXIC CHEMICALS


C. S. Godin
H. G. Wall

MANTECH ENVIRONMENTAL TECHNOLOGY, INC.
P.O. BOX 31009
DAYTON, OH 45431-0009




AUGUST 1992



FINAL REPORT FOR THE PERIOD AUGUST 1991 THROUGH OCTOBER 1991



AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

## NOTICES



## TECHNICAL REVIEW AND APPROVAL

AL-TR-1992-0116

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Lt Col, USAF, BSC
Deputy Director, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

| REPORT DOCUMENTATION PAGE | Form Approved OMB No. 0704-0188 |
|---|---|



**1. AGENCY USE ONLY (Leave Blank)**

**2. REPORT DATE**
August 1992

**3. REPORT TYPE AND DATES COVERED**
Final Report, August - October 1991

**4. TITLE AND SUBTITLE**
Structure-Activity Comparison of Hydrazine to Other Nasotoxic Chemicals

**5. FUNDING NUMBERS**
Contract F33615 90-C-0532
PE 62202F
PR 6302
TA 630214
WU 63021402

**6. AUTHOR(S)**
C.S. Godin, H.G. Wall

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45431-0009

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
AL/OET
Armstrong Laboratory
Wright-Patterson AFB, OH 45433-6573

**10. SPONSORING/MONITORING AGENCY REPORT NUMBER**
AL-TR-1992-0116

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION/AVAILABILITY STATEMENT**
Approved for public release; distribution is unlimited.

**12b. DISTRIBUTION CODE**

**13. ABSTRACT (Maximum 200 words)**

The biotransformation of 19 chemicals that have caused nasal epithelial toxicity in long-term carcinogenesis experiments in laboratory rodents was compared with the biotransformation of hydrazine, in order to determine if these chemicals share common metabolic pathways. Ten of the 19 chemicals were tumorigenic; four were epoxides or epoxide-formers; three were metabolized to reactive aldehydes; and one was metabolized to a lactone ring. The two remaining chemicals, *p*-cresidene and 2,6-xylidene, possess an amino group that can undergo biotransformation to reactive metabolites in a way similar to hydrazine, but there is no evidence to support this hypothesis. Therefore, none of the 19 chemicals are metabolized in a way similar to hydrazine.

**14. SUBJECT TERMS**
Structure-activity
Hydrazine
Nasotoxicity
Carcinogenicity

**15. NUMBER OF PAGES**

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| UNCLASSIFIED | UNCLASSIFIED | UNCLASSIFIED | UL |

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std Z39-18
298-102

# PREFACE

This document serves as a technical report describing the results of a literature search for information regarding the biotransformation of chemicals that were found to produce either nasal tumors or changes in nasal epithelial tissue when tested in 2-year bioassays by the National Toxicology Program (NTP). The research described herein began in August 1991 and was completed in October 1991. It was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F16). Lt Col James N. McDougal served as the Contract Technical Monitor for the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, located at Wright-Patterson Air Force Base, OH.

TABLE OF CONTENTS

## LIST OF FIGURES

## INTRODUCTION

Most of the classic organic carcinogens are classified as DNA-reactive, or genotoxic. This category includes chemicals that chemically react with DNA to form adducts. Biotransformation may not be required for the chemical to bind to DNA (activation-independent), but the vast majority of chemical carcinogens in the environment must first be activated by enzymes to reactive intermediates that can bind to DNA (activation-dependent). Activation-independent carcinogens usually produce tumors at the site of administration but activation-dependent carcinogens produce their effects in tissues where metabolic activation occurs.

A second category of carcinogens are those classified as epigenetic. These carcinogens include those that lack evidence of interaction with genetic material. The mechanisms involved in the production of tumors by these agents may involve chronic tissue injury and cell proliferation, generation of reactive species, immunosuppressive effects, hormonal imbalances, blocks in differentiation, breakage of gap junctions, or promotion of preexisting genetically altered cells. Although a single exposure to a low dose of a genotoxic carcinogen may be sufficient to produce tumors, epigenetic carcinogens require sustained exposure to high doses before any carcinogenic effects can be observed.

The active species involved in the interaction with DNA was identified by Miller and Miller (1981) as either an electrophile or radical cation. An electrophile is deficient in electrons and can form covalent bonds with tissue nucleophiles that have electrons to share. Electrophilic species include carbonium ions, nitrenium ions, free radicals, diazonium ions, epoxides, aziridinium ions, episulfonium ions, strained lactones, sulfonates, halo ethers, and enals (Williams and Weisburger, 1991). The effectiveness of such chemicals depends on the differences in the interaction between the chemical and DNA, reactions with other cellular nucleophiles, and competing enzymatic biotransformation reactions. In the case of most activation-dependent carcinogens, bioactivation is mediated by mixed function oxidases that are part of the cytochrome $P_{450}$ enzyme system of the endoplasmic reticulum. Such reactions result in the formation of intermediates that contain these reactive functional groups; however, in some cases subsequent conjugation reactions are required for activation.

The purpose of this report is to compare the biotransformation of several chemicals that have caused nasal epithelial toxicity in long-term carcinogenesis experiments in laboratory rodents, with the biotransformation of hydrazine (HZ), in order to determine if these chemicals share common metabolic pathways with HZ. The list of chemicals to be examined includes the following: 1,3-butadiene (BTD), 1,2-epoxybutane (EB), 1,2-propylene oxide (PO), naphthalene (NPT), vinyl

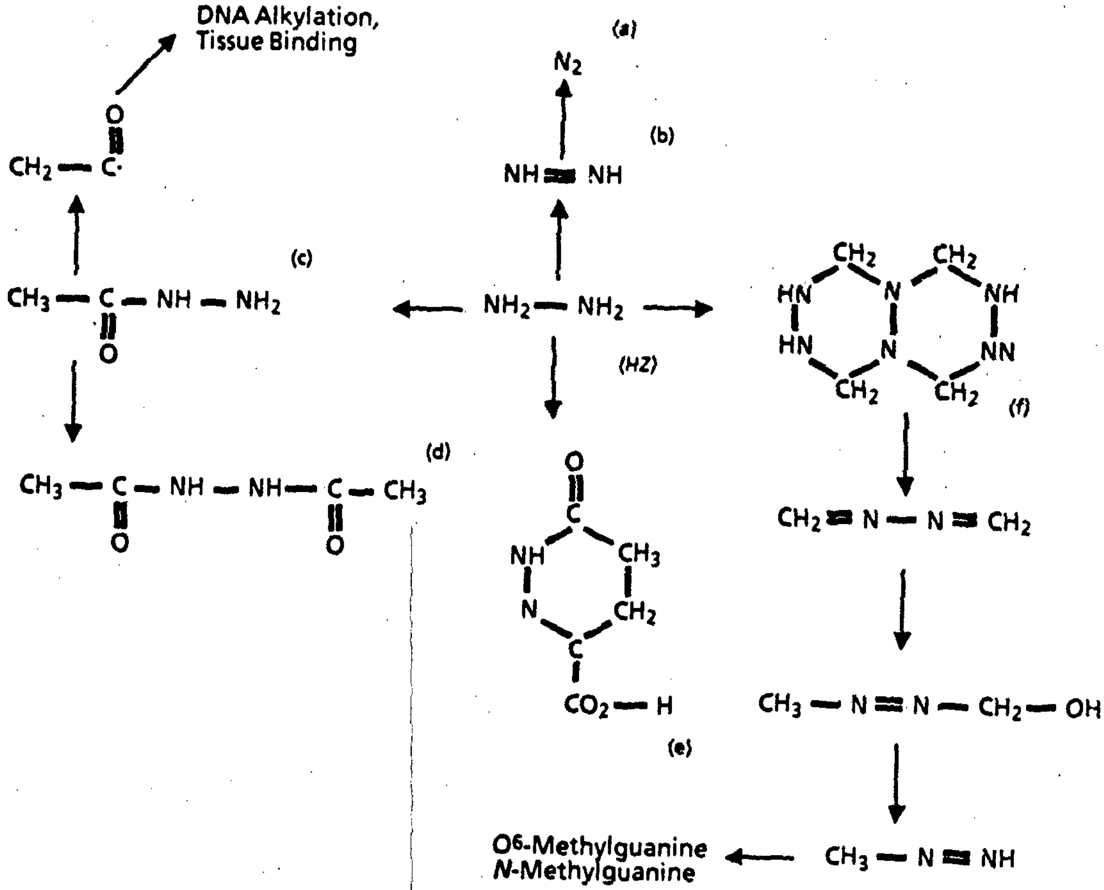
1,2-dibromoethane (DBE), 1,2-dibromo-3-chloropropane (DBCP), monochloroacetic acid (MCA), 2-chlorobenzalmalononitrile (CS), *p*-cresol and mixed cresol isomers, methyl methacrylate (MMA), 1,4-dioxane (DX), titanocene dichloride (TD), tetranitromethane (TNM), 2,6-xylidine (XL), and *p*-cresidine. These chemicals were obtained from the carcinogenicity data base of the National Toxicology Program (NTP). The biotransformation of HZ as it relates to its toxic effects wili be presented first.

## LITERATURE REVIEW

### Hydrazine

Hydrazine is widely used in industry and is used by the military as a rocket fuel. It has been shown to be the metabolite of isoniazid and hydralazine (Blair et al., 1984). Hydrazine is acutely toxic to the liver, kidney, and central nervous system (CNS), and is necrogenic at high doses (Shank, 1981). These effects are likely caused by the metabolism of HZ to reactive intermediates that can subsequently bind to tissue nucleophiles (Mitchell et al., 1976; Nelson et al., 1976), although HZ itself is a potent reducing agent and may cause toxicity directly. In a chronic inhalation study, squamous cell carcinomas, adenocarcinomas, and squamous cell papillomas of the nasal turbinates were detected in rats exposed to the highest concentration (5.0 ppm) (MacEwen et al., 1981; Vernot, 1985). Oral administration in rats has produced both hepatocellular carcinomas and pulmonary adenocarcinomas (Severi and Biancifiori, 1968; Biancifiori, 1970). The biochemical and molecular mechanism of HZ toxicity is still not clear, but is believed to result from bioactivation by cytochrome $P_{450}$.

The biotransformation of HZ is presented in Figure 1. Hydrazine is rapidly oxidized to nitrogen gas (a) and diimide (b) and is also acetylated to mono- (c) and diacetylhydrazine (d) (Dost et al., 1979; Nelson and Gordon, 1980; Springer et al., 1981). Recently Timbrell (1990) investigated the *in vitro* and *in vivo* metabolism of HZ. In this study, HZ was metabolized by rat liver microsomes, and this metabolism was inhibited by piperonyl butoxide and increased by phenobarbital. Interestingly, a second pathway believed to be responsible for the bioactivation of HZ was acetone-inducible, although further studies are required for confirmation. Shank et al. (1984) suggested that HZ and endogenous formaldehyde could form a hydrazone derivative (e) which could be oxidized to diazomethane, a known DNA alkylating agent. Although DNA alkylation could not be shown *in vivo*, *in vitro* studies clearly demonstrated that DNA alkylation by HZ required microsomal activation. Based on these studies, Shank (1987) proposed that the *in vivo* reaction of HZ and formaldehyde may be complex and that activation of this reaction product seems to require active aldehyde dehydrogenase. This activated reaction product, tetraformyltrisazine (f), has been shown to produce 4 to 5 times more 7-methylguanine and 10 times more $O^6$-methylguanine than HZ (Shank, 1987). Although alkylation of guanine is a possible mechanism to explain the toxic effects of HZ, other studies have shown that acetylated metabolites of HZ and HZ derivatives can undergo deacetylation (Sinha, 1987). This reaction results in the formation of carbon-centered radicals that have been implicated in liver necrosis (Mitchell et al., 1976; Nelson et al., 1976) and in the binding of hydralazine and procarbazine to microsomal proteins (Streeter and Timbrell, 1983, 1985).



DNA Alkylation,
Tissue Binding
(a)
$N_2$
(b)
NH = NH
(c)
$CH_3 - C(=O) - NH - NH_2$
$NH_2 - NH_2$
(HZ)
(d)
$CH_3 - C(=O) - NH - NH - C(=O) - CH_3$
(e)
(f)
$CH_2 = N - N = CH_2$
$CH_3 - N = N - CH_2 - OH$
$CH_3 - N = NH$
O6-Methylguanine
N-Methylguanine


Figure 1.

**1,3-Butadiene**

1,3-Butadiene is an industrial chemical that is used as an intermediate in chemical synthesis, or as a monomer in the synthesis of polymers. The pathway for the biotransformation of BTD is presented in Figure 2. Incubation of BTD with microsomal enzymes has resulted in the formation of butadiene oxide (a), (Malvoisin et al., 1979). In a subsequent report, Malvoisin and Roberfroid (1982) incubated butadiene oxide with rat liver microsomes and showed that it was further metabolized to diepoxybutane (b) and 3-butene-1,2-diol (c). The latter product is further biotransformed to 3,4-epoxy-1,2-butanediol (d). Butadiene oxide has also been shown to form glutathione conjugates (Malvoisin and Roberfroid, 1982). Butadiene oxide is the major *in vivo* metabolite, and has been demonstrated in the expired breath of animals exposed to BTD at concentrations between 6000 and 7000 ppm (Bolt et al., 1983).

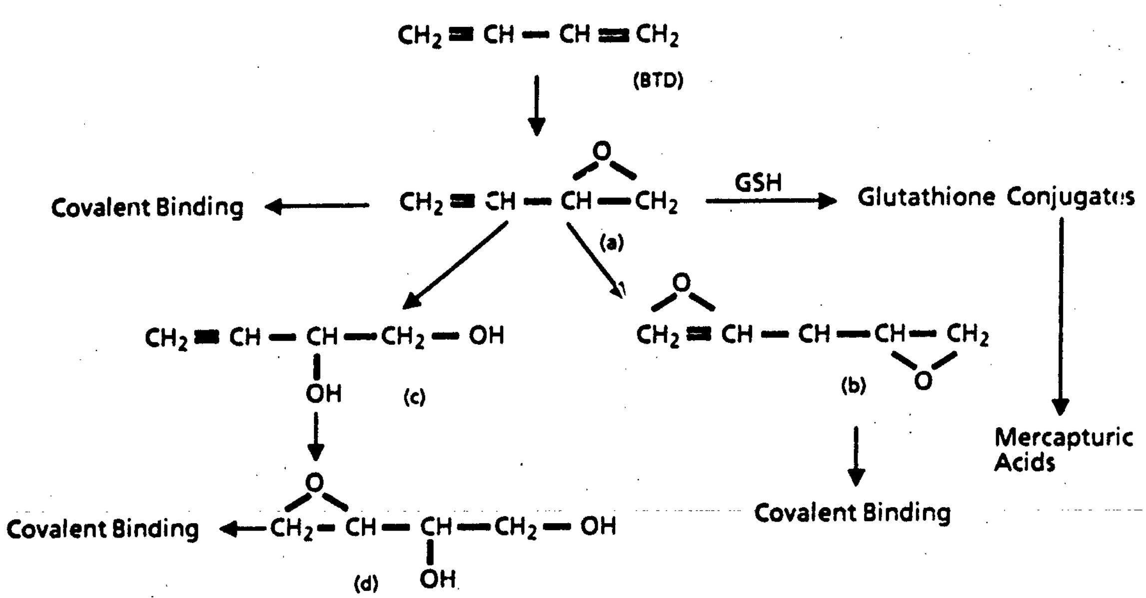



**Figure 2.**

Both butadiene oxide and diepoxybutane are mutagenic in the Ames test (deMeester et al., 1978) and carcinogenic in experimental animals (IARC, 1976). Intraperitoneal injection of diepoxybutane in mice resulted in a significant increase in lung tumors, whereas subcutaneous or intramuscular injection resulted in fibrosarcomas in rats and mice (IARC, 1976). Neither 3-butene-1,2-diol nor 3,4-epoxy-1,2-butanediol are mutagenic in the Ames test (Malvoisin and Roberfroid, 1982), which suggests that the epoxides are the reactive species involved in induction of carcinogenesis.

Recent long-term inhalation studies in rats (Loeser, 1982) and mice (NTP, 1984) have demonstrated that rats can survive higher BTD exposure levels than mice. The rats also demonstrated minimal toxicity and lung tumor formation when compared to mice, which suggested that rats may have less cytrochrome $P_{450}$ or higher levels of epoxide hydrolase and glutathione S-transferase than mice. Further species differences have been reported by Kreiling et al. (1986) that indicated that radiolabeled BTD was covalently bound to liver DNA and that the amount was the same in both species. However, the covalent binding to mouse liver nucleoproteins was twice as high as in rats. Schmidt and Loeser (1985) have further shown that butadiene oxide formation in mouse lung homogenates was 5 to 6 times higher than in rat lung preparations. In the case of both monkey and human lung homogenates, no butadiene oxide could be detected, which is consistent with the low cytochrome $P_{450}$ levels in the lungs of both species (Lorenz et al., 1979).

**1,2-Epoxybutane and 1,2-Propylene Oxide**

1,2-Epoxybutane is a short-chain epoxide closely related in structure to BTD. It is used as a stabilizer in chlorinated hydrocarbon solvents, as a gasoline additive, and for the preparation of butanediols, glycol ethers and esters, and in the preparation of surfactants. 1,2-Propylene oxide is shorter than EB by one carbon. It has been used as an intermediate in the synthesis of insecticides, repellants, and synthetic resins, and has also been used as a fumigant, principally for sterilizing packaged food products. Both compounds are highly reactive, electrophilic chemicals. The biotransformation of either EB or PO has not been described in the literature. Nevertheless, metabolic pathways have been proposed for both chemicals and are presented in Figure 3.

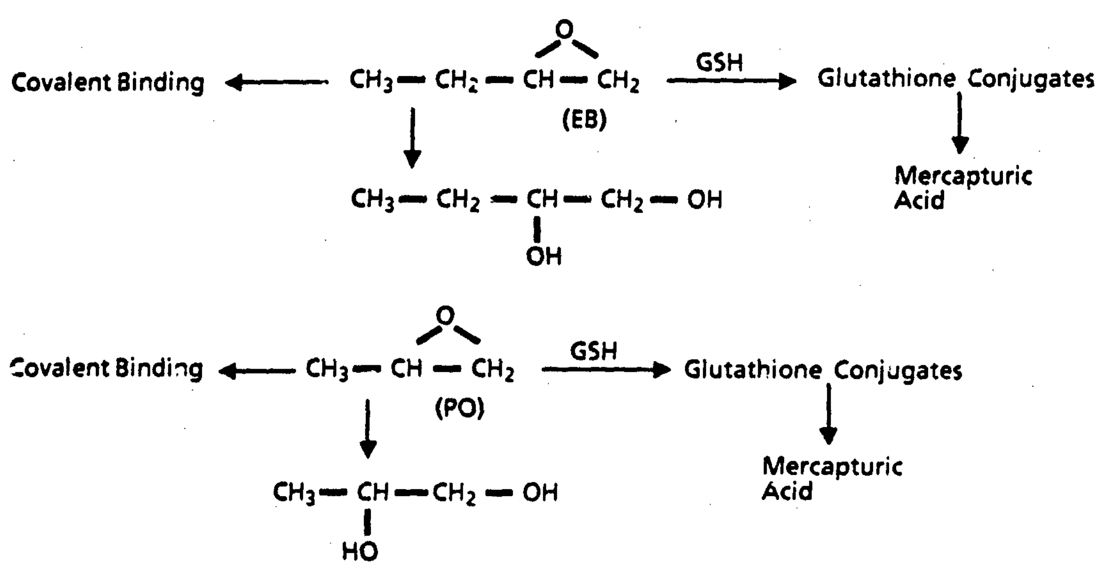



Figure 3.

