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DRINKING WATER CRITERIA DOCUMENT
FOR TRICHLOROBENZENES

Prepared for

OFFICE OF WATER

Prepared by

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1985; however, more recent data may have been added during the review process. Editorial changes were also made in 1991 when this document was finalized.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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Special Note: This document was developed from the comprehensive information found in the Health Assessment Document for Chlorinated Benzenes (EPA 600/8-84-015F).

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BUN	Blood urea nitrogen
DWEL	Drinking water equivalent level
EPN	O-ethyl-O-p-nitrophenyl phenylphosphothionate
G-6-P	Glucose-6-phosphatase
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GI	Gastrointestinal
i.p.	Intraperitoneal
i.v.	Intravenous
LDH	Lactic dehydrogenase
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
RfD	Reference dose
SAP	Serum alkaline phosphatase
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
wt	Weight

I. SUMMARY

The trichlorobenzenes are a group of three chemical isomers in which three chlorine atoms have been added to a benzene ring. The 1,2,3- and 1,3,5-trichlorobenzenes are normally solid while 1,2,4-trichlorobenzene is normally a liquid at 25°C. The trichlorobenzenes are only slightly soluble in water (6.6-34.6 mg/ℓ at 25°C). The trichlorobenzenes are produced in relatively small amounts (1.3-7 million kg/year) and are used primarily as chemical intermediates, solvents, insecticides, and coolants and insulators in electrical equipment. Analysis of the trichlorobenzenes in water normally involves a solvent extraction and cleanup method followed by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS) analysis. The water analysis methods are slightly modified for analysis of trichlorobenzenes in soil and food.

The limited comparative pharmacokinetic data available on the trichlorobenzenes prevent specification of the absorption, distribution, metabolism and excretion of the individual isomers. The trichlorobenzenes appear to enter the systemic circulation readily by inhalation, ingestion and dermal absorption; however, data were not available to quantitate the rates of these processes nor of any of the pharmacokinetic processes. Initial distribution of the trichlorobenzenes and metabolites is mainly to the liver, kidneys and adrenals, followed by migration to adipose tissue or metabolism to polar compounds that are more readily excreted. From the available data, it seems relatively clear that metabolism in at least three species has a common first step, the production of an arene oxide intermediate. Subsequent metabolic steps, however, vary among the species examined, at least for the most studied isomer, 1,2,4-trichlorobenzene.

In general, the pharmacokinetics of the trichlorobenzenes are similar to those described for the other halogenated aromatics. These compounds are lipophilic and their metabolism and excretion depends on their conversion to polar intermediates. In addition, their lipophilic character provides for ready absorption from the gastrointestinal tract and initial distribution to the more highly perfused tissues, particularly the liver, kidneys and adrenal and thyroid glands, after which they are either metabolized and excreted or redistributed to adipose tissue or skin. Additional experiments are needed to clarify the relationship of these studies to the metabolism of trichlorobenzenes in humans.

The effects in mammals of acute exposure by various routes to trichlorobenzenes include local irritation, convulsions and death. Livers, kidneys, adrenals, mucous membranes and brain ganglion cells appear to be target organs with effects including edema, necrosis, fatty infiltration of livers, increased organ weights, porphyrin induction and microsomal enzyme induction.

Quantitative data on the toxic effects of trichlorobenzene following subchronic exposure by various routes were obtained in a variety of species. In general, these studies indicate that the liver, kidney, adrenal glands and thyroid glands are target organs. Oral gavage of 1,2,4-trichlorobenzene at 53.5 mg/kg (10 ppm) for 95 days induced vacuolization of the zona fasciculata of the adrenal cortex in several rats. One study identified 14.8 mg/kg/day of 1,2,4-trichlorobenzene as a no-observed-adverse-effect level (NOAEL) in rats, while another study reported that some rats exposed by inhalation to 1,3,5-trichlorobenzene at 1000 mg/m^3 for 13 weeks showed squamous metaplasia and focal hyperplasia

of the respiratory epithelium, which appeared to be reversible. Subchronic oral studies have also shown that the trichlorobenzenes induce transient hepatic xenobiotic metabolism and porphyria. Subchronic dermal exposure resulted in mild to moderate irritation .

One chronic study, on the effects of trichlorobenzene painted on the skin of mice for 2 years, reported increased mortality in females at the low dose (30% solution in acetone) and in both sexes at the high dose (60% solution).

Results of two reports on mutagenicity tests with Salmonella typhimurium test strains were negative. However, this test system is generally insensitive to chlorinated compounds. One carcinogenicity study, a 2-year skin painting study in mice, failed to demonstrate a conclusive tumorigenic effect. A multigeneration study of the reproductive effects of oral exposure of rats to trichlorobenzene failed to show effects on reproduction. Oral teratogenicity studies in rats showed mild osteogenic changes in pups and significantly retarded embryonic development as measured by fetal growth parameters.

Human exposure to 1,2,4-trichlorobenzene at 3-5 ppm causes eye and respiratory irritation. The only other data on human exposure are individual case reports of aplastic anemia of persons exposed occupationally or domestically.

No health advisories (HAs) or lifetime drinking water equivalent levels (DWELs) are suggested for the 1,2,3- and 1,3,5-trichlorobenzene isomers because of insufficient data being available for evaluation.

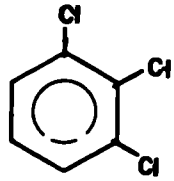
The 1-day HA for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child is based on a study in which female Wistar rats were given single oral doses of 1,2,4-trichlorobenzene and were then evaluated 24 hours later. The 10-day HA for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child is based on a study in which male CD rats were given 1,2,4-trichlorobenzene for 14 days and were then evaluated for effects. The longer-term HAs for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child and 5 mg/ℓ for a 70 kg adult are based on a study in which CD-1 rats were exposed orally to 1,2,4-trichlorobenzene for 95 days and evaluated for reproductive effects and organ weight changes. A DWEL for 1,2,4-trichlorobenzene of 0.4 mg/ℓ for a 70 kg adult is derived from an RfD of 0.01 mg/kg/day (verified 12/12/91) from the same 95-day reproductive study. The data available on 1,2,4-trichlorobenzene is inadequate for making any conclusions about its potential carcinogenicity in humans. The trichlorobenzenes are classified as U.S. EPA Group D compounds at this time, that is, available data are insufficient. This information was verified by the CRAVE workgroup in October 1988.

II. PHYSICAL AND CHEMICAL PROPERTIES

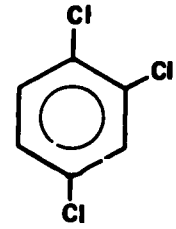
The trichlorobenzenes are a group of three chemical isomers in which three chlorine substituents have been added to a benzene ring. The trichlorobenzenes are only slightly soluble in water (6.6-34.6 mg/ℓ at 25°C). The trichlorobenzenes are produced in relatively small amounts (1.3-7 million kg/year is the estimated 1983 production) (U.S. EPA, 1983; Chlorobenzene Producers Association, 1984) and are used primarily as chemical intermediates, solvents, insecticides, and coolants and insulators in electrical equipment (Hawley, 1977; Slimak et al., 1980). Trichlorobenzenes have been detected in all environmental media including drinking water, and have been found to bioaccumulate in fish (U.S. EPA, 1985). In addition to the exposure of humans during the manufacture and use of trichlorobenzenes, exposure could result from inhalation of contaminated air and ingestion of contaminated food and water.

The chemical structures of the trichlorobenzenes are shown in Figure II-1. Synonyms, trade names and identification numbers for the trichlorobenzenes are found in Table II-1. Some of the physical and chemical properties of the trichlorobenzenes are found in Tables II-2 and II-3. 1,2,3-Trichlorobenzene is a white crystalline solid (platelets from alcohol) that is volatile with steam. It is slightly soluble (31.5 mg/ℓ) at 25°C in water, slightly soluble in alcohol, soluble in benzene and carbon disulfide, and very soluble in ether (NLM, 1981a; Yalkowsky and Valvani, 1980).

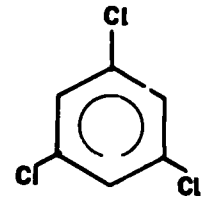
1,2,4-Trichlorobenzene is a colorless liquid at 25°C but at somewhat reduced temperatures may also take the form of rhombic crystals because its



1,2,3-TRICHLOROBENZENE



1,2,4-TRICHLOROBENZENE



1,3,5-TRICHLOROBENZENE

FIGURE II-1

Chemical Structures of the Trichlorobenzenes

TABLE II-1

Synonyms, Trade Names and Identification Numbers of the Trichlorobenzenes*

Chemical	Identification Number	Synonyms and Trade Names
1,2,3-	CAS No. 87-61-6	vic-Trichlorobenzene 1,2,6-Trichlorobenzene v-Trichlorobenzene
1,2,4-	CAS No. 120-82-1 TSL No. DC2100000	Benzene, 1,2,4-trichloro- asym-Trichlorobenzene TCB Trojchlorobenzen (Polish) 1,2,4-Trichlorobenzol Hostetex L-Pec
1,3,5-	CAS No. 108-70-3	s-Trichlorobenzene sym-Trichlorobenzene TCB TCBA Benzene, 1,3,5-trichloro-

*Source: NLM, 1981a,b, Toxicology Data Bank (TDB)

Physical Properties of the Trichlorobenzenes[a]

Chemical	Molecular Weight	Melting Point (°C)	Boiling Point[b] (°C)	Density[c] (g/mℓ)	Henry's Law Constant x 10^{-3} (atm m^3 mol^{-1})	Log P^o[d]	Water Solubility (mg/ℓ)[e]	Flash Point (°C)	Index of Refraction at (°C)
Trichlorobenzene									
1,2,3-	181.46	52.6	221	1.69	1.0[d]	4.1[f]	31.5[g]	113	1.5776(19)
1,2,4-	181.46	16.95	213.5	1.45	1.42[h]	4.12[i]	34.6[g]	110	1.5717(20)
1,3,5-	181.46	63.4	208.4	1.39(64)[j]	NA	NA	6.6[g]	107	1.5662(19)

[a]Data are from the NLM, 1981 a,b, Toxicology Data Bank (TDB), except as noted.

[b]At 760 mm

[c]At 20°C, except as noted

[d]MacKay et al., 1979

[e]At 25°C

[f]Isomer unspecified

[g]Yalkowsky and Valvani, 1980

[h]Warner et al., 1980

[i]Hansch and Leo, 1981

[j]Horvath, 1982

P^o = Octanol/water partition coefficient at 25°C

NA = Not available

TABLE II-3

Vapor Pressures and Vapor Densities of the Trichlorobenzenes

Chemical	Vapor Pressure (mm Hg)	Specific Vapor Density (air = 1)
Trichlorobenzene		
1,2,3-	0.07 at 25°C[a] 1 at 40°C[b]	6.26[b]
1,2,4-	0.29 at 25°C[a] 1 at 38.4°C[b]	6.26[b]
1,3,5-	0.15 at 25°C[a] 10 mm at 78°C[b]	6.26[b]

[a]NLM, 1981a,b; 1982

[b]Sax, 1979

melting point occurs at 16.95°C. It possesses a distinctive odor, similar to that of 1,4-dichlorobenzene, and is considered volatile with steam (NLM, 1981b). It is slightly soluble in water, 34.6 mg/ℓ at 25°C (Yalkowsky and Valvani, 1980); miscible with benzene, petroleum ether and carbon disulfide; slightly soluble in ethanol; and very soluble in diethyl ether (NLM, 1981b). The summer sunlight photolysis half-life for 1,2,4-trichlorobenzene in surface waters at 40° latitude has been calculated to be 450 years (Dulin et al., 1986). An information sheet (Dow Chemical Company, 1979-1980) listed a purity of 100% for its product. Kao and Poffenberger (1979) reported that commercial 1,2,4-trichlorobenzene may contain monochlorobenzene (<0.1 wt percent) and di- and tetrachlorobenzenes (<0.5 wt percent and <0.5 wt percent) with the 1,2,4-trichlorobenzene content being ~97%.

1,3,5-Trichlorobenzene takes the physical form of white crystals or needles. It is very slightly soluble (6.6 mg/ℓ at 25°C) in water; sparingly soluble in alcohol; and soluble in ether, benzene, petroleum ether, carbon disulfide and glacial acetic acid (NLM, 1982; Yalkowsky and Valvani, 1980).

Chemical Analysis

A solvent extraction and cleanup method followed by GC or GC/MS is the most commonly used method to isolate trichlorobenzenes from water. Methods that are slightly modified from the analytical procedures for aquatic samples are used for the analysis of trichlorobenzenes in soil and food. The usual sampling and analytical methods for airborne trichlorobenzenes involve the adsorption and concentration of airborne vapors on sorbent-packed cartridges followed by thermal desorption and GC analysis using

either flame ionization detection, electron capture (EC) detection, or photoionization detection. The following sections provide examples of these analytical methods.

Chemical Analysis in Water. The purge-trap technique does not provide quantitative recoveries for compounds with low volatilities, such as trichlorobenzenes. Therefore, a solvent extraction and cleanup method is normally used to produce organic extracts suitable for GC/MS analysis. The U.S. EPA (1982) (Method 612) has recommended the use of Florisil column chromatography as a cleanup step before the quantification of the samples by GC with EC detector. This recommended method is applicable for the determination of trichlorobenzenes in drinking water and wastewater.

Chemical Analysis in Soil, Sediment and Chemical Waste Disposal Site Samples. The solvent extraction method was used by Lopez-Avila et al. (1983) to determine trichlorobenzenes in sediment samples. In this method, the solvent extract was subjected to acid-base fractionation. The base/neutral fraction containing the trichlorobenzenes was fractionated by silica gel chromatography. The final separation and quantification was accomplished by GC/MS. The recovery of 1,2,4-trichlorobenzene by this method was 67% at a spike level of 400 ng/g of dry sediment.

Chemical Analysis in Fish and Other Foods.

Fish -- The determination of trichlorobenzenes in fish samples can be accomplished by a solvent extraction method. In one method, Kuehl et al. (1980) subjected the solvent extract to Florisil and gel permeation on chromatographic separation, followed by GC/MS identification and quantification of trichlorobenzene in fish samples.