Studies conducted by the NTP have shown that EB was mutagenic in the Ames test, induced mutations in mouse lymphoma cells, and induced both sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells (NTP, 1988). Subchronic inhalation studies with EB in both rats and mice resulted in inflammatory and degenerative lesions of the nasal cavity, neoplastic lesions of the nasal cavity and the lung in male rats, and neoplastic lesions of the nasal cavity of female mice (Dunnick et al., 1988).

1,2-Propylene oxide was mutagenic to bacteria, fungi, and insects; produced DNA damage and chromosomal aberrations in cultured mammalian cells; and produced micronuclei in bone marrow cells of mice (IARC, 1985). Chromosomal aberrations and micronuclei in lymphocytes have been observed in workers exposed to PO (Hogstedt et al., 1990) and DNA adducts of PO have been produced *in vitro* (Solomon and Segal, 1989). Nasal cavity tumors, but not lung tumors, have been observed in rats and mice following inhalation exposures to PO (NTP, 1985a). 1,3-Butadiene, a compound known to be biotransformed to reactive epoxide intermediates, apparently does not cause nasal tumors, but induces tumor formations at other sites such as the lung (NTP, 1984; Huff et al., 1985). It is possible that the difference in location of the tumors may be simply due to carbon chain length differences (Dunnick et al, 1988).

**Napthalene**

Naphthalene is a widespread environmental contaminant. Most of the NPT in industry has been used as a starting material in the synthesis of dyes and of carbaryl (an insecticide). Acute, high-dose exposures have resulted in necrosis of the nonciliated bronchiolar epithelial cells in mouse lung as well as proximal tubular cells of the mouse kidney (O'Brien et al., 1985). Napthalene was administered by inhalation in a 2-year bioassay and was shown to produce chronic inflammation of the nasal cavity, metaplasia of the olfactory epithelium, hyperplasia of the respiratory epithelium, and chronic inflammation of the lung (NTP, 1991a). This study also reported a 21% incidence of alveolar and bronchiolar adenomas at the highest exposure concentration (30 ppm). Stillwell et al. (1982) have indicated that NPT is metabolized to reactive, potentially toxic metabolites. The proposed pathway for NPT biotransformation is presented in Figure 4.

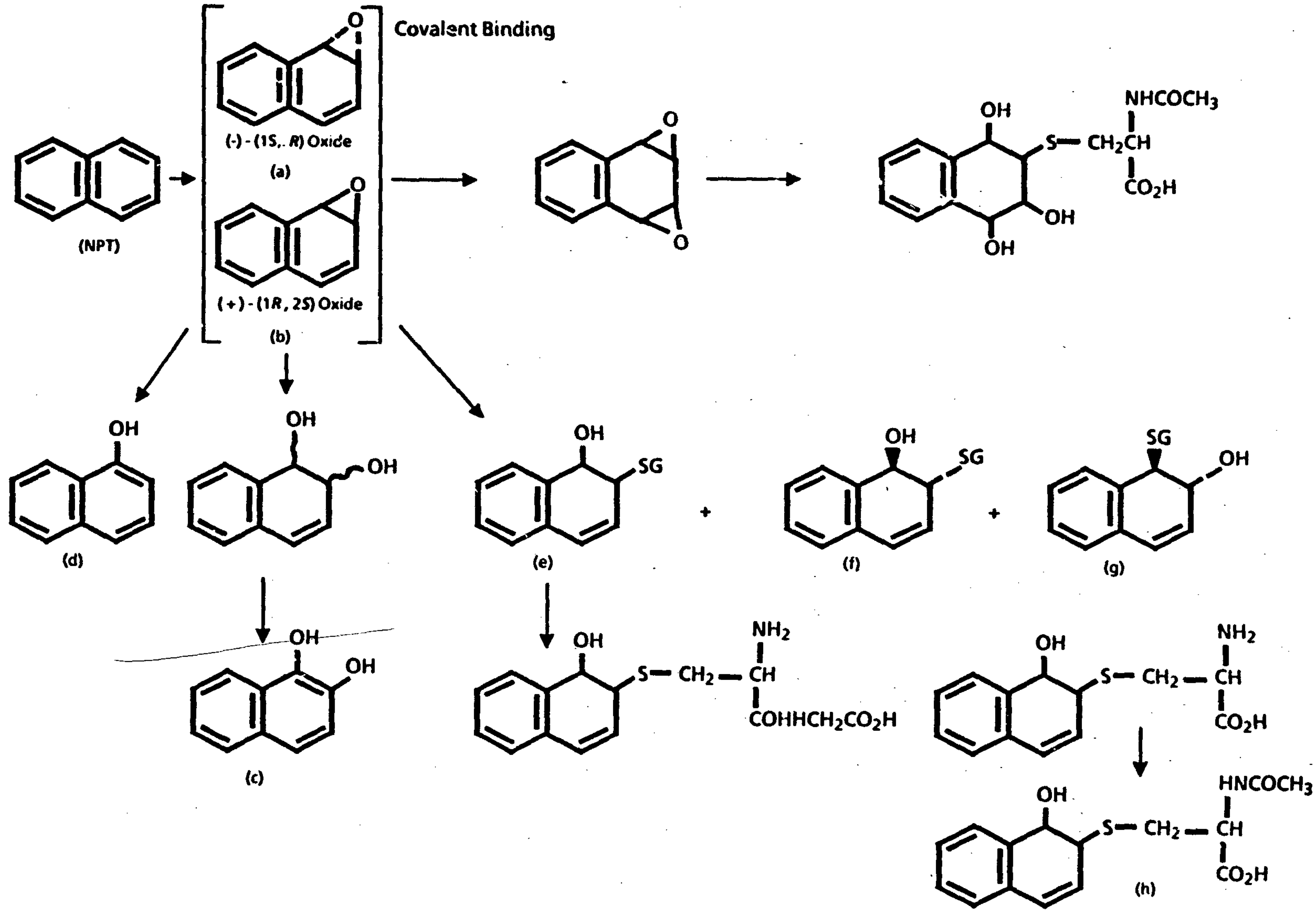

Covalent Binding
(NPT)
(-) - (1S,. R) Oxide
(a)
(+) - (1R, 2S) Oxide
(b)
OH
NHCOCH3
S— CH2CH
CO2H
(d)
(c)
(e)
SG
NH2
CH2
CH
COHHCH2CO2H
(f)
(g)
HNCOCH3
(h)

Figure 4.

*In vitro* studies by Buckpitt et al. (1987) have shown that a major portion of NPT oxides (a,b) generated in incubations with microsomal enzymes were either trapped as glutathione conjugates or were hydrated by the enzyme epoxide hydrolase to NPT dihydrodiol (c). Covalently bound metabolites and 1-naphthol (d) accounted for 2 and 12% of the metabolism of NPT, respectively. Incubation of NPT, glutathione, and glutathione S-transferase with pulmonary, hepatic, or renal microsomes from mouse, rat, or hamster tissues resulted in the formation of three enantiomeric glutathione conjugates (e,f,g) in all species, but substantial differences in the rates of formation of these conjugates were observed. In mouse lung preparations, the major target organ for acute toxicity in this species, the predominant NPT oxide formed was shown to be the enatiomer labeled "b" because of the glutathione conjugate that was isolated and identified (f). By contrast, no such stereoselectivity was observed in mouse liver or kidney preparations, although the rate of metabolism in kidney preparations was low.

Total rates of metabolism in the rat were lower than those in the mouse. In lung preparations, in contrast to the mouse, the other NPT oxide enantiomer (a) was formed in slight excess. This isomer was also apparently favored, in an approximate 4:1 ratio, in rat and hamster liver preparations. The data from the hamster study could not be relied upon because the dihydrodiol (c) was the major metabolite produced. The idea that the sterochemistry of epoxidation is related to tissue-selective injury resulting from NPT administration was supported by the data with mouse lung preparations. It is also possible that the toxicity of NPT is related to the inability of the mouse lung to biotransform the oxide to less reactive metabolites. Finally, the kidney toxicity observed after acute high-dose exposures may be due to the formation of mercapturic acids (h) from the glutathione conjugates. Mercapturic acids have been implicated in kidney toxicity and carcinogenesis (Monks and Lau, 1988).

**Vinyl Toluene**

Vinyl toluene is structurally similar to styrene and is important in the production of paints and plastics, and is also used as a solvent.

The first study that profiled the metabolism of this compound was conducted by Bergemalm-Rynell and Steen (1982), who analyzed the urine from rats that had been administered intraperitoneal (ip) injections of VT (See Figure 5). These authors concluded that the metabolism of VT was similar to that of styrene. One of the initial metabolic conversions was the formation of vinyl toluene-7,8-oxide (a); the similar pathway occurs in the case of styrene (el-Masri et al., 1958). Vinyl toluene-7,8-oxide has been shown to alkylate guanosine residues *in vitro* (Hemminki et al., 1981) and is mutagenic in bacteria and Chinese hamster V79 cells (Sugiura et al., 1978). Both VT and its epoxide metabolite induce sister chromatid exchanges in human lymphocytes (Norppa and Vainio, 1983). In spite of these findings, an *in vivo* exposure of rats to concentrations of VT ranging from 0 to 300 ppm did not produce any evidence of tumors. However, nonneoplastic lesions in the nasal cavity and metaplasia of the olfactory epithelium were observed (NTP, 1990a).

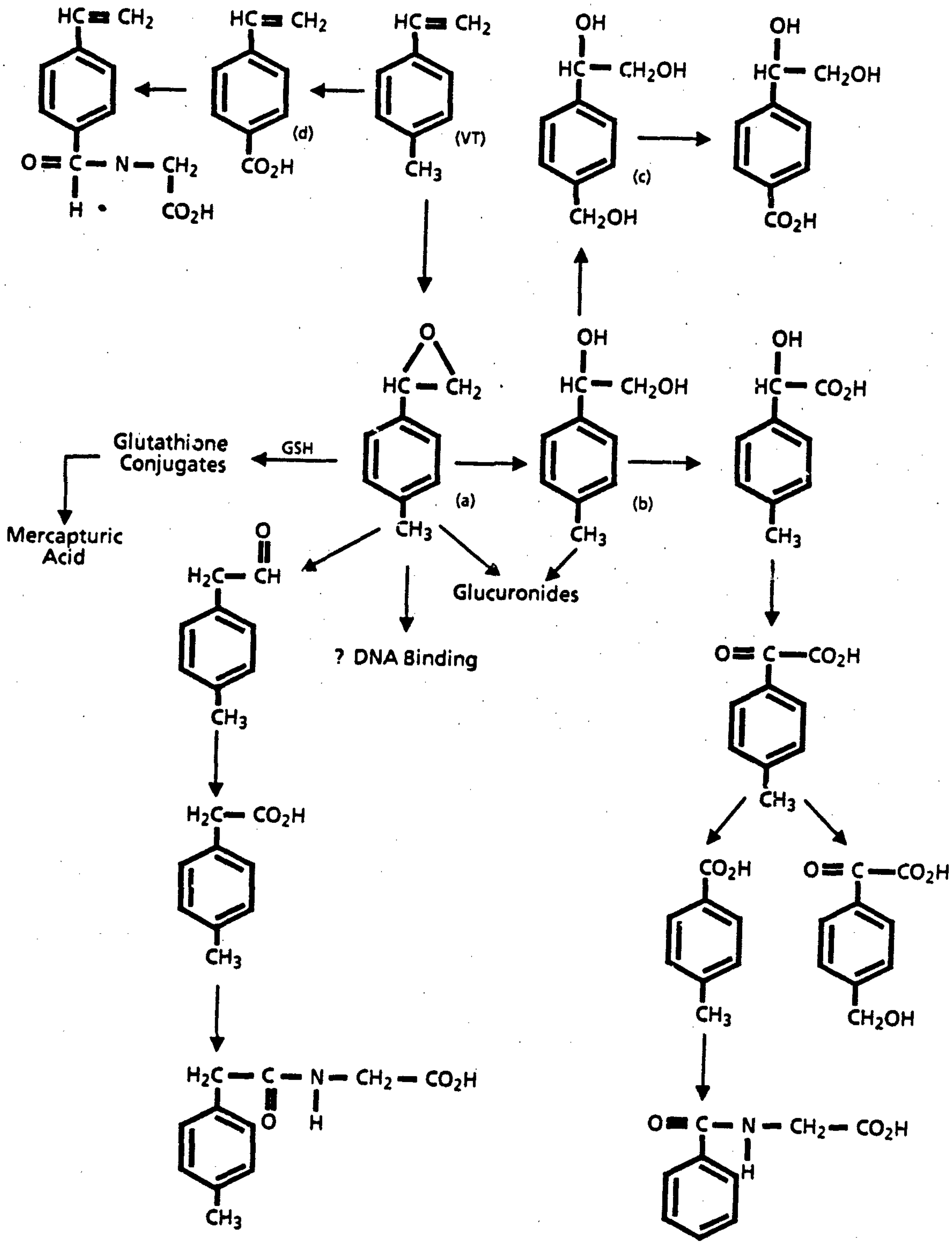

HC= CH2
O= C — N — CH2
H
CO2H
CO2H
(d)
(VT)
CH3
OH
HC — CH2OH
(c)
CH2OH
CO2H
O
HC — CH2
Glutathione
Conjugates
GSH
Mercapturic
Acid
(a)
(b)
H2C— CH
? DNA Binding
Glucuronides
HC — CO2H
O= C —CO2H
H2C— CO2H
H2C — C — N — CH2 — CO2H
O= C — N — CH2— CO2H

Figure 5.

In a subsequent article, Heinonen (1984) also characterized the metabolism of VT after injecting different doses of the material ip. This study indicated that the major detoxification pathway also results in the formation of vinyltoluene-7,8-oxide, followed by glutathione conjugation. The subsequent formation of mercapturic acids from glutathione conjugates was confirmed by Kuhler (1984). This study also indicated that the other main detoxification pathway occurs via hydration of the epoxide to diols (b,c), although these metabolites only accounted for 2.5% of the injected dose. The third metabolic route that was detected in this study was the oxidation of the methyl group of VT (d), which was also considered to be a minor route in the biotransformation of VT.

**Iodinated Glycerol**

Iodinated glycerol is used therapeutically as a mucolytic expectorant to clear bronchiolar secretions in humans and is a source of organically bound iodine. The manufactured product is described by the patent and chemical literature as a mixture of two isomeric iodopropylideneglycerols, but a study by Cannon et al. (1989) indicated that the two principal components of the product were 3-iodo-1,2-propanediol and glycerol. The two iodopropylideneglycerols were not observed.

Two-year toxicology and carcinogenesis studies were conducted by administering IDG orally to groups of rats and mice 5 days per week for 103 weeks at doses ranging from 0 to 250 mg/kg. Under these conditions, there was some evidence for carcinogenicity in male Fisher 344 (F-344) rats because adenomas of the nasal cavity were observed in two high dose male rats (NTP, 1990b). In NTP *Salmonella* studies, IDG induced a strong dose-related increase in the number of revertant colonies, but only in those *Salmonella* mutants containing base-substitutions. No increase in revertants was observed in the frame-shift mutant strains (Zeiger et al., 1987). Epstein et al. (1972) reported that 3-iodo-1,2-propanediol did not induce dominant lethal mutations, as measured by early fetal deaths and preimplantation losses in female mice mated to males dosed with 80 mg/kg.

Hoffnagle and Osol (1958) administered 2 mg radiolabeled IDG orally and intravenously to male Wistar rats. Within 2 h, 77% of the radiolabel was absorbed intact, with little or no decomposition of IDG in the gastrointestinal tract. Within 24 h after oral administration, approximately 30 to 60% of the labeled iodine was found in the thyroid gland, the remainder was excreted in the urine and feces. Barrigon et al., (1986) administered radiolabeled IDG (284 mg) orally to human volunteers and the principal urinary product was IDG (94.8% of the dose within 48 h). 3-Iodo-1,2-propanediol, the principal ingredient in IDG, was metabolized after ip administration of 100 mg/kg to male rats or 200 mg/kg to male mice (See Figure 6). In this study, two metabolites were found in the urine: *S*-(2,3-dihydroxypropyl)cysteine (a) and *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine (b) (Jones, 1975). It has been proposed that the metabolism of 3-iodo-1,2-propanediol occurs through the release of iodide and the subsequent formation of the epoxide intermediate glycidol (2,3-epoxypropanol) (c), and subsequent conjugation with gluatathione. The release of radiolabeled carbon dioxide ($CO_2$) through glycerol formation, presumably from formation of glycidol, and subsequent metabolism supports this hypothesis (Jones, 1975). Interestingly, mutagenicity studies conducted by the NTP on glycidol show it to be positive for induction of gene mutations in *Salmonella* (Canter et al., 1986), positive for induction of trifluorothymidine resistance in mouse L5178Y/TK cells (NTP, 1990c), and positive for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells (NTP, 1990c).

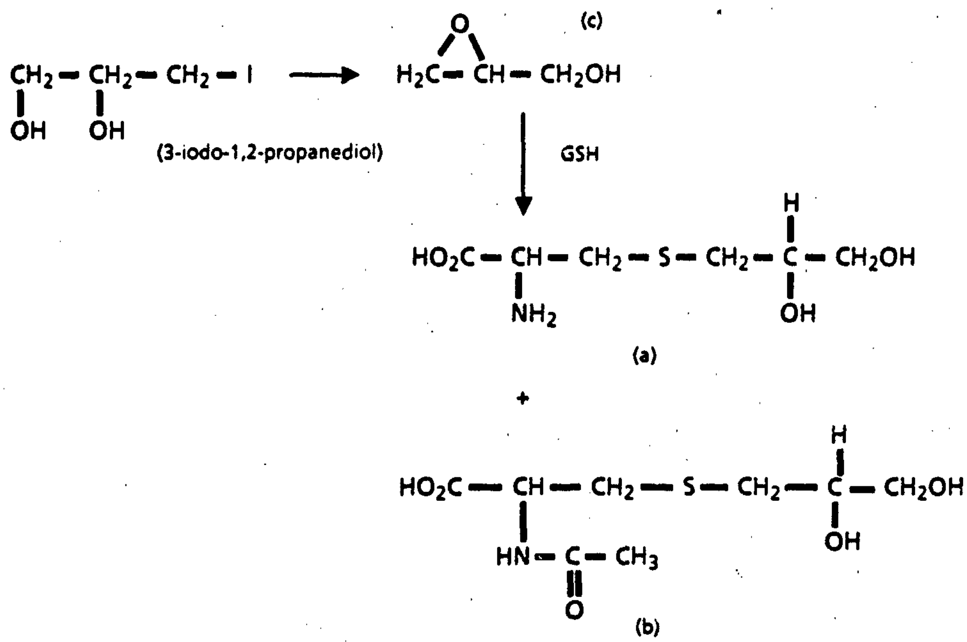



**Figure 6.**

**Allyl Glycidyl Ether**

Allyl glycidyl ether is used as a stabilizer of chlorinated compounds, vinyl resins, and rubber. It is also used as an intermediate in the synthesis of rubber. Of all the glycidyl ethers, AGE is one of the most toxic. The $LD_{50}$ in the rat and mouse are 1.6 and 0.39 g/kg, respectively, whereas the $LC_{50}$ in the rat has been reported to be 3120 mg/m$^3$ (Hine et al., 1956). Hine et al. (1956) reported that AGE produced labored breathing and CNS depression during acute oral exposures and it has been reported to produce moderate-to-severe skin and eye irritation. During repeated exposure, these authors also reported severe toxic effects at concentrations of 600 and 900 ppm. These effects included bronchopneumonic consolidation, severe emphysema, bronchiectasis, and inflammation. In more recent studies, rats and mice were exposed to AGE at concentrations ranging up to 10 ppm for 2 years (NTP, 1990d). Under these conditions, there was equivocal evidence of carcinogenic activity based on the presence of one papillary adenoma of respiratory epithelial origin, one squamous cell carcinoma of respiratory epithelial origin, and one poorly differentiated adenocarcinoma of olfactory epithelial origin, all occurring in the nasal passages of males exposed to 10 ppm. There was no evidence of carcinogenicity in female rats. Some evidence of carcinogenic activity was reported in male mice based on the presence of three adenomas of the respiratory epithelium, dysplasia in four males, and focal basal cell hyperplasia in the respiratory epithelium in seven males exposed to 10 ppm. There was no evidence of carcinogenicity in female mice. Allyl glycidyl ether was mutagenic in *Salmonella* both with and without activation (NTP, 1990d). Sister chromatid exchange, chromosomal aberrations, and sex-linked recessive mutations in *Drosophila* were also observed in this study.

Little is known about specific pathways for biotransformation of AGE but it seems reasonable to assume that it is metabolized in a way similar to other epoxides. The proposed metabolic pathway is presented in Figure 7. Allyl glycidyl ether can undergo hydrolysis of the epoxide group to form the corresponding diol (a) and also can form glutathione conjugates. Both the diol and the parent can undergo a subsequent epoxidation reaction to yield the epoxydiol (b) and the diepoxide (c), respectively. The epoxide rings are highly reactive and can undergo SN1-type reactions with proteins and DNA.

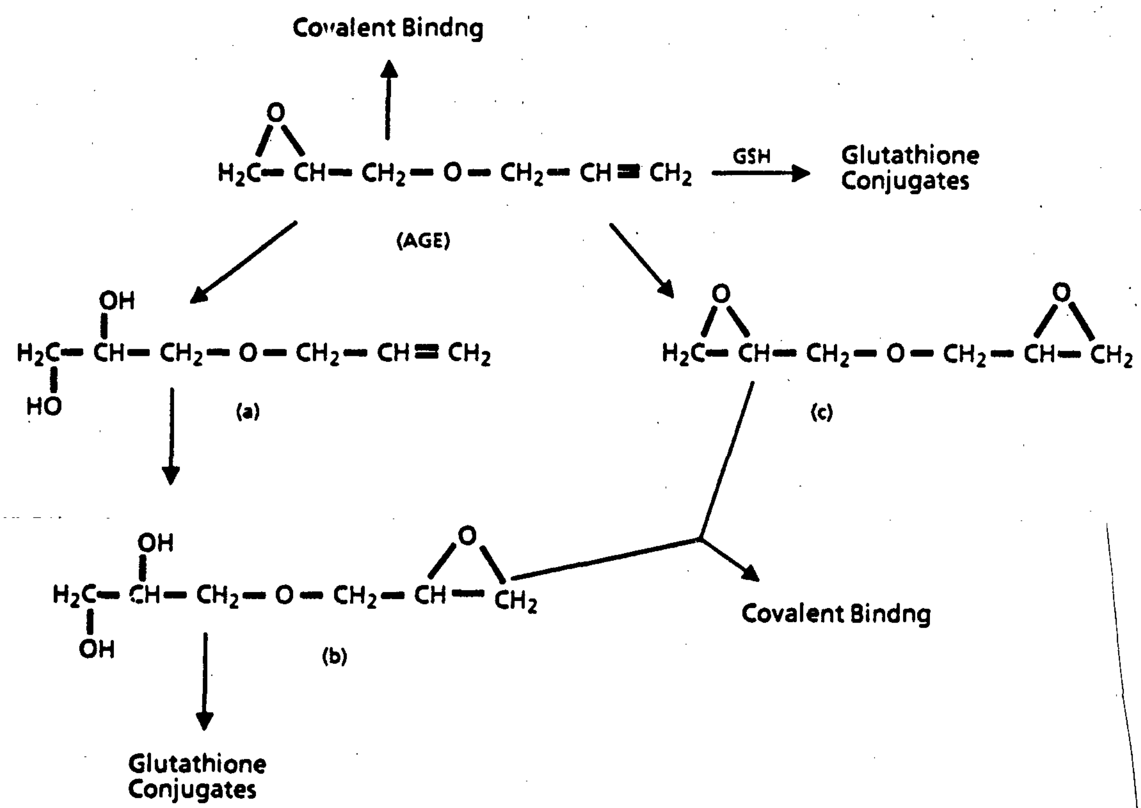



Figure 7.

**Dimethyl Vinylchloride**

Dimethyl vinylchloride is a structural analog of vinyl chloride, a documented human carcinogen (Maltoni and Selikoff, 1988). In a 2-year bioassay, DMVC was found to increase the incidence of neoplasms of the nasal cavity, oral cavity, esophagus, and forestomach of F-344 rats (NTP, 1985b). The metabolism (Figure 8) and disposition of DMVC revealed that mice and rats exhaled 25% of a dose of radiolabeled DMVC as $CO_2$ in the 24 h following administration, whereas 30% and 5% of the dose were exhaled unchanged by rats and mice, respectively. The 24-h urine of rats and mice contained 35 and 47% of the administered dose, respectively. The major urinary metabolites were cysteine and *N*-acetylcysteine conjugates (Ghanayem and Burka, 1987). The cysteine conjugate (a) was the major urinary metabolite and accounted for 23% (rat) or 35% (mouse) of the total urinary metabolites. The mercapturic acid (b) accounted for 9% (rats) and 12% (mice) of the total urinary metabolites. The nature of these metabolites indicated that DMVC was metabolized differently from vinyl chloride and other polyhalogenated ethylenes that undergo epoxidation (Henschler, 1985). Because of the presence of the methyl groups in DMVC, oxidation results in the production of E- and Z-alcohols (c,d) in a ratio of 2:1, showing that the reaction is stereoselective. This is followed by formation of an aldehyde (e,f), followed by conjugation with sulfur-containing nucleophiles and subsequent oxidation of the aldehyde functional group to a carboxylic acid (a,b).

As indicated above, approximately 30% of the administered dose of DMVC was exhaled unchanged by rats, as compared to 5% by mice. This observation may explain the presence of tumors in the nasal and oral cavity of rats but not in those of mice (NTP, 1985b). Although the mechanism is unclear, if metabolic activation is required for the initiation of these lesions, then it probably occurs in the nasal cavity. If the reactive species were generated elsewhere and eliminated via the lungs, one would not expect to see lung lesions. In the 2-year bioassay (NTP, 1985b), only 2 to 4% of nonneoplastic lesions, and no neoplastic lesions, were observed in the lungs of either mice or rats. In a subsequent study, Srinivas and Burka (1988) concluded that the reactivity of the haloenoic aldehydes makes them likely candidates for the reactive species responsible for the toxicity and carcinogenicity of DMVC.

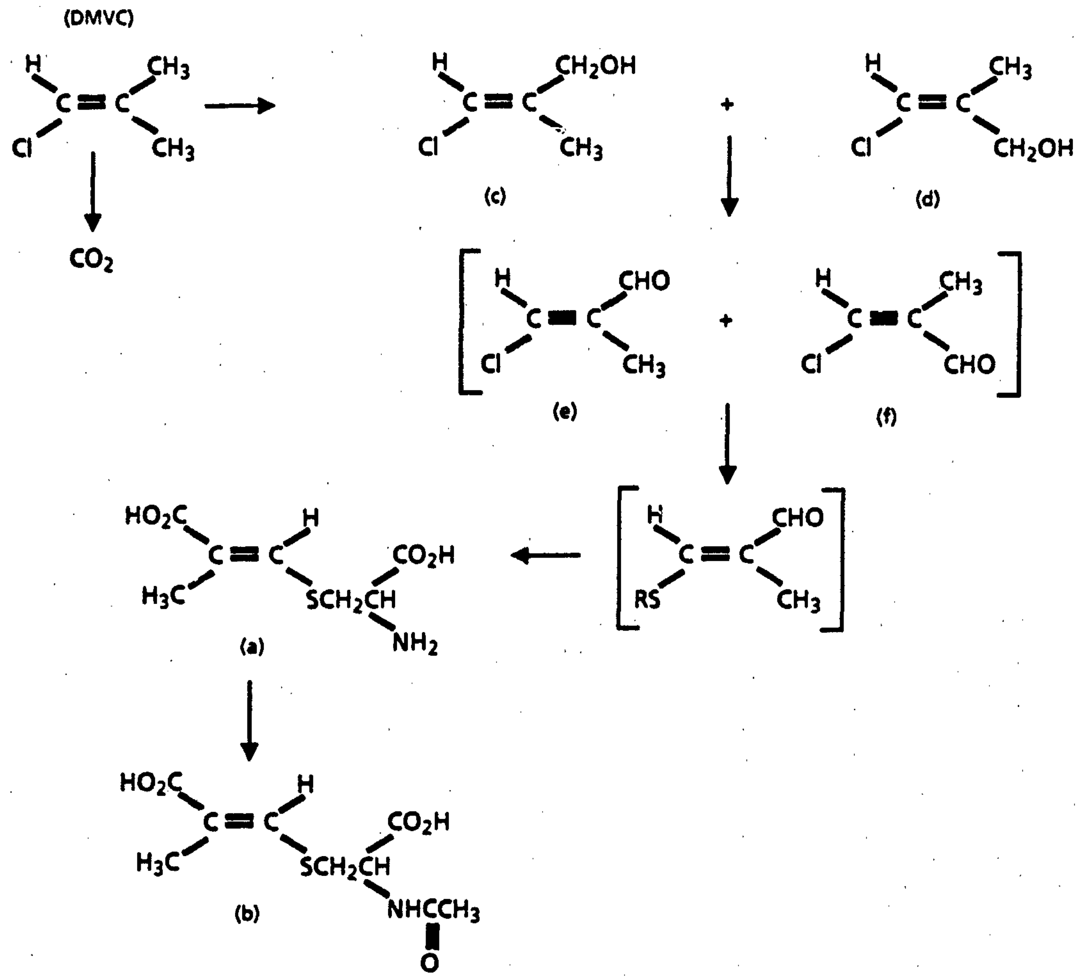


Figure 8.

**1,2-Dibromoethane**

1,2-Dibromoethane has been widely used as a grain fumigant, an industrial solvent, a gasoline additive, and an intermediate in the synthesis of dyes and pharmaceuticals.

This chemical has been shown to be mutagenic to bacteria, fungi, *Drosophila*, and mammalian cells, and carcinogenicity in rodents has been established (IARC, 1977; Fishbein, 1979). 1,2-Dibromoethane damaged spermatogenic cells when given ip to rats or orally to bulls (Edwards et al., 1970; Amir, 1973). Egg production by hens was reduced when they were administered DBE in the diet (Bondi et al., 1955). Massive hepatic centrilobular necrosis and proximal tubular epithelial damage in kidney was observed in humans following ingestion of a lethal dose of DBE (Olmstead, 1960). In an inhalation study, rats and mice were exposed to either 10 or 40 ppm DBE, and adenocarcinomas and carcinomas of the nasal cavity were observed in both male and female rats. In mice, the incidence of these lesions was greatly reduced (NTP, 1982a), but the incidence of alveolar and bronchiolar adenomas and carcinomas was greater than in the rat

1,2-Dibromoethane appears to require metabolic activation to elicit its mutagenic and carcinogenic properties. Two paths can be considered for bioactivation (Figure 9). Cytochrome $P_{450}$ can produce a gem-halohydrin, which can debrominate to form 2-bromoacetaldehyde (a) (Hill et al., 1978). 2-Bromoacetaldehyde has been shown to bind to protein irreversibly (Shih and Hill, 1981) and can react with glutathione, protein thiols, or DNA (Guengerich et al, 1981). In the second possible pathway, DBE can directly react with glutathione, undergoing an internal dehydrobromination to form ethyl GSH episulfonium ion (b), which can easily react with DNA (Ozawa and Guengerich, 1983), although this has not been proven *in vivo*. Conjugation with glutathione most likely results in the mutagenic activity (Van Bladeren et al., 1981) and the DNA binding (Sundheimer et al., 1982). MacFarland et al. (1984) concluded that glutathione-dependent metabolism may occur with those tissues associated with the *in vivo* toxicity of DBE.

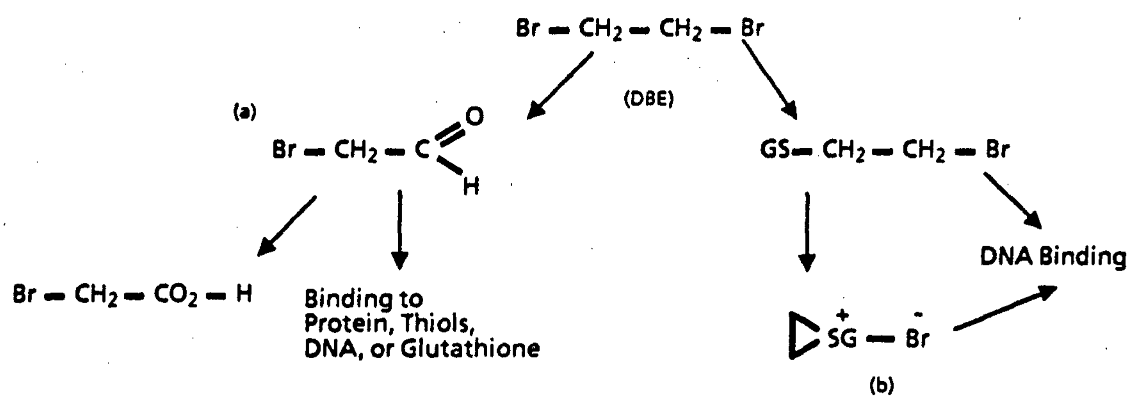



**Figure 9.**

**1,2-Dibromo-3-chloropropane**

1,2-Dibromo-3-chloropropane is used as a fumigant because of its nematocidal activity. It is both mutagenic and carcinogenic, as well as toxic to kidneys and gonads (Blum and Ames, 1977; NCI, 1980; NTP, 1982b; Kluwe et al., 1983). It has been proposed that the toxicity of DBCP is mediated by electrophilic products of metabolic activation that bind to tissue macromolecules (Kato et al., 1980; Jones et al., 1979).

The metabolism of DBCP is presented in Figure 10. A known metabolite of DBCP is 2-bromoacrylic acid (a), which can arise during oxidation of DBCP to 2-bromoacrolein (b) (Marsden and Casida, 1980). The aldehyde can then be further oxidized to the acid. 2-Bromoacrolein is a known mutagen (Segall et al., 1985). 1,2-Dibro-3-chloropropane is metabolized to 2-bromoacrolein *in vitro* and mechanistic studies have indicated that the initial oxidative dehalogenation at C-1 followed by β-elimination of bromide was the preferred pathway of formation (Omichinski et al., 1988). Inhibitors of cytochrome $P_{450}$, addition of glutathione, or incubation of DBCP with microsomes under anaerobic conditions blocked its mutagenicity. Therefore, the formation of an episulfonium ion does not appear to be involved in the toxic mechanism of DBCP, in contrast to the metabolic pathway described for DBE (Omichinksi et al., 1988). Although glutathione is not involved in the bioactivation of DBCP, MacFarland et al. (1984) have shown that glutathione-dependent debromination of DBCP occurs in cytosolic fractions prepared from a number of tissues obtained from Sprague-Dawley rats and Swiss-Webster mice.

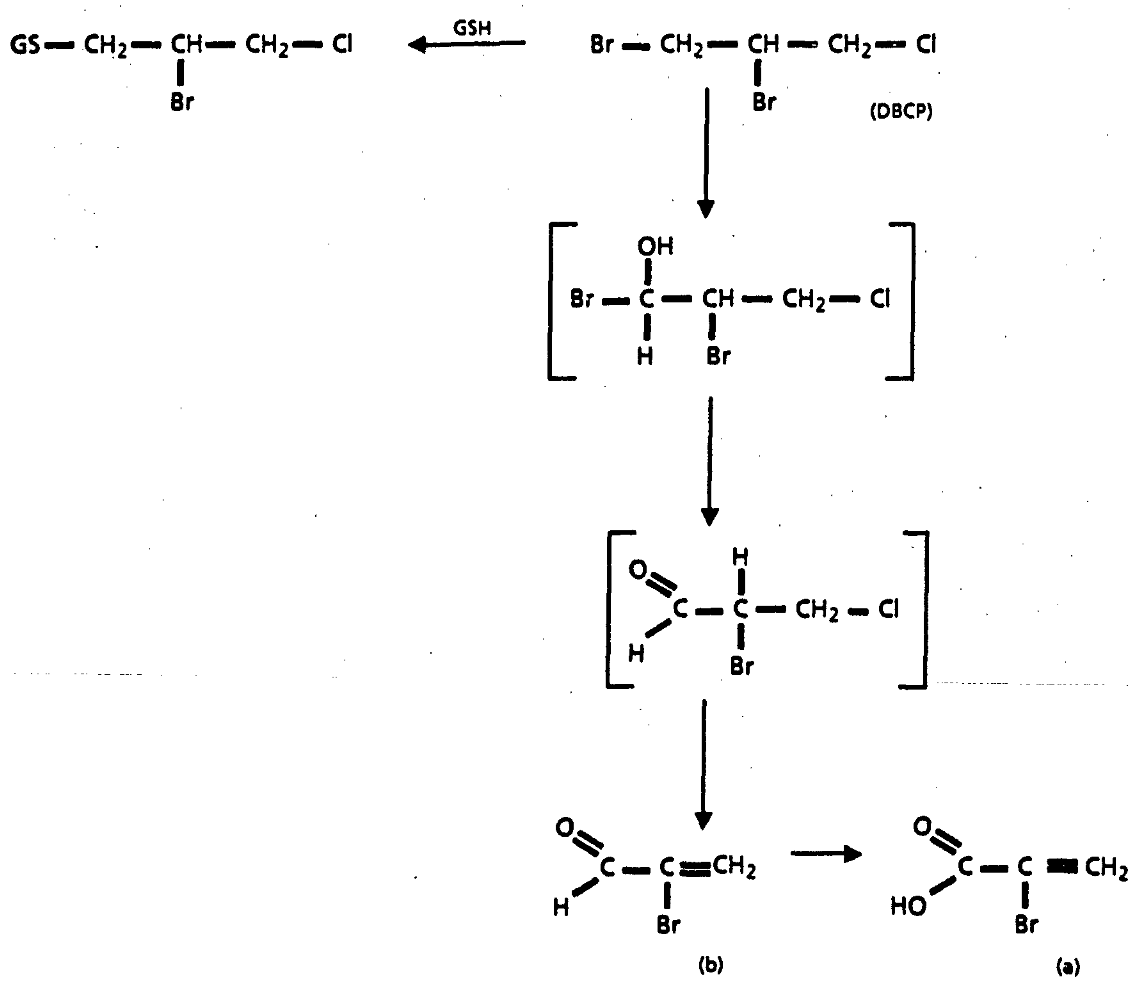



Figure 10.