Chemical Analysis in Air. Lewis and MacLeod (1982) have developed and evaluated a portable low-volume air sampling system for indoor air monitoring of semivolatile organic chemicals. Two types of sampling cartridges were tested to sample for trichlorobenzenes. The trichlorobenzenes were poorly trapped using a polyurethane foam (PUF) plug, with collection efficiencies of 6.6%. However, using a dual-sorbent trap consisting of a 0.6 g layer of Tenax-GC (35-60 mesh) sandwiched between two 3.8 cm PUF plugs, a collection efficiency of 98% was obtained. Theoretical detection limits, using GC/EC detection, are expected to be at least one order of magnitude lower (in the range of 0.06-0.1 $\mu g/m^3$). Storage stability of the PUF cartridges was tested under adverse storage conditions. The amount of trichlorobenzenes recovered from the cartridges after 15 days of storage at 32°C was 57%. Oehme and Stray (1982), however, reported high recoveries of 80, 94 and 115% for 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzenes, respectively, with PUF plugs.

Langhorst and Nestrick (1979) used an air sampling tube packed with two sections of Amberlite XAD-2 resin separated by a silanized glass wool plug to collect the trichlorobenzenes. The adsorbent was desorbed with carbon tetrachloride and analyzed by GC using a photoionization detector. Using the method described, the minimum detection limits for the trichlorobenzenes were 30 ppb (v/v). Collection and desorption efficiencies for the chlorobenzenes (air concentrations between 5 ppb and 15 ppm) were ~95% with a precision of $\pm$12%.

Summary

The trichlorobenzenes are a group of three chemical isomers in which three chlorine substituents have been added to a benzene ring. The 1,2,3- and 1,3,5-trichlorobenzenes are normally solid while 1,2,4-trichlorobenzene is normally a liquid at 25°C. The trichlorobenzenes are only slightly soluble in water (6.6-34.6 mg/ℓ at 25°C). The trichlorobenzenes are produced in relatively small amounts (1.3-7 million kg/year) and are used primarily as chemical intermediates, solvents, insecticides, and coolants and insulators in electrical equipment. Analysis of the trichlorobenzenes in water normally involves a solvent extraction and cleanup method followed by GC or GC/MS analysis. The water analysis methods are slightly modified for analysis of trichlorobenzenes in soil and food.

Absorption

No quantitative studies on the absorption of the trichlorobenzenes from the gastrointestinal tract, skin or lungs were found. Information on absorption may be obtained from data describing elimination. Male Charles River rats (16 in the group) excreted a mean of 84%, and two female rhesus monkeys excreted a mean of 40% of the orally (by gavage) administered dose of 10 mg ^{14}C-1,2,4-trichlorobenzene/kg in the 24-hour urine, while fecal elimination accounted for only 11 and 1%, respectively (Lingg et al., 1982). The results indicate that in these species, this isomer is well absorbed from the gastrointestinal tract. Two Chinchilla female rabbits given doses of 500 mg 1,3,5-trichlorobenzene/kg in arachis oil by gavage expired ~10% of the administered dose via the lungs over a period of 9 days (Parke and Williams, 1960). These investigators also observed elimination of urinary and fecal metabolites, but quantities or percentages were not reported.

That the trichlorobenzenes are absorbed by the respiratory tract and by the skin can be inferred from systemic effects observed in toxicity studies using the inhalation (Kociba et al., 1981) and dermal (Brown et al., 1969) routes of exposure. These studies, however, were not designed to give information on rates of absorption.

Distribution

Smith and Carlson (1980) examined the distribution of ^{14}C-1,2,4-trichlorobenzene in groups of four male Sprague-Dawley rats on days 1, 6, 11 and 16 after oral daily dosing with 181.5 mg/kg (1 mmol/kg) in corn oil for

7 days. Their data indicate that the adrenals initially had the highest concentration of radiolabel. This level declined rapidly; however, by day 11 it was less than twice the background of the other tissues. Abdominal fat had the highest concentration at the end of day 1 (Table III-1) and maintained detectable concentrations (20% of the day 1 level) for the duration of the observation period (16 days). The liver also maintained detectable levels throughout the recovery period, retaining ~30% of the day 1 level by day 16. These authors also found that starvation for 4 days had no observed effect on the distribution of ^{14}C-trichlorobenzene in fat or liver.

Parke and Williams (1960) reported the distribution of 1,3,5-trichlorobenzene in one rabbit on day 8 following oral administration of a single dose of 500 mg/kg as follows: 13% of the administered dose was detected in the feces, 23% (4% as monochlorobenzene) in the gut, 5% in the pelt, 5% in depot fat (exclusive of pelt) and 22% in the carcass.

Metabolism

No metabolic studies following the inhalation of trichlorobenzenes were available for review, but the metabolic fate following oral and/or intravenous (i.v.) or intraperitoneal (i.p.) administration has been characterized in rabbits (Jondorf et al., 1955; Parke and Williams, 1960; Kohli et al., 1976) and in rats and monkeys (Lingg et al., 1982).

Jondorf et al. (1955), using spectrophotometric analysis, studied the metabolism of all three isomers of trichlorobenzene in groups of 3 or 4 Chinchilla rabbits given a single oral dose of 500 mg/kg in arachis oil.

TABLE III-1

Distribution of ^{14}C-Labeled 1,2,4-Trichlorobenzene in Rat Tissues after Oral Dosing with 181.5 mg/kg/day for 7 Days[a]

Tissue	Activity (dpm/g tissue)[b]			
	Day 1	Day 6	Day 11	Day 16
Abdominal fat	2033±439	642±54	342±10	408±39
Liver	1075±87	442±22	308±21	317±18
Adrenals[c]	754±132	246±22	d/	
Muscle	400±30	d/		
Kidney	1471±167	404±43	d/	
Heart	438±14	d/		
Spleen	404±14	d/		

[a]Source: Smith and Carlson, 1980

[b]Each value is the mean ± SE for 4 rats, except for abdominal fat on day 1, which was for three rats.

[c]Total for both adrenals; they were not weighed.

[d]Value less than twice background; further analyses were not performed.

The results indicated that the 1,2,3- isomer was metabolized to 2,3,4-trichlorophenol (TCP), to 3,4,5-TCP to a lesser degree, and to small amounts of 3,4,5-trichlorocatechol. During the 5 days after administration, 50% of the dose was excreted in the urine as glucuronic acid conjugates, 12% as sulfuric acid (sulfate) conjugates and 0.3% as 2,3,4-trichlorophenylmercapturic acid. The 5-day urinary metabolites of 1,2,4-trichlorobenzene were represented by glucuronide conjugates (27%), sulfuric acid conjugates (11%) and 2,3,5- and 2,4,5- trichlorophenylmercapturic acid (0.3%). The major phenols formed were 2,4,5- and 2,3,5-TCP. For the 1,3,5- isomer, 20% was excreted as glucuronide and 3% as sulfuric acid conjugates. No mercapturic acid was found, 2,4,6-trichlorophenol was the only phenol detected in the urine, and some unchanged 1,3,5-trichlorobenzene was present in the feces. To further characterize and clarify the metabolic fate of the 1,3,5- isomer, Parke and Williams (1960) followed the 9-day urinary excretion in 2 or 3 female Chinchilla rabbits treated orally with a single dose of 500 mg of the isomer/kg. For the first 3 days, the rabbits eliminated 2,4,6-TCP along with some minor monochlorophenols, while from day 4 to 9, 4-chlorophenol was detected more prominently along with 2,4,6-TCP and ~1% of the dose as 4-chlorocatechol.

Using GC/MS analysis, Kohli et al. (1976) examined the metabolism of the three trichlorobenzene isomers following a single i.p. injection of 60-75 mg/kg doses in vegetable oil to male rabbits (number and strain not reported). In agreement with the results of Jondorf et al. (1955), the major urinary metabolites of 1,2,4-trichlorobenzene were 2,4,5- and 2,3,5-TCP. The major metabolite of 1,2,3-trichlorobenzene was 2,3,4-TCP, with 2,3,6- and 3,4,5-TCP as minor urinary metabolites. The 1,3,5- isomer

was metabolized to 2,3,5- and 2,4,6-TCP and a third, more polar metabolite, was tentatively identified as a dichlorobenzene with 2 hydroxyl and 1 methoxyl substituents.

Lingg et al. (1982) investigated the metabolism of 1,2,4-trichlorobenzene in groups of 16 male Charles River rats and groups of 2 female rhesus monkeys following a single oral or i.v. administration of 10 mg/kg doses and found similar phenolic metabolites to those observed in the rabbit. These researchers were also able to characterize some species specific conjugates. An isomeric pair of 3,4,6-trichloro-3,5-cyclohexadiene-1,2-diol glucuronides accounted for 48-61% of the 24-hour urinary metabolites in the monkeys. Also found were glucuronides of 2,4,5- and 2,3,5-TCP and unconjugated TCP, which accounted for 14-37 and 1-37% of the urinary metabolites, respectively. In the rat, the 2,4,5- and 2,3,5- isomers of N-acetyl-S-(trichlorophenyl)-L-cysteine accounted for 60-62% of the urinary metabolites. Minor urinary metabolites included 2,4,5- and 2,3,5-trichlorothiophenol and free 2,3,5- and 2,3,4-TCP, which accounted for 28-33 and 1-10% of the material excreted, respectively.

On the basis of the studies of Lingg et al. (1982) and Kohli et al. (1976), it is apparent that there may be differences among species in the metabolism of 1,2,4-trichlorobenzene. It seems likely that these differences will extend to the other isomers of trichlorobenzene as well. Both reports postulated the same first step in metabolism (i.e., initial formation of arene oxide intermediates), but indicated differences in the subsequent metabolic reactions. In the rat, conjugation of the intermediate with glutathione was postulated to account for the sulfur-containing urinary

metabolites. In the monkey, hydrolysis of the arene oxide to the dihydrodiol and the absence of sulfur-containing metabolites seemed to preclude the involvement of glutathione (Lingg et al., 1982). As proposed by Kohli et al. (1976) and illustrated in Figure III-1, formation of the isomeric trichlorophenols from the arene oxide intermediates can proceed either by direct opening of the C-O bond or by the NIH shift of chlorine.

Differences in the rate of metabolism of the different isomers within a species have been attributed to the positions of the chlorine atoms on the benzene ring, with the presence of two adjacent unsubstituted carbon atoms facilitating the formation of the arene oxide intermediate. Halogenated benzenes without adjacent unsubstituted carbons may still be metabolized via an arene oxide intermediate but at a reduced rate, and should show evidence of a NIH shift (Matthews and Kato, 1979).

Excretion

Lingg et al. (1982) measured the 24-hour excretion of radioactivity in the urine and feces of 16 male Charles River rats and 2 rhesus monkeys given a single 10 mg/kg i.v. or oral dose of ^{14}C-1,2,4-trichlorobenzene. In the rat, 84% of the oral dose and 78% of the i.v. dose were excreted in the urine by 24 hours; 11 and 7%, respectively, were the amounts identified in the feces in the same period. In the monkeys, 40% of the oral dose and 22% of the injected dose appeared in the urine and <1% in the feces. Smith and Carlson (1980) orally administered 181.5 mg/kg/day (1 mmol/kg/day) of ^{14}C-1,2,4-trichlorobenzene in corn oil to 4 Sprague-Dawley rats for 7 days and followed the excretion of radioactivity in the feces and in the urine during administration and up to 21 days after the first dose. Fecal elimination rose slightly during the first 3 days of dosing, after which it

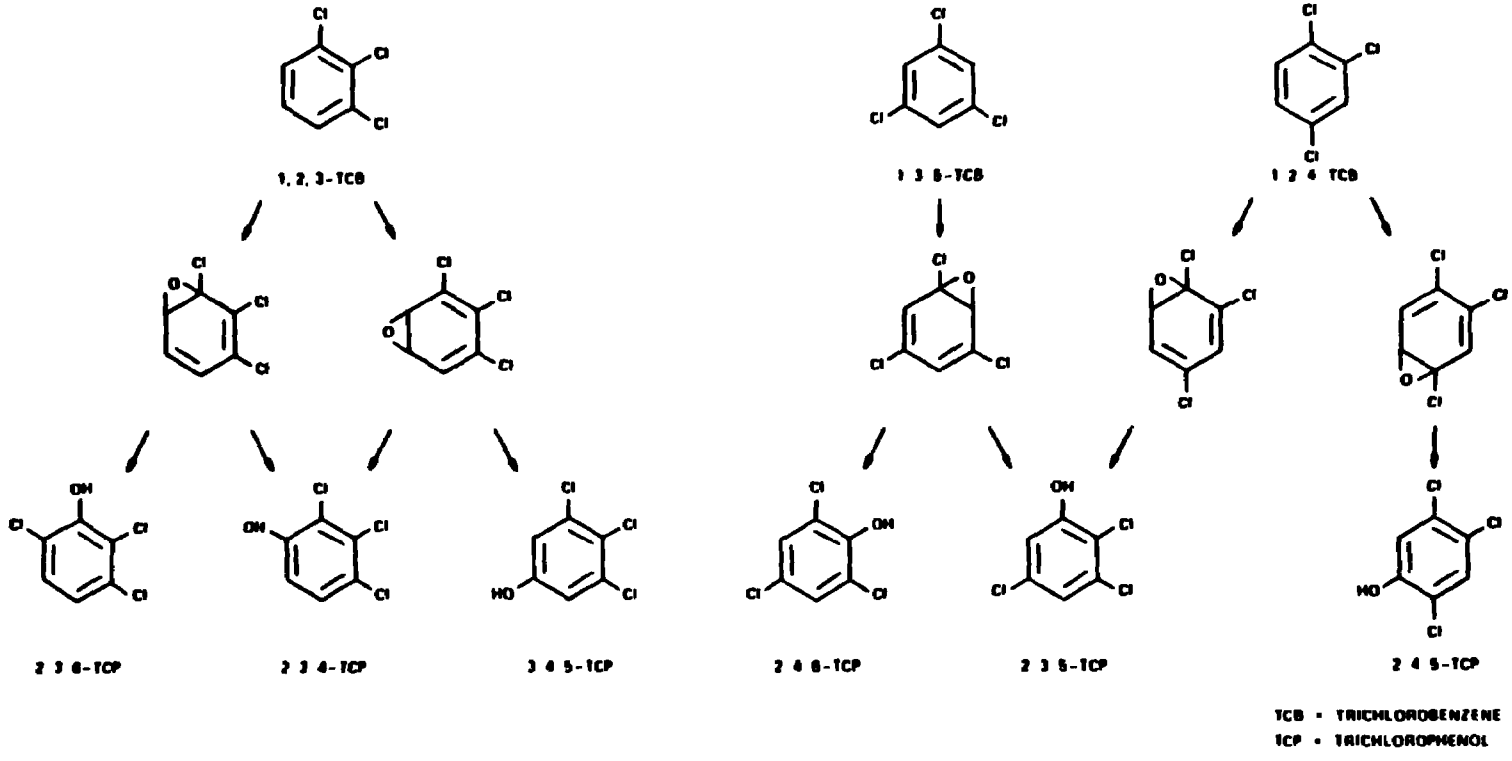


FIGURE III-1

Metabolic Pathways for Trichlorobenzene (TCB) Isomers Through Arene Oxide Intermediates in Rabbits

Source: Adapted from Kohli et al., 1976

declined rapidly and was essentially complete at 15 days of collection, accounting for ~4% of the total dose. Urinary excretion followed a similar pattern; however, at 21 days after the first dose, radioactivity was still detectable. Total urinary excretion to this time accounted for ~72% of the total administered radioactivity. As noted by Lingg et al. (1982), the differences in the excretion rate between the rat and monkey may be attributable to their different pathways of metabolism, since the monkey required two steps beyond the arene oxide to produce its urinary metabolite, while the rat required only one.