**Monochloroacetic Acid**

Monochloroacetic acid is the monochlorinated analog of acetic acid used as a post-emergence contact herbicide, and as an intermediate in the synthesis of other organic compounds. Monochloroacetic acid is a strong irritant to the skin, eyes, and mucuous membranes of humans (Sax, 1984), and is more acutely toxic to rats and mice than are acetic acid, dichloroacetic acid, or trichloroacetic acid. Signs of toxicity in male Sprague-Dawley rats have included convulsions and respiratory depression. No exposure-related gross or microscopic lesions were observed in male rats fed diets that contained up to 0.1% MCA for 30 weeks, and there was no evidence of carcinogenic activity in either male or female rats in a 2-year bioassay (NTP, 1992a). In the same 2-year bioassay, male and female mice showed evidence of inflammation of nasal mucosa. Metaplasia of olfactory epithelium was also found in female mice.

It has been suggested that the mechanism of MCA toxicity involves the inhibition of sulfhydryl groups (Dickens, 1933; Chaiken and Smith, 1969; Hayes et al., 1973) and inhibition of acetate oxidation by uncompetitive inhibition (Hayes et al., 1972, 1973). A reduction of sulfhydryl concentration has been shown in rat liver and kidney *in vivo*, but MCA does not alkylate *in vitro* cysteine sulfhydryl groups, suggesting that MCA requires bioactivation for production of sulfhydryl-alkylating metabolites (Hayes et al., 1973).

The biotransformation pathway for MCA is presented in Figure 11. Yllner (1971) reported that 3 days following ip injection of 2 mg of radiolabeled MCA in mice, 82 to 88% of the dose was eliminated in the urine, 8% was eliminated in the expired air as $CO_2$, and less than 3% was eliminated in the feces; 2 to 3% of the administered dose remained in the animal. Of the radiolabel recovered in the urine, 6 to 22% was present as the parent compound. Metabolites of MCA identified in the urine included *S*-carboxymethylcysteine (a) (33 to 43% free and 1 to 6% conjugated), thiodiglycolic acid (b) (33 to 42%), glycolic acid (c) (3 to 5%), and oxalic acid (d) (0.1 to 0.2%). In separate experiments conducted in mice, thiodiglycolic acid was found to be the major urinary metabolite of *S*-carboxymethylcysteine, and glycolic acid was largely oxidized to $CO_2$. In Wistar rats given 50 mg/kg MCA by gavage, thiodiglycolic acid was identified as the major urinary metabolite, accounting for 60% of the administered dose (Green and Hathway, 1975). A greater percentage of administered MCA was excreted as thiodiglycolic acid in rats than in mice, most of the remainder of the dose was excreted as *S*-carboxymethylcysteine (Jones and Hathway, 1978).

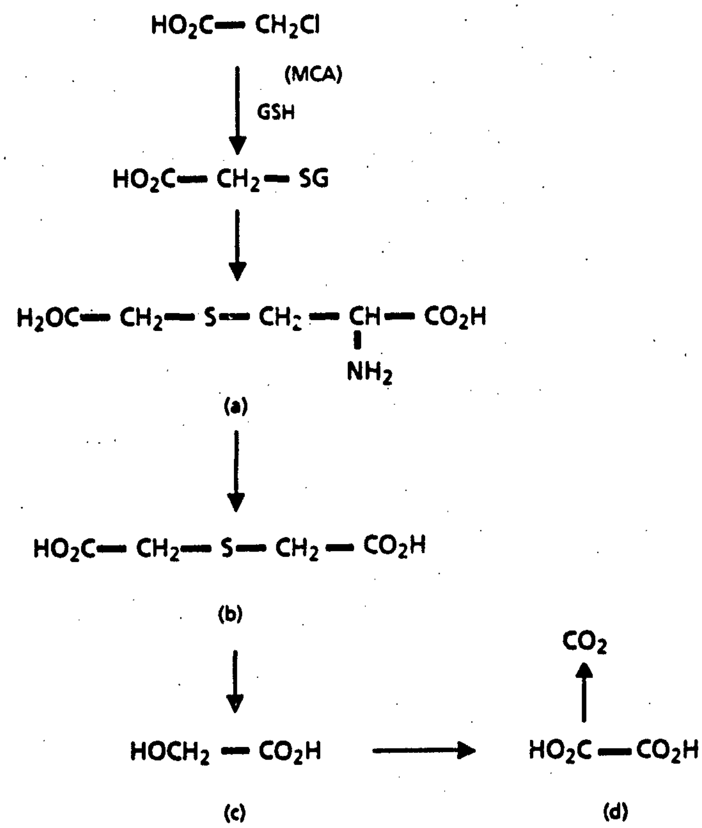



Figure 11.

**2-Chlorobenzalmalononitrile**

2-Chlorobenzalmalononitrile is a short-acting sensory irritant. Typical symptoms of exposure to aerosols of CS include eye irritation, excess lachrimation, burning sensation of the nose and throat, sneezing and coughing, and burning sensation of exposed skin (Ballantyne, 1977). Because of these effects, CS has been used for military purposes and for the control of civil disturbances.

2-Chlorobenzalmalononitrile is not teratogenic in either rats or rabbits (Upshall, 1973). The carcinogenic potential of CS has been assessed (McNamara et al., 1973; Marrs et al., 1983), but these studies did not use concentrations that were high enough and the exposure period was not long enough. Rats exposed to CS at concentrations of 21,000 $mg/m^3$ showed marked congestion of alveolar capillaries, intrapulmonary hemmorhage, and excessive broncheolar and bronchial secretions. In a more recent study, rats and mice were exposed to concentrations of CS ranging from 0.075 to 1.5 $mg/m^3$ for two years. The results of that study indicated that CS was not carcinogenic (NTP, 1991g). The only findings were inflammation of the nasal mucosa and squamous metaplasia of the olfactory epithelium. 2-Chlorobenzalmalononitrile does not bind to DNA (von Daeniken et al., 1981) and bacterial mutagenicity studies have generally been negative (Rietveld et al., 1983; Wild et al., 1983), although positive results have been reported in mouse lymphoma tests and sister chromatid exchange assays (MacGregor et al., 1988; NTP, 1990g).

The metabolic pathway is outlined in Figure 12. 2-Chlorobenzalmalononitrile was administered to rats by both intravenous (iv) and oral routes and the greatest proportion of the dose was eliminated in the urine (Brewster et al., 1987). 2-Chlorohippuric acid (b) was produced in the greatest abundance and together with its precursor, 2-chlorobenzoic acid (c), accounted for 72% of the urinary metabolites. A minor pathway was found to occur via reduction of the olefinic side chain of CS to yield 2-chlorobenzylmalononitrile (a) followed by hydrolysis via the amide to yield 2-chloro-2-cyanoproprionate (d). The finding of squamous metaplasia in the nasal cavities of rats and mice may be due to irritation effects, but the exact mechanism responsible for this effect is not understood.

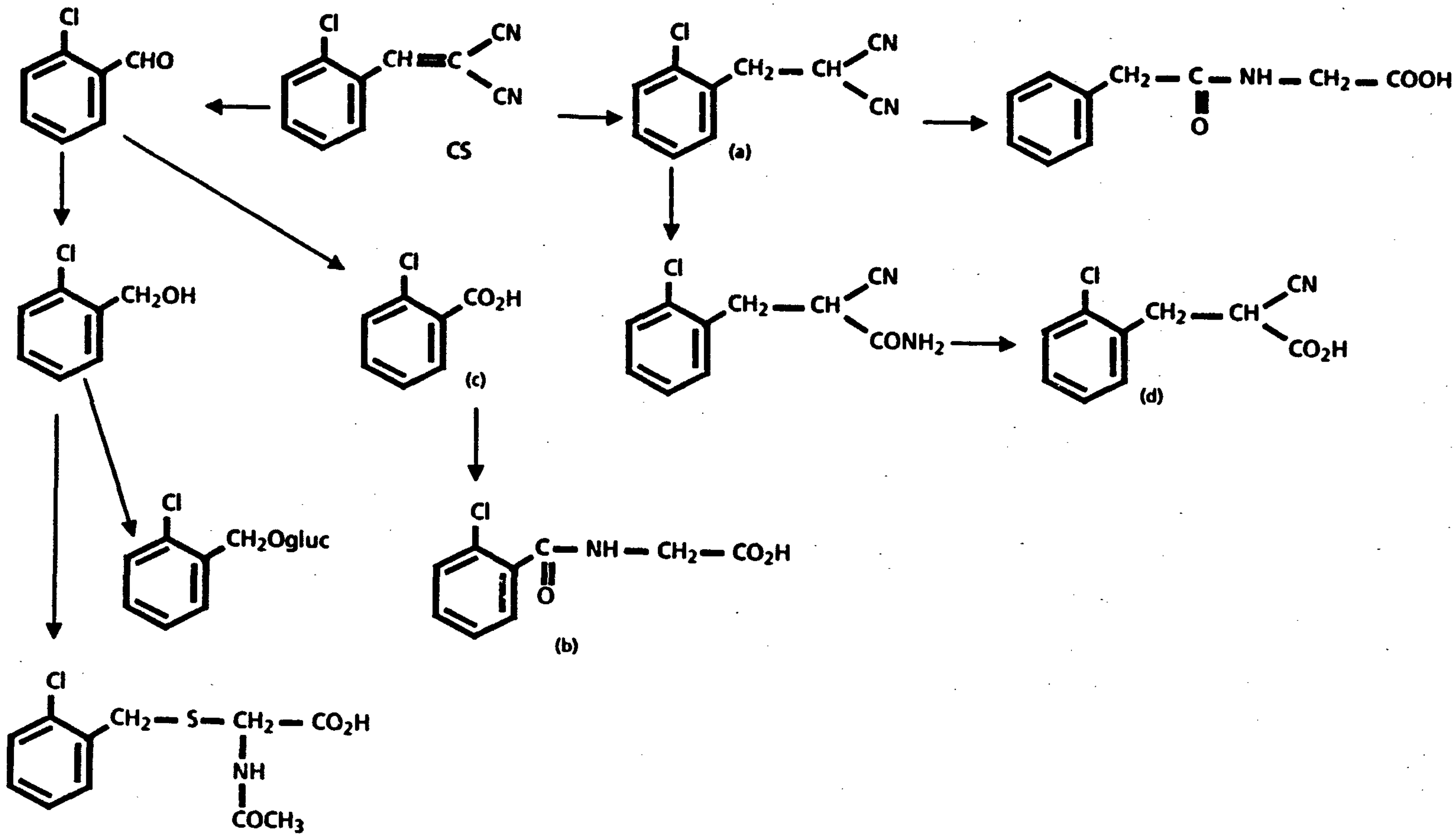

Cl
CHO
CH
C
CN
CN
CS
CH2
CH
(a)
C
NH
COOH
O
CH2OH
CO2H
(c)
CONH2
(d)
CH2Ogluc
(b)
S
COCH3

Figure 12.

### *p*-Cresol and Mixed Isomers of Cresol

Cresols are monomethyl derivatives of phenol, and are natural constituents of coal, petroleum, and wood. Coal tar products containing mixed isomers of cresol are used as pharmaceutical vehicles, and industrial and agricultural uses of cresols include the production of solvents, cleaners, and phenolic resins. *p*-Cresol is an intermediate in the production of disinfectants, explosives, perfumes, metal cleaners, and synthetic flavors. *p*-Cresol, a normal constituent of human urine with levels of excretion ranging from 16 to 74 mg/24 h, is reportedly the result of tyrosine metabolism in the gut.

Information regarding acute cresol toxicity has been obtained from suicide case studies involving Lysol®, that formerly contained mixed isomers of cresol. Symptoms following ingestion of from 1 to 60 mL have included involuntary muscle movements followed by paresis, gastrointestinal disturbances, renal toxicity, initial CNS stimulation followed by depression, tachycardia, peripheral vasoconstriction, dyspnea, acute pancreatitis, and hematological changes (Chan et al., 19[illegible]; NIOSH, 1978; Harvey, 1980; Deichmann and Keplinger, 1981; Craft, 1983; Cote et al., 1984; Gosselin et al., 1984; Arena and Drew, 1986; Plunkett, 1987). Effects of local exposure can include severe skin and eye irritation, corrosive effects upon the skin and mucuous membranes, and skin depigmentation (NIOSH, 1978; Deichmann and Keplinger, 1981; Sax and Lewis, 1989). The available data have indicated that cresol isomers are not mutagenic in bacteria (Dean, 1985). Only the ortho isomer produced a significant increase in sister chromatid exhange, but the response was weak even at the highest nontoxic concentration tested (8 mM) (Cheng and Kligerman, 1984).

In a 28-day feed study of *p*-cresol and mixed cresol isomers, a dose-related hyperplasia of the nasal respiratory epithelium occured in F-344 rats and B6C3F$_1$ mice of both sexes (NTP, 1992b), presumably a direct result of the irritant effects of the chemical or its vapors.

The proposed metabolic pathway for biotransformation of cresols is presented in Figure 13. Absorption of cresols from the gastrointestinal tract results in the formation of glucuronides or sulfates (Bray et al., 1950; Mandel, 1971; DeBruin, 1976a,b). These metabolites are highly ionized at physiological pH and are rapidly excreted from the kidney. In addition to urinary excretion, cresols undergo enterohepatic circulation (Deichmann and Keplinger, 1981), which is maintained by conjugate hydrolysis in the gut (Scheline, 1973). Rabbits exposed orally to cresols excreted 60 to 72% of all three isomers as glucuronides and 10 to 15% as sulfates in the urine (Bray et al., 1950; DeBruin, 1976b). Following oral administration of cresols to rabbits selective hydroxylation of *o*-cresol and *m*-cresol (3% of the dose) to 2,5-dihydroxytoluene (a) and *p*-cresol ($<$1% of the dose) to 3,4-dihydroxytoluene (b) was found; side chain oxidation of *p*-cresol (10% of the dose) to *p*-hydroxybenzoic acid (c) was also found (Bray et al., 1950; el-Masri et al., 1956; Hook and Smith, 1967; Kaubisch et al., 1972; Goldstein et al., 1974; DeBruin, 1976b). Hydroxybenzoic acid can then undergo conjugation with sulfates and glucuronides.

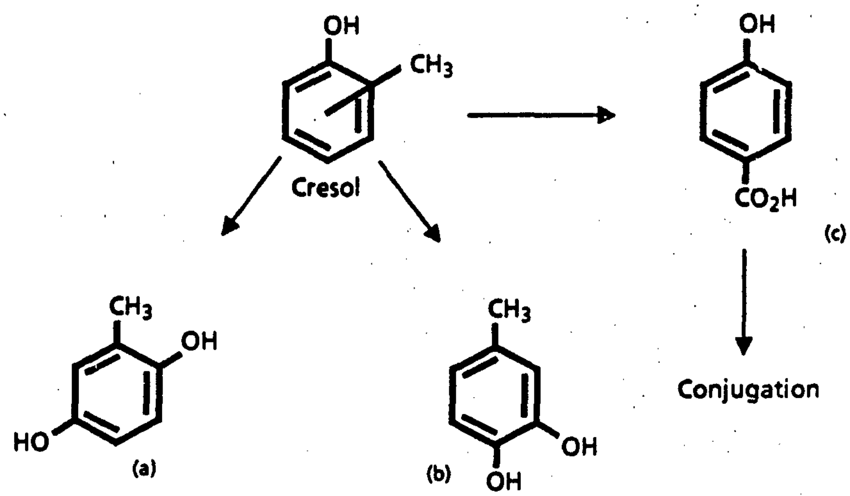



Figure 13.

**Methyl Methacrylate**

Methyl methacrylate is one of a number of acrylic acid esters that are the main ingredients in acrylic resins. Dermatitis, hypersensitivity, and blistering occur in humans after contact with acrylic esters (Delbressine et al., 1981). Animal experiments have shown that continuous absorption of small doses of these esters damages lungs, liver, and kidneys (Deichmann, 1941; Spealman et al., 1945; Treon et al., 1949; Autian, 1975). However, the $LD_{50}$ has been reported to be 5 g/kg in rats, and MMA is therefore not considered toxic in that species. Teratogenicity has been reported following ip administration (Singh et al., 1972) and an increased occurence of sarcomas has been observed in rats following subcutaneous adminstration (Laskin et al., 1954; Oppenheimer et al., 1955). Methyl methacrylate was negative in the *Salmonella* reverse-mutation assay (Lijinsky and Andrews, 1980). Forward mutations to 8-azaguanine resistance was observed when cells were exposed to concentrations ranging from 50 to 100 mM but the effect was associated with high cytotoxicity (Poss et al., 1979). In cultured Chinese hamster ovary cells, MMA produced a reproducible, dose-related increase in the frequency of sister chromatid exchanges (NPT, 1986). In a 2-year inhalation study, rats and mice were exposed to MMA at concentrations ranging from 0 to 500 ppm for female rats and mice, and from 0 to 1000 ppm for male rats and mice (NTP, 1986). Rats showed inflammation of the nasal mucosa and degeneration of the olfactory sensory epithelium at the highest doses. Mice of both sexes showed an increase in inflammation, epithelial hyperplasia, and degeneration of the olfactory epithelium at 500 ppm.

Although the use of this material is widespread, few studies of the metabolism of MMA have been reported. The proposed biotransformation pathway is presented in Figure 14. A major pathway involves carboxylesterase hydrolysis of MMA to methacrylic acid (a) (Corkill et al., 1976; Crout et al., 1979) because potentiation of MMA toxicity has been observed when the activity of carboxylesterase is inhibited (Silver and Murphy, 1978). Methyl methacrylate does not appear to involve glutathione in the process of detoxification (Hashimoto and Aldrige 1970), but Boyland and Chasseaud (1967) showed that rat liver thiol levels were reduced after ip administration, suggesting the possible formation of mercapturic acids. The isolation of mercapturic acids (b) from urine after administration of MMA to rats confirmed these findings and explains the kidney toxicity (NTP, 1986). During the biotransformation, glutathione is added to the ethylene group via a Michael addition. Methacrylic acid does not undergo this reaction (Delbressine et al., 1981), but it is detoxified by a second pathway described below. The present evidence suggests that it is unlikely that metabolism of MMA affords a DNA-reactive metabolite because the microsomal and cytoplasmic enzymes, normally involved in the bioactivation of xenobiotics, are not involved in MMA biotransformation.