Differences in the rates of excretion between the isomers of trichlorobenzene have also been reported. Jondorf et al. (1955) found that rabbits given oral doses of 500 mg/kg of 1,2,3-, 1,2,4- or 1,3,5-trichlorobenzene excreted 78, 42 or 9%, respectively, of the administered dose as monophenols in the 5-day urine collection.

U.S. EPA (1980), using data from Williams (1959) and Parke and Williams (1960), estimated the following half-lives of excretion in the rabbit: 2, 5.5 and 8.5 days for 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene, respectively. The rate of metabolism and subsequent excretion is most likely related to the position of the chlorine atoms on the benzene ring. Matthews and Kato (1979) hypothesized that two adjacent unsubstituted carbon atoms facilitate the formation of the arene oxide intermediate and increase the rate of metabolism and excretion.

Summary

The limited comparative pharmacokinetic data available on the trichlorobenzenes prevent specification of the absorption, distribution, metabolism and excretion of the individual isomers. The trichlorobenzenes appear to enter the systemic circulation readily by inhalation, ingestion and dermal absorption; however, data were not available to quantitate the rates of these processes nor of any of the pharmacokinetic processes. Initial distribution of the trichlorobenzenes and metabolites is mainly to the liver, kidneys and adrenals, followed by migration to adipose tissue or metabolism to polar compounds that are more readily excreted. From the available data, it seems relatively clear that metabolism in at least three species has a common first step, the production of an arene oxide intermediate. Subsequent metabolic steps, however, vary among the species examined, at least for the most studied isomer, 1,2,4-trichlorobenzene.

In general, the pharmacokinetics of the trichlorobenzenes are similar to those described for the halogenated aromatics by Matthews and Kato (1979). The authors observed that these compounds are lipophilic and that their metabolism and excretion depends on their conversion to polar intermediates. In addition, their lipophilic character provides for ready absorption from the gastrointestinal tract and initial distribution to the more highly perfused tissues, particularly the liver, after which they are either metabolized and excreted or redistributed to adipose tissue or skin. Additional experiments are needed to clarify the relationship of these studies to the metabolism of trichlorobenzenes in humans.

IV. HUMAN EXPOSURE

This chapter will be submitted by the Science and Technology Branch, Criteria and Standards Division, Office of Drinking Water.

V. HEALTH EFFECTS IN ANIMALS

Acute Toxicity

Studies of the acute toxicity of the trichlorobenzenes have been performed in several species using various routes of administration.

Information on the effects of acute inhalation exposure to trichlorobenzenes is limited. In an abstract of a study from the Russian literature (Gurfein and Pavlova, 1960), a single high inhalation exposure (exposures of 0.005-0.01 mg/ℓ in air or 5-10 mg/m³ were used) of an unspecified isomer of trichlorobenzene to rats resulted in immediate nervousness, and pinkness of mouth, ears and paws. These effects were followed by convulsions and death within 30 minutes, with edema of livers and kidneys observed upon necropsy. Unpublished results of a study performed by Treon (1950) were reported by Coate et al. (1977) and indicated that the target organs of non-lethal acute inhalation exposure to trichlorobenzenes (a weight-to-weight mixture of 8% 1,2,3- and 92% 1,2,4-trichlorobenzene) in cats, dogs, rats, rabbits and guinea pigs included the liver, ganglion cells at all levels of the brain, and mucous membranes. Lethal doses resulted in local irritation of the lungs and functional changes in respiration in animals dying after exposure. Levels and duration of exposure were not given.

Brown et al. (1969) reported the single-dose oral LD_{50} for 1,2,4-trichlorobenzene in CFE rats to be 756 mg/kg (95% confidence limits 556-939 mg/kg). In CF mice, the single-dose oral LD_{50} was 766 mg/kg (95% confidence limits 601-979 mg/kg). Death occurred within 5 days in rats and 3 days in mice.

Rimington and Ziegler (1963) studied the porphyria-inducing ability of 1,2,4- and 1,2,3-trichlorobenzenes administered by gavage to male albino rats for various time periods (5-15 days). Doses of the isomers were gradually increased until porphyrin excretion was high but fatalities were few. Porphyria was induced by 1,2,4-trichlorobenzene when the isomer was given for 15 days at 730 mg/kg (3 rats) as evidenced by peak elevations in urinary coproporphyrin, uroporphyrin, porphobilinogen and δ-aminolevulinic acid. At a dose of 500 mg/kg for 10 days (in 5 rats), peak liver levels of coproporphyrin, protoporphyrin, uroporphyrin and catalase were reached. For the 1,2,3-isomer, urinary excretion of these indicators peaked at 785 mg/kg for 7 days (3 rats), but to a lesser extent than for the 1,2,4-isomer. Only the liver uroporphyrin levels were increased by administration of 1,2,3-trichlorobenzene at this dose and duration. Glutathione was found to have a protective effect on trichlorobenzene-induced porphyria.

Brown et al. (1969) determined the single-dose percutaneous LD_{50} in CFE rats (4 of each sex) to be 6139 mg/kg (95% confidence limits 4299-9056 mg/kg) for 1,2,4-trichlorobenzene administered topically on the shaved dorsolumbar skin and covered with an impermeable dressing. All deaths occurred within 5 days. In skin irritation studies, 1,2,4-trichlorobenzene was applied to the skin of rabbits and guinea pigs. In the first experiment, two 2x2 cm patches of lint, each containing 1 mℓ of the compound, were applied to the shaved backs of rabbits (4 of each sex) for 6 hours/day for 3 consecutive days and covered with an impermeable dressing. For another experiment, rabbits (1 of each sex) and guinea pigs (5 of each sex) received single uncovered applications of 1,2,4-trichlorobenzene on the shaved middorsal skin (1 mℓ for rabbits, 0.5 mℓ for guinea pigs) 5

days/week for 3 weeks. The results indicated that trichlorobenzene was not very irritating, although fissuring was noted during the 3-week exposure. Some guinea pigs that died during the 3-week regimen had focal necrosis of the liver.

Hepatotoxic effects (fatty infiltration and necrosis) were reported by Cameron et al. (1937) following s.c. and/or i.v. injection of 500 mg (range of doses was 1-500 mg) trichlorobenzene in liquid paraffin to rats; the toxicity was less than that of mono- and o-dichlorobenzene. Further details of strain, number of animals or isomers were not reported.

Robinson et al. (1981), in an acute toxicity study to assess the increased adrenal weight that was noted in a multigeneration study, administered to groups composed of 9-10 preweaning female Charles River rats i.p. injections of 0, 250 or 500 mg of 1,2,4-trichlorobenzene/kg in corn oil at 22, 23 and 24 days of age. Significant changes ($p<0.05$) from control values were observed upon necropsy at 25 days of age as follows: decreased body weight and increased adrenal weight at the high dose; decreased uterus and increased liver weights at both doses.

Male Holtzman rats (number not specified) were given single intraperitoneal injections of 1,2,4- or 1,3,5-trichlorobenzene at a dose of 37 mg/kg (5 mmol/kg) as a 50% solution in sesame oil in a volume of 1 mℓ/kg (Yang et al., 1979). Controls received an equal volume of sesame oil. After 24 hours, the femoral veins and the common bile duct were cannulated. Both isomers produced significant increases ($p<0.05$) in bile duct-pancreatic fluid (BDPF) flow with the 1,2,4- isomer being 4 times more effective than

the 1,3,5- isomer. SGPT activity was elevated by treatment with 1,3,5-trichlorobenzene and bile flow was elevated by the 1,2,4- isomer. Both isomers caused a decrease in BDPF protein concentration.

Several studies have demonstrated the ability of the trichlorobenzenes to enhance xenobiotic metabolism. Carlson, in a series of reports (Carlson and Tardiff, 1976; Carlson, 1977a, 1978, 1981; Smith and Carlson, 1980), examined the ability of 1,2,4-trichlorobenzene to induce a variety of microsomal functions and enzymes including cytochrome c reductase, O-ethyl O-p-nitrophenyl phenylphosphothionate (EPN) detoxification, cytochrome P-450, glucuronyltransferase, benzopyrene hydroxylase and azoreductase. In a 14-day study by Carlson and Tardiff (1976), daily doses of 1,2,4-trichlorobenzene in corn oil were administered orally to groups of 6 male albino rats at 10, 20 and 40 mg/kg. All the above functions and enzymes increased significantly ($p<0.05$) except benzopyrene hydroxylase. In a 90-day study by the same investigators, all the functions and enzyme activities, including benzopyrene hydroxylase, increased significantly ($p<0.05$) at 10-40 mg/kg/day and remained significantly elevated after a 30-day recovery period. In a similar study, Smith and Carlson (1980) administered 1,2,4-trichlorobenzene at 181.5 mg/kg/day (1 mmol/kg/day) to rats for 7 days, and measured recovery at 1, 6, 11 and 16 days. EPN detoxification was still significantly ($p<0.05$) elevated at 11 days; p-nitroanisole demethylation at 16 days; cytochrome c reductase at 6 days; and cytochrome P-450 at 11 days. In a similar study by Carlson (1977a), 14-day administration of 1,3,5-trichlorobenzene at 100-200 mg/kg/day significantly ($p<0.05$) increased EPN detoxification, UDP glucuronyltransferase, and cytochrome c reductase, and significantly decreased hepatic G-6-P;

benzopyrene hydroxylase, azoreductase and serum isocitrate dehydrogenase were not significantly affected at 200 mg/kg/day. In the same study, in vivo hepatotoxicity of carbon tetrachloride (one dose of 0.5 mℓ/kg) was significantly ($p<0.05$) enhanced by 14-day pretreatment of rats with 1,2,4-trichlorobenzene. Glucose-6-phosphatase activity was significantly ($p<0.05$) decreased by pretreatment with 1,2,4-trichlorobenzene at 5 mg/kg/day, and isocitrate dehydrogenase was decreased by pretreatment at 20 mg/kg/day.

The 1,2,4- isomer, and to a lesser extent the 1,3,5- isomer, were also shown to induce hepatic esterases (Carlson et al., 1979; Carlson, 1980). In studies similar to those previously described, rats receiving daily oral doses of 18.2 mg isomer/kg (0.1 mmol/kg) for 14 days were killed 24 hours later and hepatic microsomes were prepared. The 1,2,4-isomer was an effective inducer of both acetanilide esterase and acetanilide hydroxylase, while the 1,3,5-isomer induced only the esterase and to a lesser degree than did 1,2,4-trichlorobenzene (Carlson et al., 1979). The 1,2,4-isomer also induced hepatic arylesterase, while 1,3,5-trichlorobenzene did not (Carlson, 1980). Pretreatment of rats with 181.5 mg/kg/day (1 mmol/kg/day) of either isomer resulted in induction of procaine esterase (Carlson et al., 1979).

In a series of experiments, Ariyoshi et al. (1975a,b,c) studied the effects of the trichlorobenzenes on induction of hepatic microsomal proteins, phospholipids and enzymes, especially in relation to the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in the biosynthesis of heme. The three trichlorobenzene isomers were administered orally to groups of 2-6 female Wistar rats at a dose of 250 mg/kg/day for 3 days, after which the rats were killed and microsomes were prepared. The

results indicated that trichlorobenzenes increased the levels of microsomal proteins, phospholipids and cytochrome P-450, and enhanced the activities of aniline hydroxylase, aminopyrine demethylase and δ-aminolevulinic acid synthetase, with the 1,2,4-isomer being the most effective (Ariyoshi et al., 1975a,b). The dose response of these effects to 1,2,4-trichlorobenzene were determined (Ariyoshi et al., 1975c) for groups of 2-6 female Wistar rats treated orally with single doses of 0, 125, 250, 500, 750, 1000 and 1500 mg/kg. The results indicated that 24 hours after the administration of the isomer, microsomal protein was elevated at ≥750 mg/kg and glycogen content was decreased at ≥500 mg/kg. The activities of aminopyrine demethylase and aniline hydroxylase and the content of cytochrome P-450 were increased at ≥250 mg/kg, as was δ-aminolevulinic acid synthetase activity.

Subchronic Toxicity

The effects of trichlorobenzene following subchronic inhalation, as well as oral and dermal exposure, have been investigated in a variety of species. Toxicity data for the trichlorobenzenes can be found in Table V-1.

Kociba et al. (1981) exposed 20 male Sprague-Dawley rats, 4 male New Zealand rabbits and 2 male beagle dogs by inhalation to 1,2,4-trichlorobenzene (99.4% pure) at levels of 0, 223 mg/m³ (30 ppm) or 742 mg/m³ (100 ppm) for 7 hours/day, 5 days/week for a total of 30 exposures in 44 days. There were no significant effects on body weight, hematologic indices or serum biochemistry tests. Upon necropsy, gross and comprehensive histologic examination revealed no significant treatment-related effects in any of the species. At the 742 mg/m³ level, increased liver weights were detected in dogs and rats and increased kidney weights in rats. Urinary excretion of

Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	inhalation	10, 100 or 1000 mg/m³ of 1,3,5-TCB	6 hr/day, 5 day/wk for up to 13 wk	No hepatotoxicity; three high-dose rats had squamous metaplasia and focal hyperplasia of respiratory epithelium, believed to be reversible	Sasmore et al., 1983
Rats, rabbits, two dogs	inhalation	223 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk; total of 30 exposures in 44 days	Increase in urinary excretion of porphyria in exposed rats; increase in liver weights in high-dose rats and dogs; increased kidney weights in high-dose rats	Kociba et al., 1981
Rat	inhalation	22.3 or 74.2 mg/m³ of 1,2,4-TCB	6 hr/day, 5 day/wk, 3 mo	Increase in urinary porphyrin excretion in high-dose rats; no effects in 22.3 mg/m³ group	Watanabe et al., 1978
Rat	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	Enlarged hepatocytes and nondose-dependent hepatocytes vacuolization, liver granuloma, biliary hyperplasia and kidney hyaline degeneration at 4 and 13 wk; no histopathology evident at 26 wk	Coate et al., 1977
Rabbits, monkeys	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hr/day, 5 day/wk, 26 wk	No treatment related changes at 26 wk	Coate et al., 1977
Monkey	oral	1, 5, 25, 90, 125 or 173.6 mg/kg/day of 1,2,4-TCB	30 days	$\leq$25 mg/kg/day - no effects observed; $\geq$90 mg/kg/day - observed toxicity and death	Smith et al., 1978
Rat	oral	50, 100 or 200 mg/kg/day of 1,2,4-TCB	30, 60, 90 or 120 days	Increases in liver weights, liver porphyrins and urine porphyrins, dose and time related	Carlson, 1977b
Rat	oral	10, 20 or 40 mg/kg/day of 1,2,4-TCB	90 days	Increase in liver-to-body weight ratio in high-dose group; changes in enzyme activation at all doses	Carlson and Tardiff, 1976
Mouse	oral	600 ppm diet (0.078 mg/kg/day) of 1,2,4-TCB	6 mo	No effects	Goto et al., 1972

TABLE V-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Guinea pig	dermal	0.5 mL/day of 1,2,4-TCB	5 day/wk, 3 wk	Death following extensor convulsion; livers showed necrotic foci	Brown et al., 1969
Mouse	dermal	0.03 mL/painting of 30 and 60% solution in acetone of 1,2,4-TCB	2 times/wk, 2 yr	Painting induced excitability, panting and epidermal thickening, inflammation and keratinization; increased organ weights and mortality	Yamamoto et al., 1982
Rats	oral (drinking water)	25, 100 or 400 mg/L of 1,2,4-TCB	F_0 to F_2 generations	Enlarged adrenals in F_0 and F_1 generations	Robinson et al., 1981
Rats	oral	36, 120, 360 or 1200 mg/kg/day of 1,2,4-TCB	days 9-13 of gestation	1200 mg/kg dose all dead by the 3rd day, 360 mg/kg dose caused 22% mortality in dams and moderate hepatocellular hypertrophy and non-significant increases in embryonic lethality and significantly retarded embryonic development, 36 and 120 mg/kg groups not observed for embryonic effects, but slight hepatocellular hypertrophy was reported in one 120 mg/kg dam	Kitchin and Ebron, 1983
Rabbits	dermal	30, 150 or 450 mg/kg/day of 1,2,3-TCB (30%) and 1,2,4-TCB (70%)	5 day/wk, 4 wk	Dose-related skin irritation; increase in urinary coproporphyrin in high-dose males and slight pallor of liver in males and females	Rao et al., 1982

1,2,3-TCB = 1,2,3-trichlorobenzene; 1,2,4-TCB = 1,2,4-trichlorobenzene; 1,3,5-TCB = 1,3,5-trichlorobenzene

porphyrin was increased in rats exposed to 1,2,4-trichlorobenzene at 223 or 742 mg/m^3, which the investigators interpreted as a compound-specific physiologic effect rather than a toxic effect. A follow-up study supported this interpretation. The same investigators exposed male and female Sprague-Dawley rats to 1,2,4-trichlorobenzene at 0, 22.3 mg/m^3 (3 ppm) or 74.2 mg/m^3 (10 ppm) for 6 hours/day, 5 days/week for 3 months. The results, which were reported in an abstract (Watanabe et al., 1978), indicated that urinary excretion of porphyrins was slightly increased in the 74.2 mg/m^3 group during exposure, but returned to control range 2-4 months postexposure. Since this appeared to be the most sensitive indicator in rats, and exposure to trichlorobenzene at 22.3 mg/m^3 did not cause increased porphyrin excretion, 22.3 mg/m^3 was considered a no-observed-adverse-effect level (NOAEL) for rats by the authors.

Sasmore et al. (1983) exposed male and female outbred albino CD rats (20/group) to 1,3,5-trichlorobenzene vapor at 0, 10, 100 or 1000 mg/m^3 for 6 hours/day, 5 days/week for up to 13 weeks. No significant effects were observed on body weights, food consumption, standard hematologic and clinical chemistry parameters or on methemoglobin and porphyrin levels. In a subgroup of five animals/sex/group killed after 4 weeks of exposure, the only altered experimental parameter was an increase in the liver-to-body weight ratios in the male 1000 mg/m^3 group, but this effect was not observed at 13 weeks. Since gross and microscopic pathologic examinations of the liver revealed no treatment-related abnormalities, the authors concluded that the exposure did not cause hepatotoxicity. Microscopic examinations, however, revealed that three high-dose rats had squamous metaplasia and focal hyperplasia of the respiratory epithelium, which the authors believed to be reversible.

Coate et al. (1977) exposed groups of 30 male Sprague-Dawley rats, 16 male New Zealand rabbits and 9 male monkeys (Macaca fascicularis) to 99.07% pure 1,2,4-trichlorobenzene vapor at levels of 0, 186 mg/m³ (25 ppm), 371 mg/m³ (50 ppm) or 742 mg/m³ (100 ppm) for 7 hours/day, 5 days/week for 26 weeks. Pulmonary function and operant behavior tests in the monkeys, ophthalmic examinations in the rabbits and monkeys, and measurements of body weight, hematologic indices and serum biochemistry parameters in all species were conducted before and during the exposure period. Subgroups of 5 rats each were killed after 4 and 13 weeks of exposure; all remaining rats were killed after 26 weeks for histologic examination of selected tissues. No treatment-related effects at any observation time were seen with respect to body weight, survival, hematology or serum chemistry for any of the species. No ophthalmic changes were observed in rabbits or monkeys. Pulmonary function and operant behavior were unaffected in monkeys. Histologic examination of rat tissues revealed that treated animals had enlarged hepatocytes that were more prominent at 4 weeks than at 13 weeks after exposure, and at 371 and 742 mg/m³ than at 186 mg/m³. Other changes in treated rats that did not appear to be dose-dependent were vacuolization of hepatocytes at 4 and 13 weeks, slightly more severe granuloma of the liver at 4 weeks and biliary hyperplasia at 4 and 13 weeks. A nondose-related increase in the severity of kidney hyaline degeneration was observed in test rats at 4 weeks. This lesion was slightly more severe in the high-dose group at 13 weeks. These effects appeared to be transient; rats necropsied after 26 weeks of exposure had none of these changes. Likewise, histologic examination of selected tissues from rabbits and monkeys revealed no treatment-related changes after 26 weeks of exposure.

Carlson and Tardiff (1976) assessed the effects of 14- or 90-day oral administration of 1,2,4-trichlorobenzene in corn oil compared with corn oil controls in male CD rats. In the 14-day studies, the effects examined were lethality, hepatotoxicity and the influence on hexabarbital sleeping time and other parameters of xenobiotic metabolism. A dose of 600 mg/kg/day, the highest dose administered, caused no deaths during the 14-day administration period. Hepatotoxicity was evaluated by dosing at 0, 150, 300 or 600 mg/kg/day and determining serum isocitrate dehydrogenase and liver glucose-6-phosphatase activities. Although no dose-related changes in serum isocitrate dehydrogenase activity was observed, liver glucose-6-phosphatase activity was significantly decreased at $\geq$300 mg/kg ($p<0.05$). Hexabarbital sleeping time was significantly decreased at 600 mg/kg/day (the only dose examined); this effect persisted through a 14-day recovery period. In rats receiving 14 daily doses at 0, 10, 20 or 40 mg/kg, there was a significant dose-related increase in liver-to-body weight ratio at $\geq$10 mg/kg/day ($p<0.05$). Significant dose-related increases were also observed in activities or contents of cytochrome c reductase (at $\geq$10 mg/kg), cytochrome P-450 (at $\geq$20 mg/kg), glucuronyltransferase (at $\geq$20 mg/kg), azoreductase (at $\geq$10 mg/kg) and the rate of detoxication of EPN (at $\geq$10 mg/kg). These results indicated that the doses, while causing a slight degree of hepatic injury, significantly enhanced xenobiotic metabolism.

In the 90-day studies by Carlson and Tardiff (1976), the effects of oral dosing of male CD rats (6 animals/group) at 0, 10, 20 or 40 mg/kg/day with 1,2,4-trichlorobenzene in corn oil on weight gain, liver weight, hemoglobin content, packed cell volume and the indicators of xenobiotic metabolism were evaluated. No effects on weight gain and no consistent alteration in hemoglobin content or packed cell volume were observed. At 40 mg/kg, there was

a statistically significant increase ($p<0.05$) in liver-to-body weight ratios that persisted throughout a 30-day recovery period. Following 90-day administration, cytochrome c reductase activity was increased at $\geq$10 mg/kg, with recovery after 30 days; cytochrome P-450 levels increased at $\geq$20 mg/kg, followed by recovery; glucuronyltransferase activity decreased at $\geq$10 mg/kg; EPN detoxication increased at $\geq$20 mg/kg; benzopyrene hydroxylase activity increased 2-fold at 40 mg/kg; and azoreductase activity increased at $\geq$10 mg/kg.

Groups of 5 female rats (strain not reported) received daily oral doses of 0, 50, 100 or 200 mg 1,2,4-trichlorobenzene/kg/day in corn oil for 30, 60, 90 or 120 days (Carlson, 1977b). Significant increases were observed in liver porphyrins at $\geq$100 mg/kg after 30 days exposure and in urinary porphyrins at 200 mg/kg after 30 days. For the 30-day study, slight but significant increases were also observed in liver weights at 200 mg/kg. When the compound was administered for 60 days, only the liver weights were increased. The administration of 1,2,4-trichlorobenzene for 90 days resulted in slight but significant increases in liver weights at $\geq$50 mg/kg, in liver porphyrins at $\geq$100 mg/kg and in urine porphyrins at 200 mg/kg. A significant increase was observed for liver porphyrins when the compound was given at $\geq$50 mg/kg for 120 days. The excretion of δ-aminolevulinic acid and porphobilinogen in the urine was not increased at any dose given for any duration. When the author compared the 1,2,4-trichlorobenzene results with the results for hexachlorobenzene, he concluded that the trichlorobenzene induced porphyria was very small compared with the hexachlorobenzene induced porphyria (Carlson, 1977b).

A 90-day oral study by Smith et al. (1978), reported in an abstract, was reviewed by U.S. EPA (1980), who gave further details of the study after communication with the authors. Rhesus monkeys (4/group) were given 1,2,4-trichlorobenzene in daily oral doses of 1, 5, 25, 90, 125 or 173.6 mg/kg. No toxic effects were observed at <25 mg/kg, while doses of ≥90 mg/kg were observed to be toxic, and the 173.6 mg/kg dose was lethal within 20-30 days. There were no deaths observed in the 1, 5 and 25 mg/kg groups; one death occurred in each of the 90 mg/kg and 125 mg/kg groups and two deaths occurred in the 173.6 mg/kg group. Animals on the highest dose exhibited severe weight loss and predeath fine tremors. All of the animals in the highest dose group had elevated BUN, Na^+, K^+, CPK, SGOT, SGPT, LDH and alkaline phosphatase as well as hypercalcemia and hyperphosphatemia from 30 days on. Smith et al. (1978) have been using the urinary pattern of chlorguanide metabolites as an indication of cytochrome P-450 dependent drug metabolism. At the high doses, monkeys showed evidence of the hepatic induction as well as increased clearance of i.v. doses of labeled 1,2,4-trichlorobenzene. Further information on the study (U.S. EPA, 1980) gave evidence of liver enzyme induction in the 90, 125 and 173.6 mg/kg animals. There were some pathologic changes noted in the livers of the high-dose groups, primarily a fatty infiltration. The point at which there was no effect related to the compound was at the 5 mg/kg level. Since only an abstract of this study was available and since the interpretation of this study was complicated by the use of other drugs and weight losses in the control animals, a valid no-observed-effect level (NOEL) cannot be derived from these data.

Two subchronic studies have assessed the dermal toxicity of the trichlorobenzenes. Powers et al. (1975) applied technical grade 1,2,4-trichlorobenzene at concentrations of 5 or 25% in petroleum ether, or 100% 1,2,4-trichlorobenzene topically in 0.2 mℓ volumes to the ventral surface of the ears of New Zealand rabbits (groups of 12 each), 3 times weekly for 13 weeks; a control group received petroleum ether only. Rabbits exposed to 5% trichlorobenzene and controls had slight redness and scaling. Dermal responses at 25 and 100% of the compound included slight to severe erythema, severe scaling, desquamation, encrustation, and some hair loss and scarring. The responses were characterized by acanthosis and keratosis, typical of moderate to severe irritation and probably attributable to degreasing (defatting) action. No overt signs of systemic toxicity were noted, body weight gain was comparable in all groups, and none of the animals showed meaningful changes in gross pathology. The investigators noted that this contrasted with the findings of Brown et al. (1969), who reported that some guinea pigs, exposed topically to 1,2,4-trichlorobenzene at 0.5 mℓ/day, 5 days/ week for 3 weeks, died following extensor convulsions and their livers showed necrotic foci. This difference in results may be attributed to the site of application (Brown et al., 1969, used the dorsal midline for application, a more extensive exposure site), the volume applied (0.5 mℓ vs. 0.2 mℓ), the species used, and the more frequent (5 times/week vs. 3 times/ week) application, although the total number of exposures was less (5x3 weeks vs. 3x13 weeks).

Rao et al. (1982) applied technical grade trichlorobenzene [1,2,4- (70%) and 1,2,3-trichlorobenzene (30%)] 5 days/week for 4 weeks, at doses of 0, 30, 150 or 450 mg/kg/day, to the dorsal skin (4x4 inch area) of groups (5 of each sex) of New Zealand rabbits weighing ~3 kg. One rabbit died after 18

applications, but the investigators were unable to determine the cause of death by either gross or histologic examination. Gross and histologic examination of the skin showed evidence of moderate irritation at the highest dose and less irritation at the lower doses. This irritation evidence consisted of epidermal scaling, thickening, fissures, ulcers and erythema. No treatment-related change was observed in clinical chemistry (BUN, glucose, SGPT, SAP) or hematology. A slight but significant increase in urinary coproporphyrin was observed in high-dose males (450 mg/kg/day) at day 24; none was seen in females. This slight porphyria and a slight generalized pallor of the liver (3/5 males, 4/4 females) were the only signs of systemic toxicity. Extensive histologic examination of numerous tissues failed to show any treatment-related abnormalities. The volume of trichlorobenzene applied at the dose levels in this study can be calculated as ≈0.06 mℓ (30 mg/kg), 0.31 mℓ (150 mg/kg) and 0.93 mℓ (450 mg/kg) by multiplying the dose in g/kg by the weight of the rabbits (3 kg) and dividing by the density of trichlorobenzene (1.45 g/mℓ).