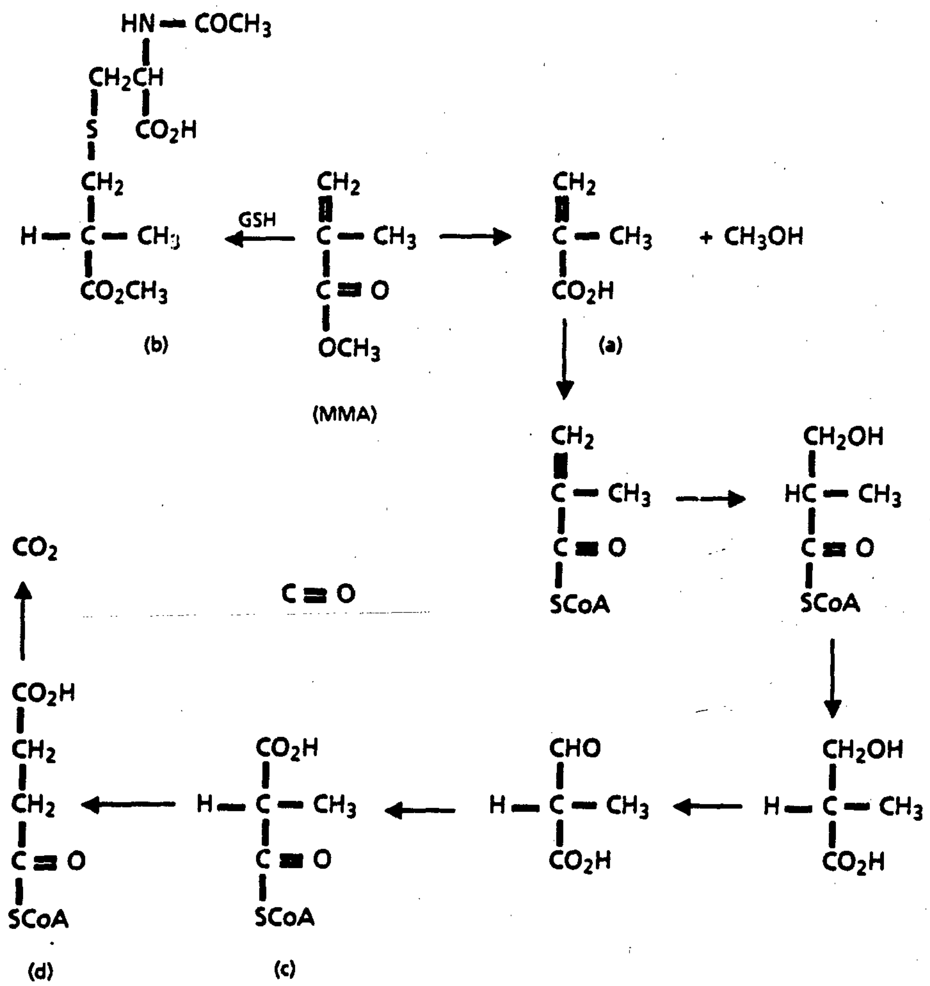



Figure 14.

When methyl(Me-$^{14}$C)methacrylate was given ip to female Wistar rats, 80% of the radioactivity was eliminated as $CO_2$ within 10 h (Crout et al., 1982). These authors concluded that MMA was metabolized by the normal pathway for valine catabolism. In this pathway, methyl malonylCoA (c), a known intermediate in valine catabolism, was converted by methylmalonyl mutase into succinyl CoA (d), a normal substrate for the Kreb's cycle.

**1,4-Dioxane**

1,4-Dioxane is a cyclic diether commonly used as an industrial and laboratory solvent. Symptoms of overexposure in humans have included irritation of the upper respiratory passages, eye irritation, drowsiness, vertigo, headache, anorexia, stomach pains, nausea and vomiting, uremia, coma, and death (Andrews and Snyder, 1991). The oral $LD_{50}$ is high, ranging from 2 to 6 g/kg in the case of rabbits and mice, respectively. 1,4-Dioxane reportedly causes hepatomas and nasal carcinomas in rats maintained on drinking water containing 0.75 to 1.8% DX for over 13 months (Argus et al., 1965; Hoch-Ligeti et al., 1970; Argus et al., 1973). These findings were confirmed by Kociba et al. (1974) who showed that DX produced variable degrees of renal and hepatic degenerative changes when rats where given 0.1% DX; no discernible effects were noted at a concentration of 0.01% DX. Rats exposed to 111 ppm of DX for 7 h/day, 5 days/week for 2 years showed neither treatment-related toxic effects nor incidence of tumors (Torkelson et al., 1974).

The metabolism of DX is presented in Figure 15. Woo et al. (1977b) showed that metabolism of DX was significantly increased by the pretreatment of rats with inducers of cytochrome $P_{450}$, and was decreased by inhibitors of cytochrome $P_{450}$. This has suggested that the biotransformation of DX is a cytochrome $P_{450}$-mediated process. Several possible metabolic pathways can be written. Braun and Young (1977) identified a-hydroxyethoxyacetic acid (HEAA) (a) as the major metabolic product in the urine of rats. Young et al. (1976) reported that both DX and HEAA were recovered from the urine of humans exposed to 1.6 ppm for 7.5 h. Woo et al. (1977a) reported that 1,4-dioxane-2-one (b) was the major urinary metabolite of DX. 1,4-Dioxane-2-one was excreted by rats given ethylene glycol, which supports Pathway I, but the absence of ethylene glycol in the urine suggests that is either rapidly biotransformed or that an alternative mechanim for the formation of the lactone exists. One possibility (Pathway II) is the formation of a keto-peroxy radical as an intermediate. This mechanism, similar to the mechanism of conversion of benzo[a]pyrene to benzo[a]pyrene dione (Terao et al., 1987), involves a direct attack by the radical followed by autoxidation. Compounds that are related to benzo[a]pyrene but less carcinogenic show little reactivity to lipid peroxy radicals. Another alternative route (Pathway III) has not been demonstrated but involves the hydroxylation of DX followed by the oxidation of the aldehyde intermediate.

Data on the acute toxicity of 1,4-dioxane-2-one indicates that it is considerably more toxic than the parent and may be the proximate carcinogen. This is certainly possible because lactones with similar stuctures, such as β-propiolactone, are known to be carcinogenic (Dickens, 1964; Van Duuren, 1969).

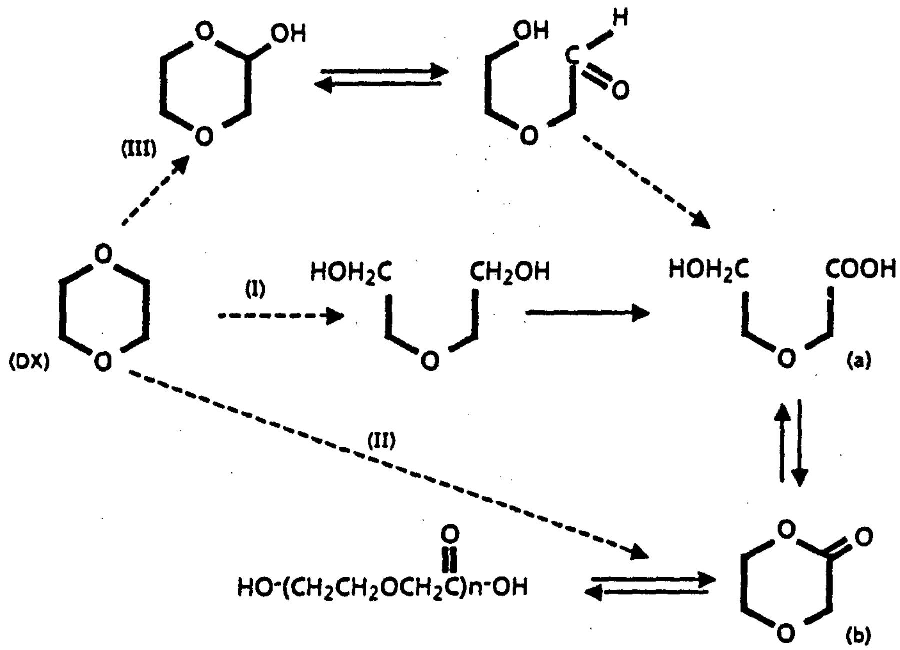



Figure 15.

**Titanocene Dichloride**

Titanocene dichloride is an organometallic compound composed of two cyclopentadienyl rings, titanium, and chloride. It has limited use as a cocatalyst for polymerization reactions. Transition metal complexes such as TD are representatives of a recently developed group of antitumor agents that include cisplatinum.

Treatment of pregnant mice with TD at doses of 30 or 60 mg/kg was associated with an increase in the incidence of cleft palate and costal malformations, and a reduction in the number of live fetuses per litter (Kopf-Maier and Erkenswick, 1984). Injections of TD in the right thigh muscle of F-344 rats (25 injections of 8 mg) caused fibrosarcomas at the injection site, and some of the animals developed hepatomas and malignant lymphomas of the spleen (Furst and Schlauder, 1971). The chemical has induced DNA damage in mammalian cells and gene mutations in *Salmonella* (NTP, 1991b). In this report, inflammation of the nasal mucosa and the lung were observed. These findings were attributed to aspiration of lavage solution caused by the irritating effects of TD on the stomach mucosa.

The mechanism of DNA damage by TD probably involves interference with cellular nucleic acid metabolism (Kopf-Maier, 1982, 1988). Model complexes have confirmed an interaction of similar compounds with nucleic acid constituents by formation of covalent linkage or hydrogen bonding to the bases and/or phosphate groups (Toney et al., 1986; Pneumatikakis et al., 1988). Organ distribution studies have revealed that the main accumulation sites of titanium are the liver and intestine (Kopf-Maier et al., 1988). A recent study confirmed that titanium-containing metabolites of TD are able to enter cells and cell nuclei in the liver, where they first appear in the nucleolus and are then extruded into the cytoplasm for incorporation into inclusion bodies (Kopf-Maier and Martin, 1989). The information regarding metabolism is scarce, but it is likely that TD is metabolized in much the same way as cisplatinum, a known chemotherapeutic agent. Cisplatinum enters cells by diffusion, and once inside the cell, the chloride atoms are hydrolyzed, forming the activated drug, which can form DNA ligands. The geometry of the *cis* compound results in the chelation of the C-6 and N positions of guanine (Williams and Weisburger, 1991). The structure and biotransformation of TD is presented in Figure 16.

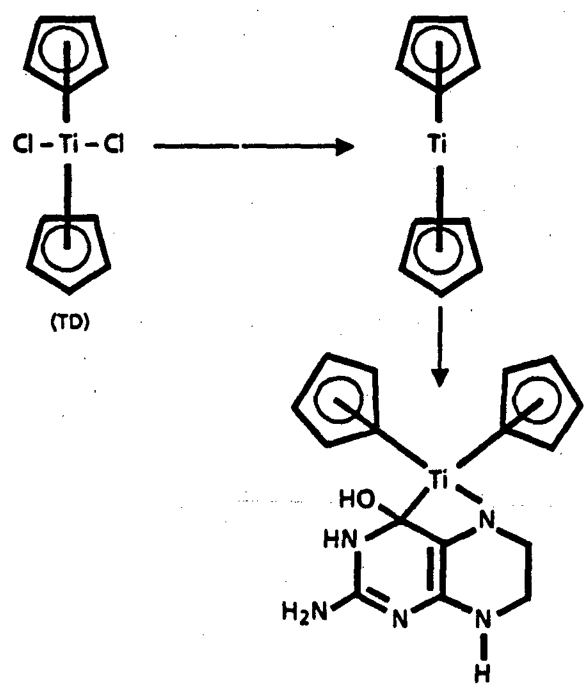



**Figure 16.**

**Tetranitromethane**

Tetranitromethane has been used as an oxidizer in rocket propellants, in explosives, and as an additive to increase the octane number of diesel fuel. It has also been used as a chemical reagent for detection of double bonds and as a mild nitrating agent because it reacts with tyrosine residues in proteins (Riordan and Vallee, 1972). Tetranitromethane is also the principal volatile contaminant of TNT (trinitrotoluene). The signs and symptoms of TNT intoxication (caused by inhalation of fumes of crude TNM) have included nasal irritation, burning of the eyes, dyspnea, cough, tightness of the chest, and dizziness. Drowsiness, headache, cyanosis, respiratory distress, and bradycardia have been reported after prolonged exposure. Deaths have resulted from severe exposure and were attributed to respiratory failure and methemoglobinemia.

The metabolism of TNM has not been described in the literature. However, from effects seen after oral administration of the chemical, certain information can be inferred. Blood samples obtained 90 min after administration of single oral doses of TNM to Sprague-Dawley rats indicated a dose-related production of methemoglobin. That finding suggested that metabolism could include formation of nitrite ions (Kinkead et al, 1977). After iv administration or inhalation exposure, methemoglobin formation was not seen, nor was it reduced when compared with that seen after an oral exposure. This suggested that nitrate oxidase activity in the gut may be involved (Kinkead et al., 1977; Vernot et al., 1977). Little is known about the mutagenic potential of TNM except that it is positive in the Ames test and induces sister chromatid exchanges and chromosomal aberrations (NTP, 1990e). Interestingly, the urine of workers exposed to TNM in a chemical plant manufacturing munitions was found to be positive in the Ames test (Ahlborg et al., 1988). The results of a 2-year bioassay have shown that the the effects of exposure to TNM are limited to the respiratory tract. Hyperplasia of the alveolar and bronchiolar epithelium and chronic nasal inflammation were observed in exposed rats. Adenomas and carcinomas of the lung were elevated in treated rats, with carcinomas occurring in nearly all animals exposed to the highest concentration (NTP, 1990e).

The mechanism of toxicity in not clear, but may involve activation of the K-*ras* oncogene. In the NTP study (NTP, 1990e), DNA isolated from alveolar/bronciolar neoplasms from mice and rats exposed to TNM was transfected into cultured fibroblasts. Morphologic transformation occurred, and the transforming gene was identified as the K-*ras* oncogene in both mice and rats. Approximately 40% of examined human pulmonary adenocarcinomas contain an activated K-*ras* oncogene (You et al., 1989) and activation of this oncogene is frequently observed in chemically induced pulmonary neoplasms in rodents (Belinsky et al., 1989). Tetranitromethane is known to nitrate hydroxyl groups of tyrosine residues and Ptitsyn et al. (1979) have shown that TNM similarly modifies tyrosine residues of deoxyribonucleoproteins *in vitro*.

**2,6-Xylidine**

2,6-Xylidine is widely used as a dye, a drug, and a cosmetic precursor. Administration of XL at a concentration of 10,000 ppm in the diet for 6 months produced liver enlargement in the rat (Lindstrom et al., 1963). Similar effects were observed when XL was administered orally for 20 days by gavage at a dose of 157.5 mg/kg (Short et al., 1983), or orally for 4 weeks by gavage at a dose of 500 to 700 mg/kg/day (Magnusson et al., 1971). No microscopic lesions have been observed except for a slight decrease in centrilobular glycogen and a slight proliferation of smooth endoplasmic reticulum. In a 2-year bioassay, XL was administered in the diet at concentrations ranging from 0 to 3000 ppm (NTP, 1990f). The incidence of nasal carcinomas was about 50% in both male and female rats in the high-dose group.

The biotransformation of XL is presented in Figure 17. Lindstrom et al. (1963) have shown that XL is metabolized to 4-hydroxy-2,6-dimethylaniline and 2-amino-3-methylbenzoic acid in the rat. A more recent investigation determined that XL was metabolized principally to 4-hydroxy-2,6-dimethylaniline (a) in the rat. No 2-amino-3-methylbenzoic acid (b) was noted in the rat, but in the dog, significant quantities of 2-amino-3-methylbenzoic acid and its glycine conjugate (c) were produced (Short et al., 1989). A minor metabolite, N,2,4-trimethylaniline (d) was also seen in the rat. Repeated administration of XL did not change the profile of the metabolites in either species, but the administration of 3-methylchloanthrene caused an increase in the amount of 4-hydroxy-2,6-dimethylaniline. This finding has suggested that hydroxylation is a cytochrome $P_{448}$ mediated process. This is an interesting finding because 3-methylcholanthrene has been shown in other studies to specifically induce *N*-oxidation of aromatic amines (Thorgeirsson et al., 1983; 1984; Atlas et al., 1977).

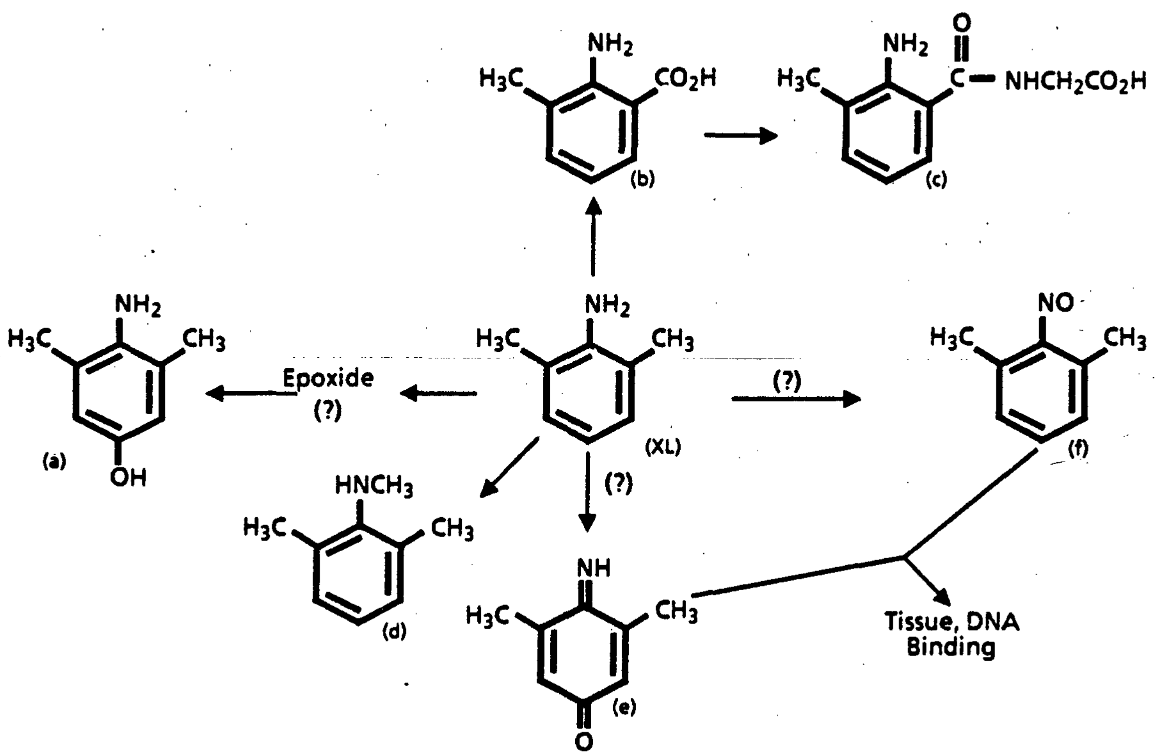



Figure 17.