Chronic Toxicity

No studies on the effects of the trichlorobenzenes following chronic oral or inhalation exposure were available for review; however, a chronic skin painting study was encountered. Goto et al. (1972) conducted a 6-month feeding study in mice using hexachlorocyclohexane isomers and their metabolites, including 1,2,4-trichlorobenzene. Male mice (20/group) of the ICR-JCL strain (age at initiation 5 weeks, average weight 26.5 g) received a diet containing 600 ppm of trichlorobenzene (78 μg of compound/kg body weight, assuming mice consume 13% of their body weight in food per day).

The weight gain of treated mice did not differ from controls during the 6-month exposure. At 26 weeks, 10 mice were killed and liver, heart and kidneys were weighed; no abnormal weight changes were observed. Macroscopic and histologic examination of the liver revealed no hepatic tumors or any other lesions.

Yamamoto et al. (1982) studied the toxicity of 1,2,4-trichlorobenzene when painted on the skin of Slc:ddy mice 2 times/week for 2 years. Groups consisted of 75 mice/sex receiving 0.03 mℓ applications of the compound as 30 or 60% solutions in acetone. Controls consisted of 50 mice/sex and received only acetone. The skin painting produced general symptoms of excitability and panting, local skin thickening, keratinization and inflammation of the epidermis. These effects were not observed in controls. For the 30% trichlorobenzene groups, mortality was increased in females (5/75 survived for 83 weeks compared with 11/50 controls). The mean survival days were 357 ± 125.4 for treated females compared with 423.8 ± 145.0 for controls ($p<0.01$). The survival of males at this exposure level was not significantly different from that of controls. Spleen weights were significantly increased ($p<0.05$) and left adrenal weights were significantly decreased ($p<0.01$) for treated males when compared with controls. Hematologic and blood chemistry indices were essentially unchanged with the exception of decreased red blood cell counts in the 30% treated males ($p<0.05$) and decreased Cl^- concentration ($p<0.01$). For the 60% solution, 6/75 treated females survived for 83 weeks. Mean survival days were 320.2 ± 147.7 for treated females compared with 423.8 ± 145.0 for controls ($p<0.001$). Eight of 75 treated males survived for 83 weeks compared with 9/50 control males.

Mean survival days were 288.0±173.7 for treated males and 363.9±173.9 for controls ($p<0.05$). Significant differences in organ weights from control values were seen in the spleens of males ($p<0.01$) and the adrenals of females ($p<0.05$). Hematologic and blood biochemistry changes were seen in increased lymphocyte counts in treated females ($p<0.05$), and in increased SGOT ($p<0.05$), SGPT ($p<0.001$) and BUN ($p<0.01$) for treated males.

Mutagenicity

Schoeny et al. (1979) and Lawlor et al. (1979) examined the mutagenic potential of 1,2,4-trichlorobenzene in Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537, using the plate incorporation technique. Schoeny et al. (1979) used 8 concentrations of trichlorobenzene ranging from 102 µg/plate to 1.4×10^5 µg/plate. The toxic dose was determined as 1599 µg/plate (killing of one or more strain on mutagenesis plates). Trichlorobenzene was negative for mutagenicity in the absence and presence of S-9 microsomal fractions from uninduced rats, from rats induced by the polychlorinated biphenyl, Aroclor 1254, and from rats homologously induced with trichlorobenzene.

The study of Lawlor et al. (1979), reported in an abstract, used the TA1538 strain of S. typhimurium in addition to the strains previously mentioned. Negative results were obtained for five unspecified concentrations tested in the presence and absence of rat liver microsomes induced by Aroclor 1254. Because these results were reported in an abstract without the details of the experimental procedures used, the results cannot be critically evaluated.

The negative results in the Salmonella histidine reversion assay are not unexpected because this test system is generally insensitive to highly chlorinated compounds (Rinkus and Legator, 1980).

Carcinogenicity

Yamamoto et al. (1982) applied 1,2,4-trichlorobenzene in acetone to the skin of Slc.ddy mice twice weekly for 2 years. The solution of 1,2,4-trichlorobenzene was 60% for the high dose and 30% for the low dose and the volume applied was 0.03 mℓ/application. Each treated group contained 75 animals and there were 50 control animals for each sex. Growth rates in treated and control mice were comparable through 83 weeks. Mean survival days were significantly reduced in the 60% 1,2,4-trichlorobenzene groups of males and females and also in the 30% treatment group of females.

Histopathology showed some organ sites had increased non-neoplastic lesions. Assuming that all 75 animals in the treated groups were examined and all 50 in the control groups were examined, there would be increases in lesions in the males in lung, liver, kidney, adrenal, spleen and lymph node at the high dose, and in all of these organs except lymph node in the females at the high dose. Unfortunately, the English translation of Japanese text is not very specific in describing the nature of the lesion making it difficult to use this information in the interpretation of the tumor findings.

No single tumor type was increased significantly over the control incidence but among males nine different tumors were found in the high-dose group as compared with two in the low-dose and two in the control group.

In females there were 11 different tumors in the high-dose group as compared with 3 in the low-dose and 8 in the control group. The authors do not state whether these tumors were all found in different individual animals or whether these were multiple tumors in the same animal. Therefore, the actual incidence in terms of the number of tumor bearing animals is not known.

Further information from this study is necessary for full interpretation. This single study is clearly inadequate for making any conclusions about carcinogenicity in humans.

Reproductive and Teratogenic Toxicity

Studies on the reproductive or teratogenic effects of trichlorobenzenes following inhalation exposure were not found in the available literature. Robinson et al. (1981) reported a multigeneration study of the reproductive effects of 1,2,4-trichlorobenzene following oral administration. Charles River rats were continuously exposed to the compound at 0, 25, 100 or 400 ppm in drinking water. The authors calculated the dosages for the F_0 generation based on water consumption data to be: for females at 29 days of age, 8.3±0.8, 28.0±1.2, 133.2±13.4 mg/kg/day, respectively; for males at 29 days of age, 8.5±0.6, 27.6±1.6, 133.6±15.6 mg/kg/day, respectively; for females at 83 days of age, 3.7±0.1, 14.8±1.0, 53.6±3.9 mg/kg/day, respectively; for males at 83 days of age, 2.5±0.1, 8.9±0.3, 33.0±1.4 mg/kg/day, respectively. The exposure period began with the birth of the F_0 generation and continued through 32 days of age for the F_2 generation. Each treatment group consisted of 17-23 litters. No treatment-related effects were noted with respect to fertility, neonatal weights, maternal weights, litter sizes, preweaning viability or postweaning growth in any generation.

Treatment-related differences were seen with respect to food intake and water consumption in F_0 males and females, but they were inconsistent and did not occur in other generations. Blood chemistry analyses and locomotor activity measurements revealed no overt hematologic or neurologic effects, and histologic examination of the livers and kidneys of the F_1 generation rats revealed no damage. At the 400 ppm dose level, significantly enlarged adrenals in both sexes of the F_0 and F_1 rats were observed at 95 days of age ($p<0.006$). A follow-up acute toxicity study showed that this effect could result from three daily i.p. injections of 500 mg 1,2,4-trichlorobenzene/kg.

Black et al. (1983) reported in an abstract a teratogenicity study in pregnant Wistar rats using 1,2,4-, 1,2,3- or 1,3,5-trichlorobenzene administered by gavage in doses of 75-600 mg/kg on days 6-15 of gestation (gestational day 0 or 1 not defined). Upon necropsy (gestational day not specified), thyroid and liver lesions and reduced hemoglobin and hematocrit values were observed in treated dams (doses not specified). No teratogenic effects were observed in the pups; however, pups exposed to the 1,2,4- and 1,3,5- isomers (doses not specified) had mild osteogenic changes.

Kitchin and Ebron (1983) conducted a maternal hepatic toxicity and embryotoxicity study where they administered 1,2,4-trichlorobenzene (>99% pure) dissolved in corn oil (2 mℓ/kg) orally to pregnant Sprague-Dawley (CD strain) rats (6 or more/group) on days 9-13 of gestation and the dams were then sacrificed on day 14 of gestation. The dosing groups were 0 (corn oil only), 36, 120, 360 and 1200 mg/kg/day 1,2,4-trichlorobenzene. All the dams in the 1200 mg/kg/day group died by the third day of dosing. The 360

mg/kg/day group were observed with a maternal mortality rate of 22% and greatly reduced body weight gains. Maternal liver weights, liver-to-body weight ratios and hepatic microsomal protein content were not affected by 1,2,4-trichlorobenzene administration. 1,2,4-Trichlorobenzene was observed to be a strong inducer of hepatic enzymes at the 120 and 360 mg/kg/day dose levels. Liver histology in the pregnant dams was unremarkable in the 36 mg/kg/day group, showed a slight degree of hepatocellular hypertrophy in 1 of 9 rats in the 120 mg/kg/day group and showed a moderate hepatocellular hypertrophy in 7 of 8 rats in the 360 mg/kg/day group. The uteri from only the 0 and 360 mg/kg/day groups were examined for 1,2,4-trichlorobenzene-induced embryonic effects. No statistically significant differences in resorption, embryolethality or abnormalities were reported, although 3/12 treated litters showed embryolethality as compared with 0/12 in the control litters. Several embryonic parameters were significantly decreased by 1,2,4-trichlorobenzene treatment. These parameters were embryonic head length, crown-rump length, somite number and total embryo protein content (reduced 23%).

Summary

The effects in mammals of acute exposure by various routes to trichlorobenzenes include local irritation, convulsions and death. Livers, kidneys, adrenals, mucous membranes and brain ganglion cells appear to be target organs with effects including edema, necrosis, fatty infiltration of livers, increased organ weights, porphyrin induction and microsomal enzyme induction.

Quantitative data on the toxic effects of trichlorobenzene following subchronic exposure by various routes were obtained in a variety of species. In general, these studies indicate that the liver and kidney are target organs. Inhalation of 1,2,4-trichlorobenzene at $\geq$74.2 mg/m^3 (10 ppm) for 6 hours/day, 5 days/week for up to 26 weeks induced hepatocytomegaly and hyaline degeneration in several species (Kociba et al., 1981; Watanabe et al., 1978; Coate et al., 1977), although these effects may be to some extent reversible. One study (Watanabe et al., 1978) identified 22.3 mg/m^3 (3 ppm) as a NOAEL in rats. Sasmore et al. (1983) reported that some rats exposed by inhalation to 1,3,5-trichlorobenzene at 1000 mg/m^3 for 13 weeks showed squamous metaplasia and focal hyperplasia of the respiratory epithelium, which appeared to be reversible. Subchronic oral studies have also found that the trichlorobenzenes induce hepatic xenobiotic metabolism (Carlson and Tardiff, 1976; Smith et al., 1978) and porphyria (Carlson, 1977b). Subchronic dermal exposure resulted in mild to moderate irritation (Powers et al., 1975; Rao et al., 1982).

One chronic study, on the effects of trichlorobenzene painted on the skin of mice for 2 years, reported increased mortality in females at the low dose (30% solution in acetone) and in both sexes at the high dose (60% solution) (Yamamoto et al., 1982). While numbers of all tumor types appeared to be increased, no significant change was detected for any individual tumor type. Thus, the carcinogenic results from the only relevant study are considered inconclusive.

Results of two reports on mutagenicity tests with Salmonella typhimurium test strains were negative (Schoeny et al., 1979; Lawlor et al., 1979). However, this test system is generally insensitive to chlorinated compounds.

A multigeneration study of the reproductive effects of oral exposure to trichlorobenzene (Robinson et al., 1981) failed to show effects on reproduction. Teratogenicity studies after administration by the oral route in rats (Black et al., 1983; Kitchin and Ebron, 1983) showed mild osteogenic changes in pups and significantly retarded embryonic development as measured by growth parameters.

VI. HEALTH EFFECTS IN HUMANS

Information on the health effects of trichlorobenzenes in humans is limited to case reports. Rowe (1975) found that an individual exposed to 1,2,4-trichlorobenzene at 3-5 ppm had eye and respiratory irritation. Girard et al. (1969) reported two cases, one in which a 68-year-old woman, who often soaked her husband's work clothes in trichlorobenzene, developed aplastic anemia, and the other in which a 60-year-old man, who had been occupationally exposed to DDT as well as to mono-, di- and trichlorobenzenes for over 30 years, developed anemia.

Summary

Limited data are available on human exposure to trichlorobenzenes. No conclusions can be drawn from this data.

VII. MECHANISMS OF TOXICITY

Several studies discussed in Chapter V on acute toxicity have demonstrated that the isomers of trichlorobenzene are capable of affecting xenobiotic metabolism by inducing a variety of the hepatic drug-metabolizing enzymes in rats. These include cytochrome c reductase, cytochrome P-450, glucuronyltransferase, benzopyrene hydroxylase, azoreductase (Carlson and Tardiff, 1976; Carlson, 1977a, 1978, 1981; Smith and Carlson, 1980; Denomme et al., 1983), acetanilide esterase and acetanilide hydroxylase, procaine esterase (Carlson et al., 1979), arylesterase (Carlson, 1980), microsomal proteins, phospholipids and aminopyrene hydroxylase (Ariyoshi et al., 1975a,b,c). That trichlorobenzenes enhance xenobiotic metabolism has been demonstrated by Smith and Carlson (1980) and Carlson (1977a), who showed that administration of 1,2,4- or 1,3,5-trichlorobenzene to groups of 4 male Sprague-Dawley rats for 7 days increased EPN detoxication. The administration of 1,2,4-trichlorobenzene to pregnant rats was also reported to induce hepatic levels of cytochrome P-450, cytochrome c reductase, UDP glucuronyltransferase and glutathione S-transferase (Kitchin and Ebron, 1983).

Townsend and Carlson (1981) demonstrated that 1,2,4-trichlorobenzene, administered by gavage in corn oil to groups of five male Swiss mice at 181.5 mg/kg (1 mmol/kg) for 7 days, increased the LD_{50} and protected the mice against the toxic effects of malathion, malaoxon, parathion and paraoxon when graded doses of these insecticides were administered on the day following the last dose of trichlorobenzene.

Experiments comparing the effects of trichlorobenzenes with the effects of phenobarbital and 3-methylcholanthrene indicated that the inductions of microsomal enzymes by trichlorobenzenes are of the phenobarbital type (Carlson, 1978).

These data suggest that the trichlorobenzenes stimulate the drug metabolizing enzyme system in animals. Thus, these compounds would be expected to increase the rate of their own metabolism, particularly with multiple exposures, resulting in increased production of reactive intermediates and potentially a greater toxic response.

The series of studies by Ariyoshi (1975a,b,c) demonstrated that trichlorobenzenes stimulated the activity of δ-aminolevulinic acid synthetase. Since this enzyme is the rate-limiting step in heme synthesis its elevated activity would be expected to result in the production of elevated levels of heme precursors. This may well account for at least part of the increased urinary excretion of coproporphyrin, porphobilinogen and uroporphyrin in rats treated with trichlorobenzenes as reported by Rimington and Ziegler (1963). These authors also reported elevated levels of these heme precursors in the livers of treated rats.

There are some indications in the report of Powers et al. (1975) that the irritant effects noted in skin may be, at least partially, related to the defatting action of the trichlorobenzenes.

Summary

The mechanism of toxicity for trichlorobenzene is not completely known. The clinical effects of porphyria are sometimes observed in animals and humans exposed to trichlorobenzene, but to a lesser extent than for hexachlorobenzene. The capacity of trichlorobenzene to stimulate the activity of δ-aminolevulinic acid synthetase is well documented. Since this enzyme is the rate-limiting step in heme synthesis, there is a direct link to excess porphyrin production.

VIII. QUANTIFICATION OF TOXICOLOGIC EFFECTS

Introduction

The quantification of toxicologic effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \_\_\_\_ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicologic effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the

U.S. EPA (1991) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{(RfD) \times (Body\ weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ \ell/day} = \_\_\_\_\ mg/\ell$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{(NOAEL\ or\ LOAEL) \times (bw)}{(UF) \times (\_\_\_\ \ell/day)} = \_\_\_\_\ mg/\ell$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicologic evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these

estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biologic mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is

uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects

A number of biologic endpoints have been identified in short-term studies with 1,2,4-TCB including transient porphyria, transient hepatic cellular changes, and increase in adrenal gland weight and reproductive effects (Coate et al., 1977; Kociba, 1981; Kitchin and Ebron, 1983; Robinson et al., 1981). In a reproductive study in rats, 25, 100 or 400 ppm of 1,2,4-trichlorobenzene, administered to the parental animals in their drinking water, produced no reproductive, hematologic or neurologic effects (Robinson et al., 1981). Increased adrenal gland weight occurred in both the parents and offspring at the highest dose level. This change was found to be associated with vacuolization of the zona fasciculata of the adrenal cortex and decreased serum corticosterone levels (Cicmanec, 1991). Retarded embryonic development was observed in pregnant rats receiving 1,2,4-trichlorobenzene 360 mg/kg/day on days 9-13 of gestation (Kitchin and Ebron, 1983).

Hepatic porphyria, porphyrinuria and hepatic cellular changes have been observed following the administration of 1,2,4-trichlorobenzene but these changes either occurred at very high doses or were transient. Coate et al. (1977) reported a study in rats, rabbits and cynomolgus monkeys (Macaca fascicularis) that involved inhalation exposure for 26 weeks. In rats hepatocytomegaly, hepatic vacuolization and biliary hyperplasia were seen at 4 weeks and 13 weeks but not at the completion of the study. No significant changes were seen in the rabbits or monkeys. Carlson (1977) reported a study in rats that investigated the potential induction of porphyia by hexachlorobenzene, trichlorobenzene and dichlorobenzene when given by oral gavage at 0, 50, 100 and 200 mg/kg/day. Only hexachlorobenzene showed a marked ability to induce porphyria and the author determined that di- and trichlorobenzene did not share this property. In the study reported by Carlson and Tardiff (1976), male CD rats received 0, 10, 20 and 40 mg/kg/day and xenobiotic metabolism was measured as well as body weight and hematologic parameters. There was a dose-response related change for all xenobiotic enzymes but liver-to-body weight ratio was only affected at 40 mg/kg/day.

Kociba et al. (1981) reported a study in which male rats, rabbits and dogs were exposed to 0, 30 or 100 ppm (0, 223 or 742 mg/m^3) of 1,2,4-trichlorobenzene for 44 days. No significant effects were observed for body weight gain, hematologic parameters, serum biochemical tests or microscopic appearance of tissues. A reversible increase in urinary porphyrins was noted but the authors interpreted this change as being a compound-specific physiologic effect rather than a sign of toxicity. In a 2-year mouse skin painting study (Yamamoto et al., 1982) a slight increase

in tumors of all sites was reported, but no conclusions can be drawn about carcinogenicity because of the lack of details in the English translation of the text.

Quantification of Noncarcinogenic Effects

Table VIII-1 presents a summary of the subchronic and chronic toxicity studies on the trichlorobenzenes that were considered for calculation of a DWEL for each trichlorobenzene. Table VIII-2 presents the toxicity threshold estimates that were determined from the studies discussed in Chapter V. As indicated by these tables and Chapter V, very little toxicity data are available to derive credible HAs or DWELs for the 1,2,3- and 1,3,5-trichlorobenzenes isomers. Therefore, no HAs or DWELs are recommended for these two trichlorobenzene isomers.

Derivation of 1-Day HA. The acute studies by Ariyoshi et al. (1975a,b,c) were selected for derivation of the 1-day HA for 1,2,4-trichlorobenzene.

In a series of experiments, Ariyoshi et al. (1975a,b,c) studied the effects of the trichlorobenzenes on induction of hepatic microsomal proteins, phospholipids and enzymes, especially in relation to the activity of δ-aminolevulinic acid synthetase, the rate limiting enzyme in the biosynthesis of heme. The three trichlorobenzene isomers were administered orally to groups of 2-6 female Wistar rats at a dose of 250 mg/kg/day for 3 days, after which the rats were killed and microsomes were prepared. The results indicated that trichlorobenzenes increased the levels of microsomal proteins, phospholipids and cytochrome P-450, and enhanced the activities of

TABLE VIII-1

Summary of Subchronic and Chronic Toxicity Studies on Trichlorobenzenes

Species	Route	Dose	Duration	Effects	Reference
Rat	inhalation	10, 100 or 1000 mg/m³ of 1,3,5-TCB	6 hours/day, 5 days/week for up to 13 weeks	No hepatotoxicity; three high-dose rats had squamous metaplasia and focal hyperplasia of respiratory epithelium, believed to be reversible	Sasmore et al., 1983
Rats, rabbits, two dogs	inhalation	223 or 742 mg/m³ of 1,2,4-TCB	7 hours/day, 5 days/week; total of 30 exposures in 44 days	Increase in urinary excretion of porphyria in exposed rats; increase in liver weights in high-dose rats and dogs; increased kidney weights in high-dose rats	Kociba et al., 1981
Rat	inhalation	22.3 or 74.2 mg/m³ of 1,2,4-TCB	6 hours/day, 5 days/week, 3 months	Increase in urinary porphyrin excretion in high-dose rats; no effects in 22.3 mg/m³ group	Watanabe et al., 1978
Rat	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hours/day, 5 days/week, 26 weeks	Enlarged hepatocytes and nondose-dependent hepatocytes vacuolization, liver granuloma, biliary hyperplasia and kidney hyaline degeneration at 4 and 13 wk; no histopathology evident at 26 wk	Coate et al., 1977
Rabbits, monkeys	inhalation	186, 371 or 742 mg/m³ of 1,2,4-TCB	7 hours/day, 5 days/week, 26 weeks	No treatment related changes at 26 wk	Coate et al., 1977
Monkey	oral	1, 5, 25, 90, 125 or 173.6 mg/kg/day of 1,2,4-TCB	30 days	≤25 mg/kg/day - no effects observed; ≥125 mg/kg/day - observed toxicity and death	Smith et al., 1978
Rat	oral	50, 100 or 200 mg/kg/day of 1,2,4-TCB	30, 60, 90 or 120 days	Increases in liver weights, liver porphyrins and urine porphyrins, dose and time related	Carlson, 1977b
Rat	oral	10, 20 or 40 mg/kg/day of 1,2,4-TCB	90 days	Increase in liver-to-body weight ratio in high-dose group; changes in enzyme activation at all doses	Carlson and Tardiff, 1976
Mouse	oral	600 ppm diet (0.078 mg/kg/day) of 1,2,4-TCB	6 months	No effects	Goto et al., 1972

TABLE VIII-1 (cont.)

Species	Route	Dose	Duration	Effects	Reference
Guinea pig	dermal	0.5 mL/day of 1,2,4-TCB	5 days/week, 3 weeks	Death following extensor convulsion; livers showed necrotic foci	Brown et al., 1969
Mouse	dermal	0.03 mL/painting of 30 and 60% solution in acetone of 1,2,4-TCB	2 times/week, 2 years	Painting induced excitability, panting and epidermal thickening, inflammation and keratinization; increased organ weights and mortality	Yamamoto et al., 1982
Rats	oral (drinking water)	25, 100 or 400 mg/L of 1,2,4-TCB	F_0 to F_2 generations	Increased adrenal weight in F_0 and F_1 generations	Robinson et al., 1981
Rats	oral	36, 120, 360 or 1200 mg/kg/day of 1,2,4-TCB	days 9-13 of gestation	1200 mg/kg dose all dead by the 3rd day, 360 mg/kg dose caused 22% mortality in dams and moderate hepatocellular hypertrophy and non-significant increases in embryonic lethality and significantly retarded embryonic development, 36 and 120 mg/kg groups not observed for embryonic effects, but slight hepatocellular hypertrophy was reported in one 120 mg/kg dam	Kitchin and Ebron, 1983
Rabbits	dermal	30, 150 or 450 mg/kg/day of 1,2,3-TCB (30%) and 1,2,4-TCB (70%)	5 days/week, 4 weeks	Dose-related skin irritation; increase in urinary coproporphyrin in high-dose males and slight pallor of liver in males and females	Rao et al., 1982

1,2,3-TCB = 1,2,3-trichlorobenzene; 1,2,4-TCB = 1,2,4-trichlorobenzene; 1,3,5-TCB = 1,3,5-trichlorobenzene

TABLE VIII-2

Toxicity Data for Threshold Estimates

Compound	Species	Route	Dose Concentration	Dose Duration	Effect Level	Reference
1,2,4-Trichlorobenzene	rat	inhalation	22.3 mg/m³, 6 hour/day, 5 day/week	3 months	NOAEL*	Watanabe et al., 1978
1,2,4-Trichlorobenzene	rabbit, monkey	inhalation	742 mg/m³, 7 hour/day, 5 day/week	26 weeks	NOEL*	Coate et al., 1977
1,3,5-Trichlorobenzene	rat	inhalation	100 mg/m³, 6 hour/day, 5 day/week	13 weeks	NOAEL*	Sasmore et al., 1983
1,2,4-Trichlorobenzene	rat	oral	14.8 mg/kg/day	95 days/generation 2 generations	NOAEL*	Robinson et al., 1981

*Estimated toxicity thresholds as determined in the respective Mammalian Toxicity Sections of this document.

NOEL = No-observed-effect level: That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

NOAEL = No-observed-adverse-effect level: That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this dose, but they are not considered to be adverse.

aniline hydroxylase, aminopyrine demethylase and δ-aminolevulinic acid synthetase, with the 1,2,4-isomer being the most effective (Ariyoshi et al., 1975a,b). The dose-response of these effects to 1,2,4-trichlorobenzene were determined (Ariyoshi et al., 1975c) for groups of 2-6 female Wistar rats treated orally with single doses of 0, 125, 250, 500, 750, 1000 and 1500 mg/kg. The results indicated that 24 hours after the administration of the isomer, microsomal protein was elevated at ≥750 mg/kg and glycogen content was decreased at ≥500 mg/kg. The activities of aminopyrine demethylase and aniline hydroxylase and the content of cytochrome P-450 were increased at ≥250 mg/kg, as was δ-aminolevulinic acid synthetase activity. A slight significant increase in δ-aminolevulinic acid synthetase activity was observed in the 125 mg/kg group with a dose-related increase in activity in the higher dose levels. The 125 mg/kg dose can be utilized as a NOAEL for the 1-day HA calculations.

The 1-day HA for a 10 kg child is calculated using the 24 hour oral NOAEL of 125 mg/kg reported by Ariyoshi et al. (1975c) as follows.

For a 10 kg child:

$$\text{1-day HA} = \frac{(125\ \text{mg/kg/day} \times 10\ \text{kg})}{1000 \times 1\ \text{ℓ/day}} = 1.25\ \text{mg/ℓ}\ \text{(rounded to 1 mg/ℓ)}$$

where:

125 mg/kg/day	= NOAEL, based on the absence of adverse effects in rats (Ariyoshi et al., 1975c)
10 kg	= assumed body weight of a child
1 ℓ/day	= assumed water consumption by a child
1000	= uncertainty factor, in accordance with NAS/ODW and Agency guidelines for use with a NOAEL from an animal study and to account for limited data.

This HA is equivalent to 1 mg/day or 0.1 mg/kg/day.

Derivation of 10-Day HA. The Carlson and Tardiff (1976) study was chosen for derivation of the 10-day HA for 1,2,4-trichlorobenzene.

Carlson and Tardiff (1976) assessed the effects of a 14-day oral administration of 1,2,4-trichlorobenzene in corn oil compared with corn oil controls in male CD rats. In the 14-day studies, the effects examined were lethality, hepatotoxicity and the influence on hexabarbital sleeping time and other parameters of xenobiotic metabolism.* A dose of 600 mg/kg/day, the highest dose administered, caused no deaths during the 14-day administration period. Hepatotoxicity was evaluated by dosing at 0, 150, 300 or 600 mg/kg/day and determining serum isocitrate dehydrogenase and liver glucose-6-phosphatase activities. Although no dose-related changes in serum isocitrate dehydrogenase activity was observed, liver glucose-6-phosphatase activity was significantly decreased at $\geq$300 mg/kg ($p<0.05$). Hexabarbital sleeping time was significantly decreased at 600 mg/kg/day (the only dose examined); this effect persisted through a 14-day recovery period. In rats receiving 14 daily doses at 0, 10, 20 or 40 mg/kg, there was a significant dose-related increase in liver-to-body weight ratio

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*The stimulation of the xenobiotic metabolizing system may be considered primarily a physiologic response, although in the case of the trichlorobenzenes increased metabolism of subsequent doses and the production of reactive intermediates and phenolic metabolites would be expected to enhance toxicity. In addition, the elevated activity of δ-aminolevulinic acid synthetase, the rate limiting step in heme synthesis, at 250 and 125 mg/kg dose levels suggests increased porphyrin synthesis. This is verified at higher dose levels in other studies where increased porphyrin excretion in the urine has been documented. Stimulation of porphyrin synthesis may be seriously detrimental to some portions of the population. The use of drugs, such as barbiturates, that enhance porphyrin synthesis are contraindicated in patients with intermittent porphyria or porphyria variegata (Goodman and Gilman, 1985). Thus, in addition to the sparse data, the potential effects of the stimulation of heme synthesis in man warrants an additional safety factor of 10 in this calculation.

at ≥10 mg/kg/day ($p<0.05$). Significant dose-related increases were also observed in activities or contents of cytochrome c reductase (at ≥10 mg/kg), cytochrome P-450 (at ≥20 mg/kg), glucuronyltransferase (at ≥20 mg/kg), azoreductase (at ≥10 mg/kg) and the rate of detoxication of EPN (at ≥10 mg/kg). These results indicated that the doses, while causing a slight degree of hepatic injury, significantly enhanced xenobiotic metabolism. The dose of 10 mg/kg/day can be considered a NOAEL since none of the effects observed at this dose can be directly considered adverse but rather adaptive responses.