There are several possibilities for the reactive species formed from XL that could be involved in the production of nasal carcinomas. 4-Hydroxy-2,6-dimethylaniline (a) could be involved if an epoxide forms as an intermediate. The formation of a quinone imine (e) could be involved because quinones have been implicated in the toxicity of a variety of aromatic compounds (Irons and Sawahata, 1985). Quinone imines are highly reactive electrophiles and can cause lipid peroxidation (Albano et al., 1985). Reduction of quinone imines also can result in the production of free radical intermediates (Rosen et al., 1985). Finally, aromatic nitroso-compounds are known to be reactive (Miller and Miller, 1981) and 2,6-dimethylnitrosobenzene (f) could be a toxic metabolite of XL.

### *p*-Cresidine

*p*-Cresidine is primarily used as in intermediate in the manufacturing of dyes. In long-term feeding studies, *p*-cresisine was carcinogenic to both rats and mice, the main sites of its action being the urinary bladder, nasal cavity, and liver (IARC, 1982). Positive results have been displayed in genotoxicity screening assays including the Ames test, but only after metabolic activation (IARC, 1982).

There have been no reports in the literature describing the metabolism of this chemical. Nevertheless, a proposed biotransformation pathway is presented in Figure 18. In this example, an initial oxidation of the methoxy group to yield 2-amino-*p*-cresol (a) is followed by oxidation of the methyl group to 4-aminohydroxybenzoic acid (b) which can then undergo either conjugation or acetylation of the amino functional group. The free amino group can also undergo *N*-methylation in an *S*-adenosylmethionine-dependent reaction to yield metabolites (c,d). The species responsible for the observed carcinogenicity of this chemical may be an aromatic-nitroso compound or, in a reaction similar to that of hydrazine, the *N*-acetylhydroxybenzoic acid (e) could undergo deacetylation, resulting in an acetyl radical.

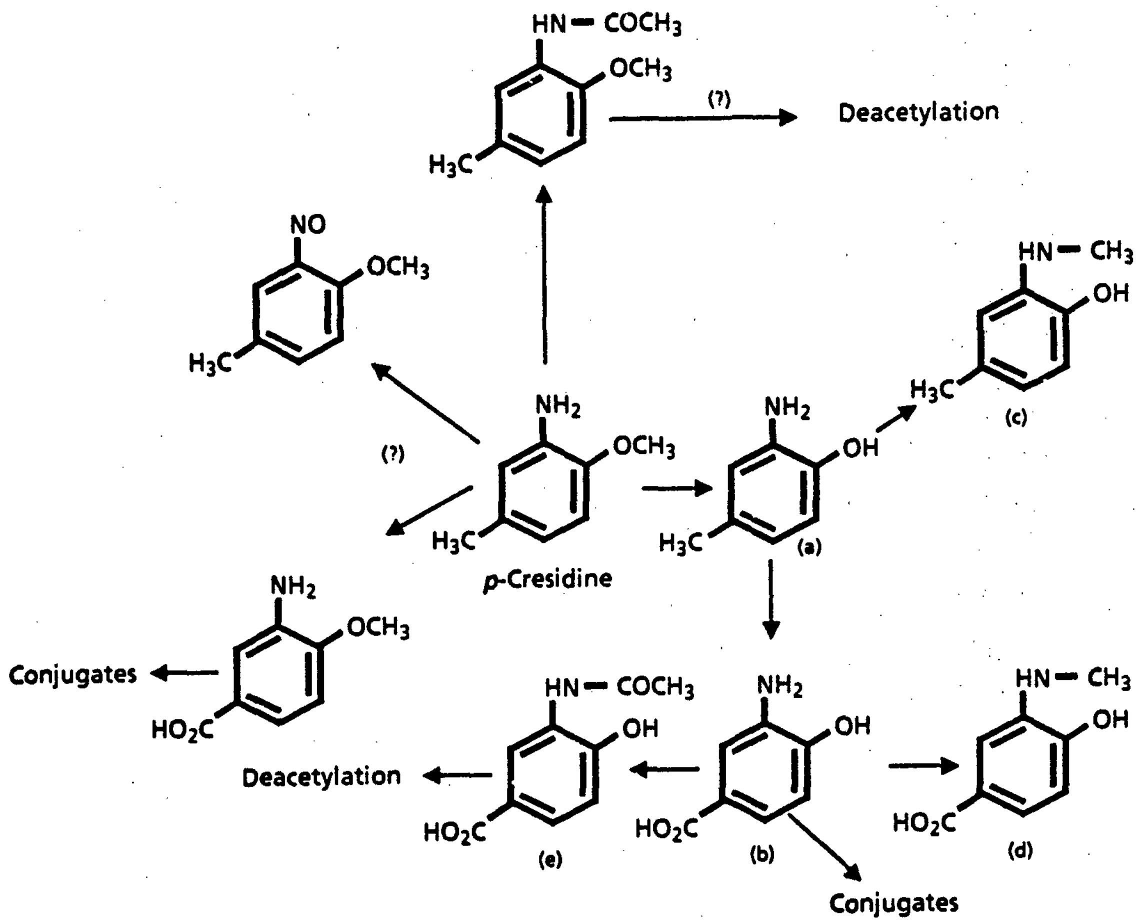

HN— COCH3
OCH3
H3C
(?)
Deacetylation
NO
OCH3
H3C
HN— CH3
OH
H3C
(c)
NH2
OCH3
(?)
H3C
p-Cresidine
NH2
OH
H3C
(a)
NH2
OCH3
Conjugates
HO2C
HN— COCH3
OH
Deacetylation
HO2C
(e)
NH2
OH
HO2C
(b)
HN— CH3
OH
HO2C
(d)
Conjugates

Figure 18.

## DISCUSSION

The report by Huff et al. (1991) has listed 10 chemicals that have been tested by the NTP and shown to produce tumors in the nasal cavity. These chemicals were AGE, *p*-cresidine, DBCP, DBE, DMVC, DX, EB, IDG, PO, and XL. The results of the *Salmonella* reverse mutation assay (Ames test) have indicated that all of these chemicals were either weakly positive or positive, except for DX, which was negative. The tumors produced by AGE and PO were limited to the nasal cavity, whereas the others produced tumors at multiple sites.

Of the 10 chemicals listed by Huff et al. (1991), 3 were epoxides (AGE, EB, and PO). One chemical of these 10 (IDG) could undergo biotransformation to form an epoxide. Nasal tumors were found in only 2 of 49 male rats exposed to IDG by oral gavage at a dose of 250 mg/kg. There was no evidence of nasal tumors in female rats or in mice of either sex. Three other chemicals reviewed in this manuscript could form epoxides during biotransformation (BTD,NPT, and VT). Both BTD and NPT produced alveolar and bronchiolar adenomas in mice, but VT produced no evidence of carcinogenicity.

Epoxides can produce tumors, and nasal tumors were produced when AGE, EB, and PO were administered by inhalation. The administration of other chemicals by inhalation that required metabolic activation to an epoxide resulted in formation of lung tumors, but not nasal tumors. The conversion of these chemicals to reactive epoxides by nasal epithelium is probably low, suggesting that the biotransformation occurred in the lungs.

Three of the 10 chemicals listed by Huff et al. (1991) were halogenated hydrocarbons that formed reactive aldehydes during biotransformation. 1,2-Dibromo-3-chloropropane and DBE were administered by inhalation and both produced positive evidence of nasal tumors in rats and mice. In both cases, metabolic activation is required for activity but the site for this activation is unknown. The formation of alveolar and bronchiolar adenomas in both species suggests that activation occurs in the lungs. Because tumors are generally found in organs that are the site for their biotransformation to reactive intermediates (Williams and Weisburger, 1991), it is possible that activation of these agents occurred in the nasal epithelium as well. Dimethyl vinylchloride metabolism also affords a reactive aldehyde intermediate. This chemical was administered by oral gavage and clear evidence for nasal tumor formation was found in male and female rats. This suggests that activation to a reactive intermediate can occur at locations distal to the site of tumor formation but does not rule out the possibility of metabolism of blood-borne levels of DMVC by the nasal epithelium. Two other chemicals reviewed in this manuscript (MCA and CS) do not form reactive metabolites during their biotransformation and neither chemical induced nasal tumor formation.

The remaining three chemicals listed by Huff et al. (1991), and found to produce nasal tumors, were DX, XL, and *p*-cresidine. 1,4-Dioxane was administered in the drinking water. This chemical has been shown to be metabolized to a strained lactone that is excreted in the urine. This implies that the lactone ring may not be quickly metabolized and so could produce tumors at sites distant to its site of formation. Alternatively, dioxane may be absorbed to such a great extent that sufficient parent compound survives the first pass through the liver to cause tumors at other sites in the body. The principal site of biotransformation is most likely in the liver because of the large number of animals with liver adenomas and carcinomas. Both XL and *p*-cresidine were administered in the food. Liver and bladder tumors were observed for *p*-cresidine indicating that metabolism may have taken place in the liver with the formation of a stable carcinogenic metabolite excreted in the urine. As with dioxane, sufficient parent chemical may also have survived the first pass through the liver to result in tumor formation in nasal epithelium. In the case of XL, the principal findings were tumors of the nasal cavity with some neoplastic nodules in the liver of female rats that may have been related to treatment. With these last two compounds, there is little understanding of the relationship between their metabolism and the formation of tumors. The presence of free amino functional groups in both of these chemical is interesting because the amino groups could undergo reactions similar to those described for hydrazine. However, because no information in the literature exists concerning the reactivity of this group for either chemical, there is no reason to expect that they are metabolized in a similar manner to hydrazine. If the distribution and type of nasal tumors produced in animals exposed to these two chemicals is similar to that reported for hydrazine, then studies might be designed to address the metabolic pathways of both chemicals in more detail.

Four additional chemicals were reviewed in this manuscript. Cresols and MMA have produced no evidence of carcinogenicity. The metabolism of MMA clearly does not produce reactive intermediates, but it is not known whether the hydroxylation of cresols involves a direct oxygen insertion or the formation of an epoxide intermediate. The fact that cresols are not carcinogenic would tend to favor a mechanism of direct insertion, but no information exists in the literature that would answer this question. Titanocene dichloride has produced tumors of the forestomach when administered by oral gavage. The carcinogenic response has been judged as equivocal. Finally, TNM administration by inhalation produced clear evidence of alveolar and bronchiolar adenomas and carcinomas, squamous cell carcinomas, and sarcomas. This chemical is unique because it is the only chemical reviewed in this manuscript that has been shown to activate oncogenes. In this respect, it is possible that HZ may also activate oncogenes, but there is no evidence to support this hypothesis at the present time.

## SUMMARY AND CONCLUSION

The biotransformation of 19 chemicals that have been shown to produce nasal toxicity in long-term carcinogenesis studies in rodents has been compared with that of HZ. The pertinent information for all 19, as well as that for HZ, has been summarized in Table 1.

Ten of the 19 chemicals reviewed produced tumors of various types in the nasal cavity. Of these, four are epoxides or can be metabolized to an epoxide, three can undergo biotransformation to reactive aldehyde intermediates, and one (DX) can be metabolized to a stained lactone ring, described as the proximate carcinogen. *p*-Cresidene and XL possess a free amino function group that may undergo reactions similar to those reported for the metabolism of HZ; however, there is no literature data to support this hypothesis. In addition, the types of tumors that arise as a consequence of exposure to HZ (squamous cell and adenocarcinoma) are unlike those reported for *p*-cresidene (neuroblastoma) or XL (carcinoma). This further suggests that HZ is metabolized to reactive intermediates that are different from those of *p*-cresidene or XL. In conclusion, there is no evidence suggesting that the mechanisms of biotransformation of the 19 chemicals reviewed in this report is similar to that described for HZ.

## TABLE 1. COMPARATIVE STRUCTURE–ACTIVITY AND NASOTOXICITY SUMMARY

| Chemical | Nasal Tumors | Tumor Types | Epoxide? | Aldehyde? | Other Reactive Metabolites? | Reference |
|---|---|---|---|---|---|---|
| 1,3-Butadiene | no-inf, met | | yes | | mercapturic acid | Loeser, 1982; |
| | yes | olf, neuroblastoma | | | | Brown, et al.,1991 |
| 1,2-Epoxybutane | yes | Ad | yes | | mercapturic acid | Dunnick et al., 1988 |
| 1,2-Propylene oxide | yes | Ad, hemangioma/ hemangiosarcoma | yes | | mercapturic acid | NTP, 1985a |
| Naphthalene | no-olf, met | | yes | | mercapturic acid | NTP,1991a |
| Vinyl toluene | no-olf, met | | yes | | mercapturic acid | NTP, 1990a |
| Iodinated glycerol | yes | Ad | yes | | | NTP, 1990b |
| Allyl glycidyl ether | yes | SCCa, Ad, olf, AdCa | yes | | possible mercapturic acid | NTP, 1990d |
| Dimethyl vinylchloride | yes | Ca, usually olf | | yes | possible mercapturic acid | NTP, 1985b |
| 1,2 Dibromoethane | yes | AdCa, Ca | | yes | possible mercapturic acid, episulfonium ion | NTP, 1982a |
| 1,2-Dibromo-3-chloropropane | yes | SCCa | | yes | | NPT, 1982b |
| Monochloroacetic acid | no-inf | | | | | NTP, 1992a |
| 2-Chlorobenzalmalonitrile (CS) | no-sq met | | | | | NTP, 1990g |
| *p*-Cresol and mixed cresol isomers | no-inf, met | | | | | NTP, 1992b |
| Methyl methacrylate | no-inf | | | | | NTP, 1986 |
| 1,4-Dioxane | yes | SCCa | | | strained lactone | Argus et al., 1965; Hoch-Ligeti et al.,1970 |
| Titanocene dichloride | no-inf | | | | | NTP, 1991b |
| Tetranitromethane | no-inf | | | | | NTP, 1990e |
| 2,6-Xylidine | yes | Ad, Ca | | | possible nitroso-compounds | NTP, 1990f |
| *p*-Cresidine | yes | neuroblastoma | | | possible nitroso-compounds | IARC, 1982 |
| Hydrazine | yes | SCCa, AdCa, adenomatous polyp | | | free radical | Vernot et al., 1985 |

Ad = adenoma  AdCa = adenocarcinoma  Ca = carcinoma  inf = inflammation  met = metaplasia  olf = olfactory  Sq = squamous  SCCa = squamous cell cearcinoma

## BIBLIOGRAPHY


**Ahlborg, G., Jr., P. Einisto, and M. Sorsa.** 1988. Mutagenic activity and metabolites in the urine of workers exposed to trinitrotoluene (TNT). *Br. J. Ind. Med.* 45:353-358.

**Albano, E., M. Rundgren, P.J. Harvison, S.D. Nelson, and P. Moldeus.** 1985. Mechanisms of *N*-acetyl-*p*-benzo-quinone imine cytotoxicity. *Mol. Pharmacol.* 28:306-311.

**Amir, D.** 1973. Sites of spermicidal action of ethylene dibromide in bulls. *J. Reprod. Fert.* 35:519-525.

**Andrews, L.S. and R. Snyder.** 1991. In: M.O. Amdur, J. Doull, and C.D. Klaassen, eds. *Toxicology: The Basic Science of Poisons*, p. 707. New York: Pergamon Press.

**Arena, J.M. and R.H. Drew.** 1986. *Poisoning: Toxicology, Symptoms, Treatments*, 5th Ed. Springfield, IL: Thomas.

**Argus, M.F., J.C. Arcos, and C. Hoch-Ligeti.** 1965. Studies on the carcinogenic activity of protein-denaturing agents: Hepatocarcinogenicity of dioxane. *J. Natl. Cancer Inst.* 35:949-958.

**Argus, M.F., R.S. Sohal, G.M. Bryant, C. Hoch-Ligeti, and J.C. Arcos.** 1973. Dose-response and ultrastructural alterations in dioxane carcinogenesis. *Eur. J. Cancer* 9:237-243.

**Atlas, S.A., A.R. Boobis, J.S. Felton, S.S. Thorgeirsson, and D.W. Nebert.** 1977. Ontogenic expression of polycyclic aromatic compound-inducible monooxygenase activities and forms of cytochrome $P_{450}$ in rabbit. *J. Biol. Chem.* 252:4712-4721.

**Autian, J.** 1975. Structure-toxicity relationship of acrylic monomers. *Environ. Health Perspect.* 11:141-152.

**Ballantyne, B.** 1977. Biomedical and health aspects of the use of chemicals in civil disturbances. In: R.B. Scot and J. Frazier, eds. *Medical Annual 1977*, p. 7. Bristol, UK: Wright and Sons.

**Barrigon, S., A. Moreno, D. Rebollo, and P.D. Garcia de Jalon.** 1986. Parametros farmacocineticos del $^{125}$I-yodopropilidenglicerol (IPG), en voluntarious sanos. *Rev. Parmacol. Clin. Exp.* 3:73-79.

**Belinksy, S.A., T.R. Devereux, R.R. Maronpot, G.D. Stoner, and M.W. Anderson.** 1989. Relationship between the formation of promutagenic adducts and the activation of the K-*ras* protooncogene in lung tumors from A/J mice treated with nitrosamines. *Cancer Res.* 49:5303-5311.

**Bergemalm-Rynell, K. and G. Steen.** 1982. Urinary metabolites of vinyltoluene in the rat. *Toxicol. Appl. Pharmacol.* 62:19-31.

**Biancifiori, C.** 1970. Hepatomas in CBA/Cb/Se mice and liver lesions in golden hamsters induced by hydrazine sulfate. *J. Natl. Cancer Inst.* 44:943-953.

**Blair, I.A., R. Mansilla-Tinoco, M.J. Brodie, R.A. Clare, C.T. Dollery, J.A. Timbrell, and I.A. Beever.** 1984. Plasma hydrazine concentrations in man after isoniazid and hydralazine administration. *Human Toxicol.* 4:195-202.

**Blum, A. and B.N. Ames.** 1977. Flame retardant additives as possible cancer hazards. *Science* 195:17-23.

Bolt, H.M., G. Schmiedel, J.G. Filser, H.P. Rolzhuser, K. Lieser, D. Wistuba, and V. Schurig. 1983. Biological activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolite by exposed rats. *J. Cancer Res. Clin. Oncol.* 106:112-116.

Bondi, A., E. Olomucki, and M. Calderon. 1955. Problems connected with ethylene dibromide fumigation of cereals. II. Feeding experiments with laying hens. *J. Sci. Food. Agric.* 6:600-602.

Boyland, E. and L.F. Chasseaud. 1967. Enzyme-catalyzed conjugations of glutathione with unsaturated compounds. *Biochem. J.* 104:95-102.

Braun, W.H. and J.D. Young. 1977. Identification of hydroxyethoxyacetic acid as the major urinary metabolite of 1,4-dioxane in the rat. *Toxicol. Appl. Pharmacol.* 39:33-38.

Bray, H.G., W.V. Thorpe, and K. White. 1950. Metabolism of derivatives of toluene. *Biochem. J.* 46:275-278.