The 10-day HA for a 10 kg child is calculated using the 14-day oral NOAEL of 10 mg/kg/day reported by Carlson and Tardiff (1976) as follows.

For a 10 kg child:

$$\text{10-day HA} = \frac{(10\ \text{mg/kg/day} \times 10\text{kg})}{100 \times 1\ \text{ℓ/day}} = 1\ \text{mg/ℓ}$$

where:

10 mg/kg/day = NOAEL, based on the absence of adverse effects in rats (Carlson and Tardiff, 1976)

10 kg = assumed body weight of a child

1 ℓ/day = assumed water consumption by a child

100 = uncertainty factor, in accordance with NAS/ODW and Agency guidelines for use with a NOAEL from an animal study.

This HA is equivalent to 1 mg/day or 0.1 mg/kg/day.

Derivation of Longer-term HA. Three studies were evaluated as the possible basis for the derivation of longer-term HAs for a 10 kg child or a 70 kg adult. Two of the studies were oral studies (Carlson and Tardiff, 1976; Robinson et al., 1981) and one was an inhalation study (Kociba et al., 1981).

In the 90-day study by Carlson and Tardiff (1976), the effects of oral dosing of male CD rats (6 animals/group) at 0, 10, 20 or 40 mg/kg/day with 1,2,4-trichlorobenzene in corn oil on weight gain, liver weight, hemoglobin content, packed cell volume and the indicators of xenobiotic metabolism were evaluated. No effects on weight gain and no consistent alteration in hemoglobin content or packed cell volume were observed. At 40 mg/kg, there was a statistically significant increase ($p<0.05$) in liver-to-body weight ratios that persisted throughout a 30-day recovery period. Following 90-day administration, cytochrome c reductase activity was increased at $\geq$10 mg/kg, with recovery after 30 days; cytochrome P-450 levels increased at $\geq$20 mg/kg, followed by recovery; glucuronyltransferase activity decreased at $\geq$10 mg/kg; EPN detoxication increased at $\geq$20 mg/kg; benzopyrene hydroxylase activity increased 2-fold at 40 mg/kg; and azoreductase activity increased at $\geq$10 mg/kg.

The Robinson study (1981) was a multigeneration reproductive study in which dams received 0, 25, 100 or 400 ppm of 1,2,4-trichlorobenzene (TCB) in the drinking water. Following birth of the F_0 generation, litters of the F_0 and F_1 generations were dosed with 0, 25, 100 or 400 ppm (0, 3.7, 14.8 or 53.6 mg/kg/day) of 1,2,4-trichlorobenzene for 95 days. Subsequently these rats were bred and the F_1 generation received similar dosing. Seventeen to 23 litters/dose group were used for the study. During the study maternal weights, litter size, neonatal sex, and weights were recorded, as well as food and water intake. Serum chemistry determinations for glucose, BUN, creatinine, Na, K, Cl, uric acid, CaP, cholesterol, triglyceride, bilirubin, alkaline phosphatase, ALT, AST, LDH, CPK, protein, globulin and albumin were made. When the rats were killed, organ weights for liver, kidney, uterus, adrenal glands, lung, heart and gonads were

recorded. The study ended when the F_2 generation was 32 days old. A significant increase in adrenal gland weight (11% for males and 13% for females) was noted in the F_0 and F_1 groups. Further investigation of this effect found the adrenal gland increase to be associated with moderate vacuolization of the zona fasciculata of the cortex and decreased levels of corticosterone in the 53.6 mg/kg/day level of exposure in male rats (Cicmanec, 1991).

Kociba et al. (1981) reported a study in which male rats, rabbits and dogs were exposed to 0, 30 or 100 ppm (0, 223 or 742 mg/m^3) of 1,2,4-trichlorobenzene for 44 days. No significant effects were observed for body weight gain, hematologic parameters, serum biochemical tests or microscopic appearance of tissues. A reversible increase in urinary porphyrins was noted but the authors interpreted this change as being a compound-specific physiologic effect rather than a sign of toxicity.

The study by Carlson and Tardiff (1976) used the oral route of exposure, which is preferred for deriving drinking water HAs, but this study was primarily a study of 1,2,4-trichlorobenzene's ability to induce xenobiotic metabolizing enzymes and to alter related parameters such as liver weights. The critical adverse effect induced by 1,2,4-trichlorobenzene of porphyria related effects was not evaluated in this study and therefore, makes this study inappropriate for deriving HAs.

The longer-term HAs for a 10 kg child and a 70 kg adult are calculated on the basis of a NOAEL of 14.8 mg/kg/day as established in the Robinson study.

The longer-term HA for a 10 kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(14.8\ \text{mg/kg/day}) \times 10\ \text{kg}}{1\ \text{ℓ/day} \times 100} = 1.48\ \text{mg/ℓ}$$

(rounded to 1 mg/ℓ)

where:

14.8 mg/kg/day = NOAEL, based on the absence of adverse effects in rats (Robinson et al., 1981)

10 kg = assumed body weight of a child

1 ℓ/day = assumed water consumption by a child

100 = uncertainty factor, in accordance with NAS/ODW and Agency guidelines for use with a NOAEL from an animal study.

The longer-term HA for a 70 kg adult is calculated as follows:

$$\text{Longer-term HA} = \frac{(14.8\ \text{mg/kg/day}) \times 70\ \text{kg}}{2\ \text{ℓ/day} \times 100} = 5.18\ \text{mg/ℓ}$$

(rounded to 5 mg/ℓ)

where:

14.8 mg/kg/day = NOAEL, based on the absence of adverse effects in rats (Robinson et al., 1981)

70 kg = assumed body weight of an adult

2 ℓ/day = assumed water consumption by an adult

100 = uncertainty factor, in accordance with NAS/ODW and Agency guidelines for use with a NOAEL from an animal study.

Assessment of Lifetime Exposure and Derivation of a DWEL. As discussed in the longer-term HA section the Robinson et al. (1981) study is the most appropriate to derive a lifetime DWEL. The lifetime DWEL for a 70 kg adult is calculated as follows.

Step 1 - RfD Derivation

$$\text{RfD} = \frac{14.8\ \text{mg/kg/day}}{1000} = 0.0148\ \text{mg/kg/day}$$ (rounded to 0.01 mg/kg/day)

where:

14.8 mg/kg/day = NOAEL, based on the absence of adverse effects in rats (Robinson et al., 1981)

1000 = uncertainty factor, in accordance with NAS/ODW and Agency guidelines for use with a NOAEL from a subchronic animal study.

Step 2 - DWEL Derivation

$$DWEL = \frac{0.01 \text{ mg/kg/day} \times 70 \text{ kg}}{2 \text{ ℓ/day}} = 0.35 \text{ mg/ℓ}$$

(rounded to 0.4 mg/ℓ)

where:

0.01 mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 ℓ/day = assumed water consumption by an adult

Carcinogenic Effects

One study reported by Yamamato et al. (1982), where 30% or 60% solutions of 1,2,4-trichlorobenzene in acetone were applied to the skin of Slc.ddy mice twice weekly for 2 years, was evaluated by the Human Health Assessment Group (HHAG) of the U.S. EPA. The HHAG evaluation of this study determined that insufficient information was presented in this study to allow an acceptable interpretation of the data to be made. Also, this single study was found to be clearly inadequate for making any conclusions about carcinogenicity in humans. Therefore, the trichlorobenzenes are classified as U.S. EPA Group D compounds (U.S. EPA, 1991).

Existing Guidelines, Recommendations and Standards

Occupational. There are no U.S. workplace standards for the trichlorobenzenes.

The ACGIH has recommended a ceiling of 5 ppm (40 mg/m^3) for 1,2,4-trichlorobenzene (ACGIH, 1982), and NIOSH classified it as a Group III pesticide. Group III pesticides are less toxic than Group II pesticides and the recommended criteria for workplace standards are less stringent than those recommended for Group II pesticides (NIOSH, 1978). The British Journal of Industrial Medicine reported a provisional operational limit of 25 ppm for 1,2,4-trichlorobenzene (Verschueren, 1977). The 1971 TLV for 1,2,3-trichlorobenzene is 1.3 ppm [10 mg/m^3 (n.s.i.)] for the USSR (Verschueren, 1977).

Trichlorobenzenes have been designated by the ITC as TSCA Section 4(e) priority chemicals (44 FR 70666). Preliminary Assessment Information Manufacturers Reports were to be submitted to the U.S. EPA Office of Toxic Substances by November 19, 1982, for each of the trichlorobenzenes (40 CFR 712).

The U.S. EPA determined that, on the basis of present information, TCB is not classifiable as a human carcinogen; it therefore is placed in Class D. This decision was verified by CRAVE in October 1988 (U.S. EPA, 1991).

Transportation and Regulations. The export of 1,2,3- and 1,2,4-trichlorobenzene is regulated by DOT through the use of the Commodity Control List (15 CFR 399).

Solid Waste Regulations. The trichlorobenzenes are designated as hazardous constituents of hazardous wastes from specific sources subject to RCRA disposal regulations (40 CFR 261.32). The hazardous waste in which the

trichlorobenzenes are controlled as part of the hazardous constituents is from the distillation or fractionation column bottoms from the production of chlorobenzene and is designated as EPA Hazardous Waste No. K085.

Water. The U.S. EPA (1980), in an Ambient Water Quality Criteria Document for Chlorinated Benzenes, determined that "Reliable toxicological data on which to base a defensible water quality criterion do not exist for the trichlorobenzenes." As a result of this determination no criterion was recommended for any trichlorobenzene isomer.

Special Groups at Risk

Only anecdotal information regarding human exposure to trichlorobenzene is available. These data do not indicate special groups that might be at risk.

Summary

Health advisories and a DWEL for 1,2,4-trichlorobenzene in drinking water, based on noncarcinogenic toxicity data, are given in Table VIII-3. No HAs or DWELs are suggested for the 1,2,3- and 1,3,5-trichlorobenzene isomers because of insufficient data being available for evaluation. The 1-day HA for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child is based on a study by Ariyoshi et al. (1975c) in which female Wistar rats were given single oral doses of 1,2,4-trichlorobenzene and were then evaluated. The 10-day HA for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child is based on a study by Carlson and Tardiff (1976) in which male CD rats were given 1,2,4-trichlorobenzene for 14 days and were then evaluated. The longer-term

TABLE VIII-3

Summary of the Data for 1,2,4-Trichlorobenzene Used to Derive HAs and DWEL

Health Advisory	Species/Route	Dose (mg/kg bw/day)	Duration	Basis	Uncertainty Factors	Value (mg/ℓ)		Reference
						Child	Adult	
1-Day	rat/oral	125	single dose	NOAEL, higher doses cause a greater number of alterations	1000	1	NA	Ariyoshi et al., 1975c
10-Day	rat/oral	10	14 days	NOAEL, higher doses cause a greater number of dose-related hepatic changes	100	1	NA	Carlson and Tardiff, 1976
Longer-term	rat/oral	14.8	95 days/gen. 2 generations	NOAEL, higher dose caused increased adrenal gland weights	100	1	5	Robinson et al., 1981
DWEL	rat/oral	14.8	95 days/gen. 2 generations	NOAEL, higher dose caused increased adrenal gland weights	1000	NA	0.4	Robinson et al., 1981
Cancer potency						NR		

[a]For a 70 kg adult

[b]For a 10 kg child

NA = Not applicable; NR = none recommended

HAs for 1,2,4-trichlorobenzene of 1 mg/ℓ for a 10 kg child and 5 mg/ℓ for a 70 kg adult are based on a study by Robinson et al. (1981) in which CD-1 rats were exposed by gavage to 1,2,4-trichlorobenzene for 95 days/generation for 2 generations and evaluated for reproductive effects and adrenal gland changes. An oral RfD of 1E-2 mg/kg/day was derived from the Robinson study. A DWEL for 1,2,4-trichlorobenzene of 0.4 mg/ℓ for a 70 kg adult was calculated from the same Robinson et al. (1981) study. The data available on 1,2,4-trichlorobenzene are inadequate for making any conclusions about its potential carcinogenicity in humans.

IX. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1982. Threshold Limit Values for Chemical Substances in Work Air Adopted by ACGIH for 1982. Cincinnati, OH. ISBN: 0-936712-39-2.

Ariyoshi, T., K. Ideguchi, Y. Ishizuka, K. Iwasaki and M. Arakaki. 1975a. Relationship between chemical structure and activity. I. Effects of the number of chlorine atoms in chlorinated benzenes on the components of drug-metabolizing system and the hepatic constituents. Chem. Pharm. Bull. 23(4): 817-823.

Ariyoshi, T., K. Ideguchi, K. Iwasaki and M. Arakaki. 1975b. Relationship between chemical structure and activity. II. Influences of isomers in dichlorobenzene, trichlorobenzene, and tetrachlorobenzene on the activities of drug-metabolizing enzymes. Chem. Pharm. Bull. 23(4): 824-830.

Ariyoshi, T., K. Ideguchi, K. Iwasaki and M. Arakaki. 1975c. Relationship between chemical structure and activity. III. Dose-response or time-course of induction in microsomal enzymes following treatment with 1,2,4-trichlorobenzene. Chem. Pharm. Bull. 23(4): 831-836.

Berger, D.A. 1987. 1,2,4-Trichlorobenzene health-based maximum contaminant level support document. In: Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. Appendix B. Health-Based Maximum Contaminant Level Support Documents. March 26, 1987. Section P.

Black, W.D., V.E.O. Valli, J.A. Ruddick and D.C. Villeneuve. 1983. The toxicity of three trichlorobenzene isomers in pregnant rats. The Toxicologist. 3(1): 30. (Abstr.)

Brown, V.K.H., C. Muir and E. Thorpe. 1969. The acute toxicity and skin irritant properties of 1,2,4-trichlorobenzene. Ann. Occup. Hyg. 12: 209-212.

Cameron, G.R., J.C. Thomas, S.A. Ashmore, J.L. Buchan, E.H. Warren and A.W. McKinney Hughes. 1937. The toxicity of certain chlorine derivatives of benzene, with special reference to o-dichlorobenzene. J. Pathol. Bacteriol. 44(2): 281-296.

Carlson, G.P. 1977a. Halogenated benzenes, effect on xenobiotic metabolism and the toxicity of other chemicals. Ann. N.Y. Acad. Sci. 298: 159-169.

Carlson, G.P. 1977b. Chlorinated benzene induction of hepatic porphyria. Experientia. 33(12): 1627-1629.

Carlson, G.P. 1978. Induction of cytochrome P-450 by halogenated benzenes. Biochem. Pharmacol. 27(3): 361-363.

Carlson, G.P. 1980. Effects of halogenated benzenes on arylesterase activity *in vivo* and *in vitro*. Res. Commun. Chem. Pathol. Pharmacol. 30(2): 361-364.

Carlson, G.P. 1981. Effects of halogenated aromatic compounds on the metabolism of foreign organic compounds. U.S. EPA Health Effects Res. Lab., Cincinnati, OH. EPA-600/1-81-010. NTIS PB81-152522.

Carlson, G.P. and R.G. Tardiff. 1976. Effect of chlorinated benzenes on the metabolism of foreign organic compounds. Toxicol. Appl. Pharmacol. 36: 383-394.

Carlson, G.P., J.D. Dziezak and K.M. Johnson. 1979. Effect of halogenated benzenes on acetanilide esterase, acetanilide hydroxylase and procaine esterase in rats. Res. Commun. Chem. Pathol. Pharmacol. 25(1): 181-184.

Chlorobenzene Producers Association. 1984. Comments of the Chlorobenzene Producers Association on the EPA Review Draft Health Assessment Document for Chlorinated Benzenes. 49 Fed. Reg. 18616 (May, 1984) Part II. Submitted to U.S. EPA, Environmental Criteria and Assessment Office, Cincinnati, OH on July 30, 1984.

Cicmanec, J.L. 1991. Report of in-house research with 1,2,4-trichlorobenzene; an acute animal study. Memorandum to the U.S. EPA RfD/RfC Work Group. December, 1991.

Coate, W.B., W.H. Schoenfisch, T.R. Lewis and W.M. Busey. 1977. Chronic, inhalation exposure of rats, rabbits, and monkeys to 1,2,4-trichlorobenzene. Arch. Environ. Health. 32(6): 249-255.

S.T., G.F. Wolfe and C.C. Smith. 1978. Toxicity of 1,2,4-trichloro-
in Rhesus monkeys: Comparison of two in vivo methods for estimating
ctivity. Toxicol. Appl. Pharmacol. 45(1): 340. (Abstr.)

A., B. Leece, J. Gyorkos and K. Homonko. 1983. Polychlorinated
phenol congeners as inducers of rat hepatic drug-metabolizing
n immature male Wistar rats. Can. J. Physiol. Pharmacol. 61:

l Company. 1979-1980. Material safety data sheets - mono-
e, o-dichlorobenzene, p-dichlorobenzene, 1,2,4-trichlorobenzene,
achlorobenzene. Midland, MI.

. Drossman and T. Mill. 1986. Products and quantum yields for
f chloroaromatics in water. Environ. Sci. Technol. 20: 72-77.

jerova, V. and H.C. Hughes. 1983. Species differences on
ity of inhaled vapors and gases. Chapter 4. In: Modeling of
Exposure to Vapors: Uptake, Distribution and Elimination
Press, Boca Raton, FL.

F. Tolot, P. Martin and J. Bourret. 1969. Serious blood dis-
exposure to chlorine derivatives of benzene (A report of 7
ed. Lyon. 50(1164): 771-773. (Fre.)

Gilman. 1985. Goodman and Gilman's The Pharmacological Basis
cs, 7th ed. MacMillan Publishing Company, New York. p. 358.

Goto, M., M. Hattori, T. Miyagawa and M. Enomoto. 1972. Beiträge zur ökologischen chemie. II. Hepatoma-bildung in mäusen nach verabreichung von HCH-isomeren in hohen dosen. Chemosphere. 6: 279-282. (Ger.)

Gurfein, L.N. and Z.K. Pavlova. 1960. Maximum allowable concentration of chlorinated benzene in water supplies. Sanit. Okhrana Vod. Zagryazneniya Prom. Stochraymi Vodami. (4): 117-127. (Cited in CA 56:70601)

Hansch, C. and A.J. Leo. 1981. Medchem Project. Issue No. 19. Pamona College, CA.

Hawley, G.G. 1977. Condensed Chemical Dictionary, 9th ed. Van Nostrand Reinhold Company, NY.

Horvath, A.L. 1982. Halogenated Hydrocarbons. Solubility - Miscibility with Water. Marcel Dekker, Inc., NY. p. 530.

Jondorf, W.R., D.V. Parke and R.T. Williams. 1955. Studies in detoxication. 66. The metabolism of halogenobenzenes, 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzenes. Biochem. J. 61: 512-521.

Kao, C.I. and N. Poffenberger. 1979. Chlorinated benzenes. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol. 5. John Wiley and Sons, New York. p. 797-808.

Kitchin, K.T. and M.T. Ebron. 1983. Maternal hepatic and embryonic effects of 1,2,4-trichlorobenzene in the rat. Environ. Res. 31: 362-373.

Kociba, R.J., B.K.J. Leong and R.E. Hefner, Jr. 1981. Subchronic toxicity study of 1,2,4-trichlorobenzene in the rat, rabbit and beagle dog. Drug Chem. Toxicol. 4(3): 229-249.

Kohli, J., D. Jones and S. Safe. 1976. The metabolism of higher chlorinated benzene isomers. Can. J. Biochem. 54(3): 203-208.

Kuehl, D.W., E.N. Leonard, K.J. Welch and G.D. Veith. 1980. Identification of hazardous organic chemicals in fish from the Ashtabula River, Ohio, and Wabash River, Indiana. J. Assoc. Off. Anal. Chem. 63(6): 1238-1244.

Langhorst, M.L. and T.J. Nestrick. 1979. Determination of chlorobenzenes in air and biological samples by gas chromatography with photoionization detection. Anal. Chem. 51(12): 2018-2025.

Lawlor, T., S.R. Haworth and P. Voytek. 1979. Evaluation of the genetic activity of nine chlorinated phenols, seven chlorinated benzenes, and three chlorinated hexanes. Environ. Mutagen. 1: 143. (Abstr.)

Lewis, R.G. and K.E. MacLeod. 1982. Portable sampler for pesticides and semivolatile industrial organic chemicals in air. Anal. Chem. 54: 310-315.

Lingg, R.D., W.H. Kaylor, S.M. Pyle et al. 1982. Comparative metabolism of 1,2,4-trichlorobenzene in the rat and Rhesus monkey. Drug Metabol. Dispos. 10(2): 134-141.

Lopez-Avila, V., R. Northcutt, J. Onstot, M. Wickham and S. Billets. 1983. Determination of 51 priority organic compounds after extraction from standard reference materials. Anal. Chem. 55(6): 881-889.

MacKay, D., W.Y. Shiu and R.P. Sutherland. 1979. Determination of air-water Henry's Law constants for hydrophobic pollutants. Environ. Sci. Technol. 13(3): 333-337.

Matthews, M.B. and S. Kato. 1979. The metabolism and disposition of halogenated aromatics. In: Ann N.Y. Acad. Sci., Vol. 320. Health Effects of Halogenated Aromatic Hydrocarbons, Int. Symp., NY, June 24-27, 1978, W.J. Nicholson and J.A. Moore, Ed. N.Y. Acad. Sci. p. 131-137.

NAS (National Academy of Sciences). 1977. Drinking Water and Health. Safe Drinking Water Committee, NAS, Washington, DC. p. 667-673, 798-799.

NAS (National Academy of Sciences). 1980. Drinking Water and Health. Vol. 3, p. 25-67.

NIOSH (National Institute for Occupational Safety and Health). 1978. Criteria for a Recommended Standard...Occupational Exposure During the Manufacture and Formulations of Pesticides. Cincinnati, OH. DHEW (NIOSH) Publ. No. 78-174.

NLM (National Library of Medicine). 1981a. 1,2,3-Trichlorobenzene. Toxicology Data Base, Bethesda, MD. TDB No. 1502.

NLM (National Library of Medicine). 1981b. 1,2,4-Trichlorobenzene. Toxicology Data Base, Bethesda, MD. TDB No. 1105.

NLM (National Library of Medicine). 1982. 1,3,5-Trichlorobenzene. Toxicology Data Base, Bethesda, MD. TDB No. 0132.

Oehme, M. and H. Stray. 1982. Quantitative determination of ultra-traces of chlorinated compounds in high-volume air samples from the Arctic using polyurethane foam as collection medium. Fresenius Z. Anal. Chem. 311(7): 665-673.

Parke, D.V. and R.T. Williams. 1960. Studies in detoxication. LXXXI. The metabolism of halogenobenzenes: (a) penta- and hexa-chlorobenzenes, and (b) further observations on 1,3,5-trichlorobenzene. Biochem. J. 74: 5-9.

Powers, M.B., W.B. Coate and T.R. Lewis. 1975. Repeated topical applications of 1,2,4-trichlorobenzene: Effects on rabbit ears. Arch. Environ. Health. 30: 165-167.

Rao, K.S., K.A. Johnson and J.W. Henck. 1982. Subchronic dermal toxicity study of trichlorobenzene in the rabbit. Drug. Chem. Toxicol. 5(3): 249-263.

Rimington, C. and G. Ziegler. 1963. Experimental porphyria in rats induced by chlorinated benzenes. Biochem. Pharmacol. 12: 1387-1397.

Rinkus, S.J. and M.S. Legator. 1980. The need for both in vitro and in vivo systems in mutagenicity screening. In: Chemical Mutagens, Vol. 6, A. Hollander, Ed. Plenum Press, New York. p. 365-473.

Robinson, K.S., R.J. Kavlock, N. Chernoff and L.E. Gray. 1981. Multigeneration study of 1,2,4-trichlorobenzene in rats. J. Toxicol. Environ. Health. 8(3): 489-500.

Rowe, V.K. 1975. Written communication. (Cited in U.S. EPA, 1980)

Sasmore, D.P., C. Mitoma, C.A. Tyson and J.S. Johnson. 1983. Subchronic inhalation toxicity of 1,3,5-trichlorobenzene. Drug. Chem. Toxicol. 6(3): 241-258.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY. p. 716.

Schoeny, R.S., C.C. Smith and J.C. Loper. 1979. Non-mutagenicity for Salmonella of the chlorinated hydrocarbons Arochlor 1254, 1,2,4-trichlorobenzene, mirex and kepone. Mutat. Res. 68(2): 125-132.

Slimak, K., P. Johnson and V. Hodge. 1980. Materials balance-task α4 chlorobenzenes. U.S. EPA, Office of Toxic Substances, Washington, DC. EPA-560/13-80-001.

Smith, E.N. and G.P. Carlson. 1980. Various pharmacokinetic parameters in relation to enzyme-inducing abilities of 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene. J. Toxicol. Environ. Health. 6(4): 737-749.

Smith, C.C., S.T. Cragg and G.F. Wolfe. 1978. Subacute toxicity of 1,2,4-trichlorobenzene (TCB) in subhuman primates. Fed. Proc. 37(3): 248.

Townsend, B.A. and G.P. Carlson. 1981. Effect of halogenated benzenes on the toxicity and metabolism of malathion, malaoxon, parathion and paraoxon in mice. Toxicol. Appl. Pharmacol. 60(1): 52-61.

Treon, J. 1950. The toxicity of trichlorobenzene. Kettering Lab., Univ. of Cincinnati. (Unpubl. rep.) (Cited in Coate et al., 1977)

U.S. EPA. 1980. Ambient Water Quality Criteria Document for Chlorinated Benzenes. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA 440/5-80-028. NTIS PB 81-117392.

U.S. EPA. 1982. Test Methods: Methods of Organic Chemical Analysis of Municipal and Industrial Wastewater, J.E. Longbottom and J.J. Lichtenberg, Ed. Environ. Monit. Sup. Lab., Cincinnati, OH. EPA 600/4-82-057. NTIS PB83-20-1798.

U.S. EPA. 1983. Chlorinated benzenes aggregates derived from information reported under TSCA section 8(a). Preliminary assessment information rule (47 FR 26992) using the techniques for aggregating data described by 48 FR 27041. Office of Toxic Substances, Washington, DC.

U.S. EPA. 1985. Health Assessment Document for Chlorinated Benzenes. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. EPA 600/8-84/015F. NTIS PB 85-150332.

U.S. EPA. 1986. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1991. Integrated Risk Information System (IRIS). Online. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

Verschueren, K. 1977. Handbook of Environmental Data on Organic Chemicals. Von Nostrand Reinhold Co., New York.

Warner, H.P., J.M. Cohen and J.C. Ireland. 1980. Determination of Henry's Law Constants of Selected Priority Pollutants. U.S. EPA Research Report. Cincinnati, OH. EPA/600/D-87/229. NTIS PB87-212684.

Watanabe, P.G., R.J. Kociba, R.E. Hefner, Jr., H.O. Yakel and B.K.J. Leong. 1978. Subchronic toxicity studies of 1,2,4-trichlorobenzene in experimental animals. Toxicol. Appl. Pharmacol. 45(1): 332-333.

Williams, R.T. 1959. The metabolism of halogenated aromatic hydrocarbons. In: Detoxication Mechanisms, 2nd ed. John Wiley and Sons, New York. p. 237-277.

Yalkowsky, S.H. and S.C. Valvani. 1980. Solubility and partitioning. I. Solubility of nonelectrolytes in water. J. Pharm. Sci. 69(8): 912-922.

Yamamoto, H., Y. Ohno, K. Nakamori, T. Okuyama, S. Imai and Y. Tsubura. 1982. Chronic toxicity and carcinogenicity test of 1,2,4-trichlorobenzene on mice by dermal painting. J. Nara. Med. Assoc. 33: 132-145. (Jap.)

Yang, K.H., R.E. Peterson and J.M. Fujimoto. 1979. Increased bile duct-pancreatic fluid flow in benzene and halogenated benzene-treated rats. Toxicol. Appl. Pharmacol. 47(3): 505-514.