Brewster, K., J.M. Harrison, L. Leadbeater, J. Newman, and D.G. Upshall. 1987. The fate of 2-chlorobenzylidenemalononitrile (CS) in rats. *Xenobiotica* 17:911-924.

Brown, H.R., T.M. Monticello, R.R. Maronpat, H.W. Randall, J.P. Hotchkiss, and K.T. Morgan. 1991. Proliferative and neoplastic lesions in the rodent nasal cavity. *Toxicol. Pathol.* 19:358-372.

Buckpitt, A.R., N. Castagnoli, Jr., S.D. Nelson, A.D. Jones, and L.S. Bahnson. 1987. Stereoselectivity of naphthalene epoxidation by mouse, rat, and hamster pulmonary, hepatic, and renal microsomal enzymes. *Drug Metab. Dispos.* 15:491-498.

Cannon, J.M., R.D. Brown, E.M. Murrill, and C.W. Jameson. 1989. Identification of components in iodinated glycerol. *J. Pharm. Sci.* 78:48-51.

Canter, D.A., E. Zeiger, S. Haworth, T. Lawlor, K. Mortelmans, and W. Speck. 1986. Comparative mutagenicity of aliphatic epoxides in *Salmonella*. *Mutat. Res.* 172:105-138.

Chaiken, I.M. and E.L. Smith. 1969. Reaction of the sulfhydryl groups of papain with chloroacetic acid. *J. Biol. Chem.* 244:5095-5099.

Chan, T.K., L.W. Mak, and R.P. Ng. 1971. Methemoglobinemia, Heinz bodies, and acute massive intravascular hemolysis in Lysol® poisoning. *Blood* 38:739-744.

Cheng, M. and A.D. Kligerman. 1984. Evaluation of the genotoxicity of cresols using sister-chromatid exchange (SCE). *Mutat. Res.* 137:51-55.

Corkill, J.A., E.J. Lloyd, P. Hoyle, D.H.G. Crout, R.S.M. Ling, M.L. James, and E.J. Piper. 1976. Toxicology of methyl methacrylate: The rate of disappearance pf methyl methacrylate in human blood *in vitro*. *Clin. Chim. Acta* 68:141-146.

Cote, M.A., J. Lyonnais, and P.F. Leblond. 1984. Acute Heinz-body anemia due to severe cresol poisoning: Successful treatment with erythrocytapheresis. *Can. Med. Assoc. J.* 130:1319-1322.

Craft, B.F. 1983. Solvents and related compounds. In: W.N. Rom, ed. *Environmental and Occupational Medicine*, pp. 511-533. Boston, MA: Little, Brown and Co.

**Crout, D.H.G., J.A. Corkill, M.L. James, and R.S.M. Ling.** 1979. Methylmethacrylate metabolism in man. The hydrolysis of methylmethacrylate to methacrylic acid during total hip implantation. *Clin. Orthop. Relat. Res.* 141:90-95.

**Crout, D.H.G., E.J. Lloyd, and J. Singh.** 1982. Metabolism of methyl methacrylate: Evidence for metabolism by the valine pathway of catabolism in rat and in man. *Xenobiotica* 12:821-829.

**Dean, B.J.** 1985. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res.* 154:153-181.

**DeBruin, A.** 1976a. Metabolic fate of xenobiotic compounds. In: *Biochemical Toxicology of Environmental Agents*, pp. 1-85. Amsterdam: Elsevier/North-Holland Biomedical Press.

**DeBruin, A.** 1976b. Metabolism of occupational agents. In: *Biochemical Toxicology of Environmental Agents*, pp 87-170. Amsterdam: Elsevier/North-Holland Biomedical Press.

**Deichmann, W.** 1941. Toxicity of methyl, ethyl, and *N*-butyl methacrylates. *J. Ind. Hyg. Toxicol.* 23:343-351.

**Deichmann, W. and M.L. Klepinger.** 1981. Phenols and phenolic compounds. In: G.D. Clayton and F.E. Clayton, eds. *Patty's Industrial Hygiene and Toxicology,* 3rd Ed. 2:2567-2627. New York: Wiley and Sons.

**Delbressine, L.P.C., F. Seutter-Berlage, and E. Seutter.** 1981. Identification of urinary mercapturic acids formed from acrylate, methacrylate and crotonate in the rat. *Xenobiotica* 11:241-247.

**de Meester, C., F. Poncelet, M. Roberfroid, and M. Mercier.** 1978. Mutagenicity of butadiene and butadiene monoxide. *Biochem. Biophys. Res. Commun.* 80:298-305.

**Dickens, F.** 1933. Interaction of halogenacetates and SH compounds. *Biochemistry* 27:1141-1151.

**Dickens, F.** 1964. Carcinogenic lactones and related substances. *Br. Med. Bull.* 20:96-101.

**Dost, F.N., D.L. Springer, B.M. Drivak, and D.J. Reed.** 1979. Metabolism of hydrazine. AFAMRL-TR-79-43. Wright-Patterson Air Force Base, OH: Air Force Aerospace Medical Research Laboratory.

**Dunnick, J.K., S.L. Eustis, W.W. Piegorsch, and R.A. Miller.** 1988. Respiratory tract lesions in F344/N rats and $B6C3F_1$ mice after inhalation exposure to 1,2-epoxybutane. *Toxicology* 50:69-82.

**Edwards, K., H. Jackson, and A.R. Jones.** 1970. Studies with alkylating esters. II. A chemical interpretation through metabolic studies of the antifertility effects of ethylene dimethanesulphonate and ethylene dibromide. *Biochem. Pharmacol.* 19:1783-1789.

**el-Masri, A.M., J.N. Smith, and R.T. Williams.** 1956. The metabolism of alkylbenzenes: *n*-propylbenzene and *n*-butylbenzene with further observations on ethylbenzene. *Biochem. J.* 64:50-56.

**el-Masri, A.M., J.N. Smith, and R.T. Williams.** 1958. The metabolism of alkylbenzenes: Phenylacetylene and phenylethylene (styrene). *Biochem. J.* 68:199-204.

**Epstein, S.S., E. Arnold, J. Andrea, W. Bass, and Y. Bishop.** 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. Appl. Pharmacol.* 23:288-325.

Fishbein, L. 1979. Potential halogenated industrial carcinogenic and mutagenic chemicals. III. Alkane halides, alkanols, and esters. *Sci. Total. Environ.* 11:223-257.

Furst, A. and M.C. Schlauder. 1971. The hamster as a model for metal carcinogenesis. *Proc. West. Pharmacol. Soc.* 14:68-71.

Ghanayem, B.I. and L.T. Burka. 1987. Comparative metabolism and disposition of 1-chloro- and 3-chloro-2-methylpropene in rats and mice. *Drug Metab. Dispos.* 15:91-96.

Goldstein, A., L. Aronow, and S.M. Kalman. 1974. *Principles of Drug Action: The Basis of Pharmacology, 2nd Ed.* New York: Wiley and Sons.

Gosselin, R.E., R.P. Smith, and H.C. Hodge. 1984. *Clinical Toxicology of Commercial Products, 5th Ed.* Baltimore, MD: Williams and Wilkins.

Green, T. and D.E. Hathway. 1975. The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem. Biol. Interact.* 11:545-562.

Guengerich, F.P., P.S. Mason, W. Stott, T.R. Fox, and P.G. Watanabe. 1981. Roles of 2-haloethylene oxides and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in irreversible binding to protein and DNA. *Cancer Res.* 41:4391-4398.

Harvey, S.C. 1980. Antiseptics and disfectants; fungicides; ectoparasiticides. In: A.G. Gilman, L.S. Goodman, and A. Gilman, eds. *The Pharmacological Basis of Therapeutics,* 6th Ed., pp. 964-987. New York: Macmillan.

Hashimoto, K. and W.N. Aldridge. 1970. Biochemical studies on acrylamide, a neurotoxic agent. *Biochem. Pharmacol.* 19:2591-2604.

Hayes, F.D., P.J. Gehring, and J.E. Gibson. 1972. Studies on the acute toxicity of monochloroacetic acid in rats. *Toxicol. Appl. Pharmacol.* 22: 303.

Hayes, F.D., R.D. Short, and J.E. Gibson. 1973. Differential toxicity of monochloroacetate, monofluoroacetate, and monoiodoacetate in rats. *Toxicol. Appl. Pharmacol.* 26:93-102.

Heinonen, T.H.H. 1984. Metabolism of vinyltoluene in the rat: effect of induction and inhibition of the cytochrome $P_{450}$. *Biochem. Pharmacol.* 33:1585-1593.

Hemminki, K., T. Heinonen, and H. Vainio. 1981. Alkylation of guanine and 4-(*p*-nitrobenzyl)pyridine by styrene analogs *in vitro*. *Arch. Toxicol.* 49:35-41.

Henschler, D. 1985. Halogenated alkenes and alkynes. In: M.W. Anders, ed. *Bioactivation of Foreign Compounds*, pp. 318-323. Orlando, FL: Academic Press, Inc.

Hill, D.L., T.W. Shih, T.P. Johnston, and R.F. Struck. 1978. Macromolecular binding and metabolism of the carcinogen 1,2-dibromoethane. *Cancer Res.* 38:2438-2442.

Hine, C.H., J.K. Kodama, J.S. Wellington, M.K. Dunlap, and H.H. Anderson. 1956. The toxicology of glycidol and some glycidyl ethers. *AMA Arch. Ind. Health* 14:250-264.

Hoch-Ligeti, C., M.F. Argua, and J.C. Arcos. 1970. Induction of carcinomas in the nasal cavity of rats by dioxane. *Br. J. Cancer* 24:164-170.

**Hoffnagle, G.P. and A. Osol.** 1958. The distribution and fate of iodine in iodopropylidene-glycerol in the rat. *J. Am. Pharm. Assoc.* 47:149-153.

**Hogstedt, B., E. Bergmark, M. Tornqvist, and S. Osterman-Golkar.** 1990. Chromosomal aberrations and micronuclei in lymphocytes in relation to alkylation of hemoglobin in workers exposed to ethylene oxide and propylene oxide. *Hereditas* 113:133-138.

**Hook, G.E.R. and J.N. Smith.** 1967. Oxidation of methyl groups by grass grubs and vertebrate liver enzymes. *Biochem. J.* 402:504-510.

**Huff, J.E., R.L. Melnick, H.A. Solleveld, J.K. Haseman, M. Powers, and R.A. Miller.** 1985. Multiple organ carcinogenecity of 1,3-butadiene in $B6C3F_1$ mice after 60 weeks of inhalation exposure. *Science* 227:548-549.

**Huff, J., J. Cirvello, J. Haseman, and J. Bucher.** 1991. Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. *Environ. Health Perspect.* 93:247-270.

**International Agency for Research on Cancer (IARC).** 1976. Diepoxybutane. *IARC Monogr.* 11:115-123.

**International Agency for Research on Cancer (IARC).** 1977. Ethylene dibromide. *IARC Monogr.* 15:195-209.

**International Agency for Research on Cancer (IARC).** 1982. *meta*-and *para*-Cresidine. *IARC Monogr.* 27:91-101.

**International Agency for Research on Cancer (IARC).** 1985. Propylene oxide. *IARC Monogr.* 36:227-243.

**Irons, R.D. and T. Sawahata.** 1985. Phenols, catechols, and quinones. In: M. Anders, ed. *Bioactivation of Foreign Compounds*, p. 259. New York: Academic Press.

**Jones, A.R.** 1975. The metabolism of 3-chloro-, 3-bromo-, 3-iodopropan-1,2-diol in rats and mice. *Xenobiotica* 5:155-165.

**Jones, A.R., G. Fakhour, and P. Gadiel.** 1979. The metabolites of the soil fumigant 1,2-dibromo-3-chloropropane in the rat. *Experientia* 35:1432-1434.

**Jones, B.K. and D.E. Hathaway.** 1978. Differences in metabolism of vinylidene chloride between rats and mice. *Br. J. Cancer* 37:411-417.

**Kato, Y., K. Sato, O. Matano, and S. Goto.** 1980. Alkylation of cellular macromolecules by reactive metabolic intermediates by DBCP. *J. Pest. Sci.* 5:45-53.

**Kaubisch, N., J.W. Daly, and D.M. Jerina.** 1972. Arene oxidates as intermediates in the oxidative metabolism of aromatic compounds. Isomerization of methyl-substituted aren oxides. *Biochemistry* 11:3080-3088.

Kinkead, E.R., J.D. MacEwen, and E.H. Vernot. 1977. Acute toxicity of five atmospheric pollutants from army ammunition plants. Section I. In: E.R. Kinkead, J.D. MacEwen, C.C. Haun, E.H. Vernot, and J.C. Dacre, eds. *Toxic Hazard Evaluation of Five Atmospheric Pollutants from Army Ammunition Plants.* AAMRL-TR-77-25, pp. 5-17. Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory, Frederick, MD: U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick.

Kluwe, W.M., B.N. Gupta, and J.C. Lamb. 1983. The comparative effects of 1,2-dibromo-3-chloropropance (DBCP) and its metabolites, 3-chloro-1,2-propaneoxide (epichlorohydrin), 3-chloro-1,2-propanediol (`-chlorohydrin), and oxalic acid, on the urogenital system of male rats. *Toxicol. Appl. Pharmacol.* 70:67-86.

Kociba, R.J., S.B. McCollister, C. Park, T.R. Torkelson, and P.J. Gehring. 1974. 1,4-Dioxane. I. Results of a 2-year ingestion study in rats. *Toxicol. Appl. Pharmacol.* 30: 275-286.

Kopf-Maier, P. 1982. Development of necroses, virus activation, and giant cell formation after treatment of Ehrlich ascites tumor with metallocene dichlorides. *J. Cancer Res. Clin. Oncol.* 103:145-164.

Kopf-Maier, P. 1988. Histologic and ultrastructural alterations of a xenografted human colon adenocarcinoma after treatment with titanocene dichloride. *J. Cancer Res. Clin. Oncol.* 114: 250-258.

Kopf-Maier, P. and P. Erkenswick. 1984. Teratogenicity and embryotoxicity of titanocene dichloride in mice. *Toxicology* 33: 171-181.

Kopf-Maier, P. and R. Martin. 1989. Subcellular distribution of titanium in the liver after treatment with the antitumor agent titanocene dichloride: A study using electron spectroscopic imaging. *Virchow's Arch. B Cell Pathol.* 57:213-222.

Kopf-Maier, P., U. Brauchle, and A. Henssler. 1988. Organ distribution and pharmacokinetics of titanium after treatment with titanocene dichloride. *Toxicology* 51: 291-298.

Kreiling, R., R.J. Laib, and H.M. Bolt. 1986. Alkylation of nuclear proteins and DNA after exposure of rats and mice to [1,4-$^{14}$C] 1,3-butadiene. *Toxicol. Lett.* 30:131-136.

Kuhler, T. 1984. Mercapturic acid metabolites from 2-, 3-, and 4-ethenyl-methylbenzenes in the rat. *Xenobiotica* 14:417-428.

Laskin, D.M., I.B. Robinson, and J.P. Weinmann. 1954. The experimental production of sarcomas by methylmethacrylate implants. *Proc. Soc. Exp. Biol. Med.* 87:329-332.

Lijinsky, W. and A. Andrews. 1980. Mutagenicity of vinyl compounds in *Salmonella typhimurium. Teratol. Carcinog. Mutag.* 1:259-267.

Lindstrom, H.V., W.H. Hansen, A.A. Nelson, and O.G. Fitzhugh. 1963. The metabolism of FD & C Red No. 1. I. The fate of 2,5-para-xylidine and 2,6-meta-xylidine in rats and observations on the toxicity of xylidine isomers. *J. Pharmacol. Exp. Ther.* 142:256.

Loeser, E. 1982. IISRP Sponsored Toxicity studies on 1,3-Butadiene. Paper presented at the Twenty-Third Meeting of the International Institute of Synthetic Rubber Producers.

**Lorenz, J., H. Schmassmann, E. Ohnhaus, and F. Oesch.** 1979. Activities of polycyclic hydrocarbon activating and inactivating enzymes in human lungs of smokers, non-smokers, lung cancer, and non-cancer patients. *Arch. Toxicol. (Suppl).* 2:483-489.

**MacEwen, J.D., E.H. Vernot, C.C. Haun, E.R. Kinkead, and A. Hull.** 1981. Chronic inhalation toxicity of hydrazine: Oncogenic effects. AFAMRL-TR-81-56. Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory.

**MacFarland, R.T., A.J. Gandolfi, and I.G. Sipes.** 1984. Extra-hepatic GSH-dependent metabolism of 1,2-dibromoethane (DBE) and 1,2-dibromo-3-chloroporpane in the rat and mouse. *Drug Chem. Toxicol.* 7:213-227.

**Magnusson, G., N.O. Bodin, and E. Hansson.** 1971. Hepatic changes in dogs and rats induced by xylidine isomers. *Acta Pathol. Microbiol. Scand.* 79: 639-648.

**Maltoni, C. and I.J. Selikoff, eds.** 1988. Living in a chemical world: occupational and environmental significance of industrial carcinogens. *Ann. N.Y. Acad. Sci.* 534:1-1045.

**Malvoisin, E., G. Lhoest, F. Poncelet, M. Roberfroid, and M. Mercier.** 1979. Identification and quantitation of 1,2-epoxybutene-3 as the primary metabolite of 1,3-butadiene. *J. Chromatogr.* 178:419-425.

**Malvoisin, E. and M. Roberfroid.** 1982. Hepatic microsomal metabolism of 1,2-butadiene. *Xenobiotica* 12:137-144.

**Mandel, H.G.** 1971. Pathways of drug biotransformation: biochemical conjugations. In: B.N. LaDu, H.G. Mandel, and E.L. Way, eds. *Fundamentals of Drug Metabolism and Drug Disposition*, pp. 149-186. Baltimore, MD: Williams and Wilkins.

**Marrs, T.C., H.F. Colgrane, N.L. Cross, M.F. Jazzard, and R.F.R. Brown.** 1983. A repeated dose study of the toxicity of 2-chlorobenzilidene malononitrile (CS) aerosol in three species of laboratory animals. *Arch. Toxicol.* 52:183-198.

**Marsden, P.J. and J.E. Casida.** 1980. 2-Haloacrylic acids as indicators of mutagenic 2-haloacrolein intermediates in mammalian metabolism of selected promutagens and carcinogens. *J. Agric. Food Chem.* 30: 627-631.

**McGregor, D.B., A. Brown, P. Catlonuch, I. Edwards, D. McBride, and W.J. Caspary.** 1988. Responses of the L5178Y$_{t}$k$^{+}$/tk-mouse lymphoma cell forward mutation assay: II. 18 coded chemicals. *Environ. Molec. Mutagen.* 11:91-118.

**McNamara, B.P., R.A. Renne, H. Rozmiarek, D.F. Ford, and E.J. Owens.** 1973. CS: A study of carcinogenicity. Iss. No. 4D 770:365/5 GA. U.S. Department of Commerce, National Technical Information Service.

**Miller, E.C. and J.A. Miller.** 1981. Mechanisms of chemical carcinogenesis. *Cancer* 47:1055-1064.

**Mitchell, J.R., H.J. Zimmerman, K.G. Ishak, V.P. Thorgiersson, J.A. Timbrell, W.R. Snodgrass, and S.D. Nelson.** 1976. Isoniazid liver injury: Clinical spectrum, pathology, and probable pathogenesis. *Ann. Intern. Med.* 84:181-192.

**Monks, T.J. and S.S. Lau.** 1988. Reactive intermediates and their toxicological significance. *Toxicology* 52:1-53.

National Cancer Institute (NCI). 1980. Bioassay of dibromochloropropane (inhalation) for possible carcinogenicity. Publication No. (NIH) 80-1762. Bethesda, MD: U.S. Department of Health and Human Services.

National Institute for Occupational Safety and Health (NIOSH). 1978. Criteria for a recommended standard – occupational exposure to cresol. DHEW (NIOSH) Publication No. 78-133. Cincinnati, OH: Department of Health, Education, and Welfare.

National Toxicology Program (NTP). 1982a. Carcinogenesis bioassay of 1,2-dibromoethane (CAS No. 106-93-4) in F344 rats and B6C3F$_1$ mice (inhalation study). Technical Report No. 210. NIH Publication No. 82-1766.

National Toxicology Program (NTP). 1982b. Carcinogenesis bioassay of 1,2-dibromo-3-chloropropane (CAS No. 96-12-8) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 206. NIH Publication No. 82-1762.

National Toxicology Program (NTP). 1984. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in B6C3F$_1$ mice (inhalation studies). Technical Report No. 288. NIH Publication No. 84-2544.

National Toxicology Program (NTP). 1985a. Toxicology and carcinogenesis studies of propylene oxide (CAS No. 75-56-9) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 267. NIH Publication No. 85-2527.

National Toxicology Program (NTP). 1985b. Toxicology and carcinogenicity studies of dimethylvinyl chloride (1-chloro-2-methyl-propene) in F344/N rats and B6C3F$_1$ mice (gavage studies). Technical Report No. 316. NIH Publication No. 86-2572.

National Toxicology Program (NTP). 1986. Toxicology and carcinogenesis studies of methyl methacrylate (CAS No. 80-62-6) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 314. NIH Publication No. 87-2570.

National Toxicology Program (NTP). 1988. Toxicology and carcinogenesis studies of 1,2-epoxybutane (CAS No. 106-88-7) in F344/N rats and B6C3F$_1$ mice (gavage studies). Technical Report No. 329. NIH Publication No. 88-2585.

National Toxicology Program (NTP). 1990a. Toxicology and carcinogenesis studies of vinyl toluene (mixed isomers) (65%-71% meta-isomer and 32%-35% para-isomer) (CAS No. 25013-15-4) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 375. NIH Publication No. 90-2830.

National Toxicology Program (NTP). 1990b. Toxicology and carcinogenesis studies of iodinated glycerol (organidin) (CAS No. 5634-39-9) in F344/N rats and B6C3F$_1$ mice (gavage studies). Technical Report No. 340. NIH Publication No. 90-2596.

National Toxicology Program (NTP). 1990c. Toxicology and carcinogenesis studies of glycidol in F344/N rats and B6C3F$_1$ mice. Technical Report No. 374.

National Toxicology Program (NTP). 1990d. Toxicology and carcinogenesis studies of allyl glycidyl ether (CAS No. 106-92-3) in Osborne-Mendel rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 376. NIH Publication No. 90-2831.

**National Toxicology Program (NTP).** 1990e. Toxicology and carcinogenesis studies of tetranitromethane (CAS No. 509-14-8) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 386. NIH Publication No. 90-2841.

**National Toxicology Program (NTP).** 1990f. Toxicology and carcinogenesis studies of 2,6-xylidine ((2,6-dimethylaniline) (CAS No. 87-62-7) in Charles River CD Rats (feed studies). Technical Report No. 278. NIH Publication No. 90-2534.

**National Toxicology Program (NTP).** 1990g. Toxicology and carcinogenesis studies of CS2 (94% o-chlorobenzalmalononitrile (CAS No. 2698-41-1) in F344/N rats and B6C3F$_1$ mice (inhalation studies). Technical Report No. 377. NIH Publicaton No. 90-2832.

**National Toxicology Program (NTP).** 1991a. Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in B6C3F$_1$ mice (inhalation studies). Technical Report No. 410. NIH Publication No. 91-3141.

**National Toxicology Program (NTP).** 1991b. Toxicology and carcinogenesis studies of titanocene dichloride (CAS No. 1271-19-8) in F344/N rats (gavage studies). Technical Report No. 399. NIH Publication No. 91-2854.

**National Toxicology Program (NTP).** 1992a. Toxicology and carcinogenesis studies of monochloroacetic acid (CAS No. 79-11-8) in F344/N rats and B6C3F$_1$ mice (gavage studies). Technical Report No. 396. NIH Publication No. 92-2851.

**National Toxicology Program (NTP).** 1992b. Toxicology studies of cresols (CAS Nos. 95-48-7, 108-39-4, 106-44-5) in F344/N rats and B6C3F$_1$ mice (feed studies). Technical Report No. TOX-09. Draft report.

**Nelson, S.D., J.R. Mitchell, J.A. Timbrell, W.R. Snodgrass, and G.B. Corcoran.** 1976. Isoniazid and iproniazid: Activation of metabolites to toxic intermediates in man and rat. *Science* 193:901-903.

**Nelson, S.D. and W.P. Gordon.** 1980. Metabolic activation of hydrazines. In: R. Snyder, D.V. Parke, J. Kocsis, D.J. Jollow, and G.G. Gibson, eds. *Biological Reactive Intermediates. 2. Chemical Mechanisms and Biological Effects*, pp. 971-981. New York: Plenum Press.

**Norppa, H. and H. Vainio.** 1983. Induction of sister chromatid exchanges by styrene analogs in cultured human lymphocytes *Mutat. Res.* 116:379-387.

**O'Brien, K.A.F., L. Smith, and G.M. Cohen.** 1985. Differences in naphthalene-induced toxicity in the mouse and rat. *Chem. Biol. Interact.* 55:109-122.

**Olmstead, E.V.** 1960. Pathological changes in ethylene dibromide poisoning. *Arch. Ind. Health* 21:525-529.

**Omichinski, J.G., E.J. Soderlund, E. Dybing, P.G. Pearson, and S.D. Nelson.** 1988. Detection and mechanism of formation of the potent direct-acting mutagen 2-bromoacrolein from 1,2-dibromo-3-chloropropane. *Toxicol. Appl. Pharmacol.* 92:286-294.

**Oppenheimer, B.S., E.T. Oppenheimer, A.P. Danishefsky, and A.P. Stout.** 1955. Further studies of polymers of carcinogenic agents in animals. *Cancer Res.* 15:333-340.

**Ozawa, N., and F.P. Guengerich.** 1983. Evidence for formation of an S-[2-(N$^7$-guanyl)ethyl] glutathione adduct in glutathione-mediated binding of the carcinogen 1,2-dibromoethane to DNA. *Proc. Natl. Acad. Sci. U.S.A.* 80:5266-5270.

Plunkett, E.R. 1987. *Handbook of Industrial Toxicology*, 3rd Ed. New York: Chemical Publishing.

Pneumatikakis, G., A. Yannopoulos, and J. Markopoulos. 1988. Interactions of dichloro-*bis*($\eta^5$-cyclopentadienyl)titanium(IV) with nucleosides. *Inorg. Chim. Acta* 151:125-128.

Poss, R., W. Thilly, and D. Raden. 1979. Methylmethacrylate is a mutagen for *Salmonella typhimurium*. *J. Bone Jt. Surg.* 61A:1203-1207.

Ptitsyn, L.A., M.A. Chepyzheva, G.Y. Kolomiitseva, and E.P. Senchenkov. 1979. Interaction of tetranitromethane with deoxyribonucleoproteins. *Biokhimya* 43:1435-1441.

Rietueld, E.C., L.P.C. Delbressine, T.H.J.M. Waegemaekers, and F. Seutter-Berlage. 1983. 2-Chlorobenzylmercapturic acid, a metabolite of the riot control agent 2-chlorobenzilidene malononitrile (CS) in the rat. *Arch. Toxicol.* 54:139-144.

Riordan, J.F. B.L. and Vallee. 1972. Nitration with tetranitromethane. *Methods Enzymol.* 25:515-521.

Rosen, G.M., E.J. Rauckman, S.P. Ellington, D.C. Dahlin, J.L. Christie, and S.D. Nelson. 1985. Reduction and glutathione conjugation reactions of *N*-acetyl-*p*-benzoquinone imine and two dimethylated analogues. *Mol. Pharmacol.* 25: 151-157.

Sax, N.I. 1984. *Dangerous Properties of Industrial Materials*, 6th ed, p. 676. New York: Van Nostrand Reinhold Co.

Sax, N.I. and R.J. Lewis, Sr. 1989. *Dangerous Properties of Industrial Materials, 7th* Ed., Vol. *2*. New York: Van Nostrand Reinhold.

Scheline, R.R. 1973. Metabolism of foreign compounds by gastrointestinal microorganisms. *Pharmacol. Rev.* 25:451-523.

Schmidt, U. and E. Loeser. 1985. Species differences in the formation of butadiene monoxide from 1,3-butadiene. *Arch. Toxicol.* 57:222-225.

Segall, Y., E. Kimmel, D.R. Dohn, and J.E. Casida. 1985. 3-Substituted 2-halopropenals: Mutagenicity, detoxification, and formation from 3-substituted 2,3-dihalopropenal promutagens. *Mutat. Res.* 158:61-68.

Severi, L. and C. Biancifiori. 1968. Hepatic carcinogenesis in CBA/Cb/Se mice and Cb/Se rats by isonicotinic acid hydrazine and hydrazine sulfate. *J. Natl. Cancer Inst.* 41:331-349.

Shank, R.C. 1981. Comparative biochemistry and metabolism, Part 1: Carcinogenesis. AFAMRL-TR-81-83, Wright Patterson Air Force Base, OH.

Shank, R.C., W.S. Bosan, and C.E. Lambert. 1984. Studies on the mechanism of action of hydrazine-induced methylation of DNA guanine. Air Force Aerospace Medical Research Laboratory Technical Report. AFAMRL-TR-84-057. Wright-Patterson Air Force Base, OH.

Shank, R.C. 1987. Comparative study on the indirect methylation of liver DNA guanine by the 1-carbon pool in hepatotoxicity. *Arch. Toxicol. (Suppl.)* 10, 204-216.

Shih, T.W. and D.L. Hill. 1981. Metabolic activation of 1,2-dibromoethane by glutathione transferase and by microsomal mixed function oxidase: Further evidence for formation of two reactive metabolites. *Res. Commun. Chem. Pathol. and Pharmacol.* 33:449-461.

Short, C.R., C. King, P.W. Sistrunck, and K.M. Kerr. 1983. Subacute toxicity of several ring-substituted dialkylanilines in the rat. *Fundam. Appl. Toxicol.* 3:285-292.

Short, C.R., M.L. Hardy, and S.A. Barker. 1989. The *in vivo* oxidative metabolism of 2,4- and 2,6-dimethylaniline in the dog and rat. *Toxicology* 57:45-58.

Silver, E.H. and S.D. Murphy. 1978. The effects of carboxylesterase inhibition on the toxicity of methylmethacrylate, ethyl acrylate, and acrylic acid. *Toxicol. Appl. Pharmacol.* 45:312.

Singh, A.R., W.H. Lawrence, and J. Autian. 1972. Embryonic-fetal toxicity and teratogenic effects of a group of methacrylate esters in rats. *J. Dent. Res.* 51:1632-1638.

Sinha, B. 1987. Activation of hydrazine derivatives to free radicals in the perfused rat liver: A spin-trapping study. *Biochim. Biophys. Acta* 924:261-269.

Solomon, J.J. and A. Segal. 1989. DNA adducts of propylene oxide and acrylonitrile epoxide: Hydrolytic deamination of 3-alkyl-dCyd to 3-alkyl-dUrd. *Environ. Health Perspect.* 81:19-22.

Spealman, C.R., R.J. Main, H.B. Haag, and P.S. Larson. 1945. Monomeric methyl methacrylate: Studies on toxicity. *Ind. Med.* 14:292.

Springer, D.L., B.M. Krivak, D.J. Broderick, D.J. Reed, and F.N. Dost. 1981. Metabolic fate of hydrazine. *J. Toxicol. Environ. Health* 8:21-29.

Srinivas, P. and L.T. Burka. 1988. Metabolism of 1-chloro-2-methylpropene: Evidence for reactive chloroaldehyde intermediates. *Drug Metab. Disposit.* 16:449-454.

Stillwell, W.G., M.G. Horning, G.W. Griffin, and W.S. Tsang. 1982. Identification of new sulfur containing metabolites of naphthalene in mouse urine. *Drug Metab. Dispos.* 10:624-631.

Streeter, A.J. and J.A. Timbrell. 1983. Enzyme-mediated covalent binding of hydrazine to rat liver microsomes. *Drug Metab. Dispos.* 11:179-183.

Streeter, A.J. and J.A. Timbrell. 1985. The *in vitro* metabolism of hydrazine. *Drug Metab. Dispos.* 13:255-259.

Sugiura, K., S. Yamanaka, S. Fukasawa, and M. Goto. 1978. The mutagenicity of substituted and unsubstituted styrene oxides in *Escherichia coli*: Relationship between mutagenesis and physicochemical properties. *Chemosphere* 9:737-742.

Sundheimer, D.W., R.D. White, and K. Brendel. 1982. The bioactivation of 1,2-dibromoethane in rat hepatocytes: Covalent binding to nucleic acids. *Carcinogenesis* 3:1129-1133.

Terao, J., B.P. Lim, H. Murakami, and S. Matsushita. 1987. Quinone formation from carcinogenic benzo[*a*]pyrene mediated by lipid peroxidation in phosphatidylcholine liposomes. *Arch. Biochem. Biophys.* 254:472-481.

Thorgeirsson, S.S., I.B. Glowinski, and M.E. McManus. 1983. Metabolism, mutagenicity, and carcinogenicity of aromatic amines. *Rev. Biochem. Toxicol.* 5:349-386.

Thorgeirsson, S.S., M.E. McManus, and I.B. Glowinski. 1984. Metabolic processing of aromatic amines. In: J.R. Mitchell and M.G. Horning, eds., *Drug Metabolism and Drug Toxicity*, p. 183. New York: Raven Press.

Timbrell, J.A. 1990. *U.S. Air Force Funded Study of Hydrazine Metabolism and Toxicity*. 2nd Annual Report, Grant #AFOSR-88-0313.

Toney, J.H., C.P. Brock, and T.J. Marks. 1986. Aqueous coordination chemistry of vanadocene dichloride with nucleotides and phosphoesters. Mechanistic implications for a new class of anti-tumor agents. *J. Am. Chem. Soc.* 108:7263-7274.

Torkelson, T.R., B.J.K. Leong, R.J. Kociba, W.A. Richter, and P.J. Gehring. 1974. 1,4-Dioxane. II. Results of a 2-year inhalation study in rats. *Toxicol. Appl. Pharmacol.* 30:287-298.

Treon, J.F., H. Sigmon, H. Wright, and K.V. Kitzmiller. 1949. The toxicity of methyl and ethyl acrylates. *J. Ind. Hyg. Toxicol.* 31:317-326.

Upshall, D.G. 1973. Effects of o-chlorobenzylidene malononitrile (CS) and the stress of aerosol inhalation upon rat and rabbit embryonic development. *Toxicol. Appl. Pharmacol.* 24:45-59.

Van Duuren, B.L. 1969. Carcinogenic epoxides, lactones, and haloethers and their mode of action. *Ann. N.Y. Acad. Sci.* 163:633-651.

Van Bladeren, P.J., D.D. Breimer, J.A.T.C.M. van Huijgevoort, N.P.E. Vermeulen, and A. van der Gen. 1981. The metabolic formation of *N*-acetyl-*S*-2-hydroxyethyl-L-cysteine from tetradeutero-1,2-dobromoethane. Relative importance of oxidation and glutathione conjugation *in vivo*. *Biochem. Pharmacol.* 30:2499-2502.

Vernot, E.H., J.D. MacEwen, E.R. Kinkead, and C.C. Haun. 1977. Comparative subchronic toxicity of tetranitromethane and nitrogen dioxide: Acute toxicity of methyl nitrate to guinea pigs. Section II. pp 18-36. In: E.R. Kinkead, J.D. MacEwen, C.C. Haun, E.H. Vernot, and J.C. Dacre, eds. *Toxic Hazards Evaluation of Five Atmospheric Pollutants from Army Ammunition Plants*. AAMRL-TR-77-25. Wright-Patterson Air Force Base, OH: Frederick, MD. Aerospace Medical Research Laboratory, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick.

Vernot, E.H., J.D. MacEwen, R.H. Bruner, C.C. Haun, E.R. Kinkead, D.E. Prentice, A. Hall, III, R.E. Schmidt, R.L. Eason, G.B. Hubbard, and J.T. Young. 1985. Long-term inhalation toxicity of hydrazine. *Fundam. Appl. Toxicol.* 5:1050-1064.

von Daeniken, A., U. Friederich, W.K. Lutz, and C. Schlatter. 1981. Tests for mutagenicity in *Salmonella* and covalent binding to DNA and protein in the rat of the riot control agent o-chlorobenzylidene malononitrile (CS). *Arch. Toxicol.* 49:15-27.

Wild, D., K. Eckhardt, D. Harnasch, and M.-T. King. 1983. Genotoxicity study of CS (ortho-chlorobenzylidene-malononitrile) in *Salmonella, Drosophila*, and mice: Failure to detect mutagenic effects. *Arch. Toxicol.* 54:167-170.

Williams, G.M. and J.H. Weisburger. 1991. In: M.O. Amdur, J. Doull, and C.D. Klaassen, eds. *Toxicology: The Basic Science of Poisons.*, pp. 127-200. New York: Pergamon Press.

Woo, Y., J.C. Arcos, and M.F. Argus. 1977a. Metabolism *in vivo* of dioxane: Identification of *p*-dioxane-2-one as the major urinary metabolite. *Biochem. Pharmacol.* 26:1535-1538.

**Woo, Y., M.F. Argus, and J.C. Arcos.** 1977b. Metabolism *in vivo* of dioxane: Effects of inducers and inhibitors of hepatic mixed function oxidases. *Biochem. Pharmacol.* 26:1539-1542.

**Yllner, S.** 1971. Metabolism of chloroacetate-1-14C in the mouse. *Acta Pharmacol. Toxicol.* 30:69-80.

**You, M., U. Candrian, R.R. Maronpot, G.D. Stoner, and M.W. Anderson.** 1989. Activation of the *K-ras* protooncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse. *Proc. Natl. Acad. Sci. USA* 86:3070-3074.

**Young, J.D., W.H. Braun, P.J. Gehring, B.S. Horvath, and R.L. Daniel.** 1976. 1,4-Dioxane and beta-hydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors. *Toxicol. Appl. Pharmacol.* 38:643-646.

**Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, and W. Speck.** 1987. *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.* (Suppl. 9):1-10.



☆U.S. Government Printing Office: 1993 — 750-061/60250

# END

# FILMED

DATE: 4-93

# DTIC