NATIONAL TOXICOLOGY PROGRAM

EXECUTIVE SUMMARY OF SAFETY AND TOXICITY INFORMATION

3,3',4,4'- TETRACHLOROAZOBENZENE

CAS Number 14047-09-7

3,3',4,4'-TETRACHLOROAZOXYBENZENE

CAS Number 21232-47-3

April 10, 1991

Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

Arthur D. Little, Inc.

Board of Scientific Counselors Draft Report

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OVERVIEW[1]

Nomination History: *3,3',4,4'-Tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) were nominated for reproductive and developmental testing and a two-year carcinogenicity bioassay with a high priority by the Environmental Protection Agency (EPA) in 1988. The nomination was based on the potential for human exposure, particularly among pesticide workers and consumers. The nomination was also based on preliminary data indicating that TCAB and TCAOB are isosteric to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and bind to the same liver receptor site as TCDD in laboratory animals, and the prediction that the toxicity of TCAB and TCAOB should be similar to that of TCDD, which is a known teratogen, hepatoxin, and chloracnegen.*

Chemical and Physical Properties: *Limited data were found on the physical characteristics of these compounds. TCAB is a bright orange crystalline solid, with a melting point of 158.0–158.5 °C (316.4–317.3 °F). TCAOB is a yellowish-orange crystalline solid, with a melting point of 142.5–143.0 °C (288.5–289.4 °F). TCAB is practically insoluble in water.*

Production/Uses/Exposure: *TCAB and TCAOB are contaminants derived during the synthesis of 3,4-dichloroaniline and dichloroaniline derivative pesticides. TCAB and TCAOB are not manufactured commercially. However, they are synthesized by the reduction of dichloronitrobenzene with lithium aluminum hydride. TCAB and TCAOB are present in the environment as a result of microbial breakdown or photolysis of propanil to 3,4-dichloroaniline, followed by transformation of 3,4-dichloroaniline to TCAB and TCAOB. No information was found concerning total U.S. annual production of TCAB or TCAOB. These compounds are not listed in the National Occupational Exposure Survey. TCAB and TCAOB are speculated to be environmentally persistent, leading to crop accumulation and potential consumer exposure. OSHA has not established a permissible exposure limit for TCAB or TCAOB. ACGIH has not*

[1]The information contained in this Executive Summary of Safety and Toxicity Information (ESSTI) is based on data from current published literature. The summary represents information provided in selected sources and is not claimed to be exhaustive.

recommended a threshold limit value and NIOSH has not recommended an exposure limit for TCAB or TCAOB.

Toxicological Effects:

Human: No data were found concerning the chemical disposition, acute, chronic/carcinogenic, reproductive or teratogenic effects of TCAB or TCAOB in humans. One of the major concerns regarding the toxicity of TCAB and TCAOB is their acnegenic potential. Occupational chloracne has been reported among employees working at plants which manufacture herbicides containing TCAB or TCAOB. Chloracne is characterized by comedone formation, inflammation, cysts, and papules.

Animal: *An oral rat LD_{50} value of > 5000 mg/kg has been reported for TCAB. Numerous studies conducted on rats, mice, and rabbits indicate that TCAB and TCAOB are potent acnegens. In prechronic skin application studies, TCAB and TCAOB were found to induce dose-dependent skin irritation leading to chloracne. Systemic toxicity was demonstrated by adverse effects on the liver, with death occurring at high doses due to liver toxicity. In addition, an increase in methemoglobin, a decrease in other blood parameters, and a decrease in body weights were observed. Similar adverse effects were observed in chronic feeding studies in rats, including a decrease in body weight, hematocrit value, white blood cell count, hemolysin level, and an increase in liver, spleen, and testicular weights.*

TCAB and TCAOB were found to be teratogenic in mice and chicken embryos. In Ah-responsive mice, TCAOB caused an increase in fetal malformations (including cleft palate, hydronephrosis, and hydrops), an increase in fetal resorption, and death. The mechanism of cleft palate formation in mice was determined to be the same for TCAOB and TCDD. TCAB and TCAOB caused rump edema in chicken fetuses. In mice, prechronic oral adminstration of TCAOB caused a decrease in thymus weight, lymphocyte number, and leukocytes. In rats, prechronic intraperitoneal injection of TCAB and TCAOB

caused thymic atrophy and an increase in the number of macrophage. TCAOB also caused a severe depression in T-cell dependent humoral response, a decrease in macrophage phagocytic ability, and bone marrow depression. In vitro and in ovo, TCAOB caused a decrease in lymphocyte formation.

Genetic Toxicology : TCAB and TCAOB have been found to cause mutation induction at the HGPRT locus in Chinese hamster ovary cells. TCAB caused a 20.3% induction of DNA repair in rat liver cells but this response was not considered to be statistically significant. In weanling mice, TCAOB administered in the diet caused chromosomal abberations and sister chromatid exchanges. TCAB was weakly mutagenic to two strains of Salmonella typhimurium in the presence of metabolic activation under aerobic conditions. However, the investigators indicated that the statistical significance of these results were debatable. Other authors note that these compounds were non-mutagenic to Salmonella typhimurium under aerobic conditions in the presence and absence of metabolic activation. TCAB was mutagenic to Aspergillus nidulans. TCAB and TCAOB caused an inhibition of cell division and deformed nuclei in Allium cepa L roots.

Structure Activity Relationships : TCAB and TCAOB are isosteric to TCDD, a potent teratogen, hepatotoxin, and chloracnegen. TCAB and TCAOB have been shown to bind to TCDD receptors with high affinity and to have similar, but less potent, toxic potential.

I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: U.S. Environmental Protection Agency (EPA) [USEPA, 1988]

2. Date: February 1988

3. Recommendations:
   - Reproductive/developmental toxicity
   - Two-year cancer bioassay

4. Priority: High

5. Rationale/Remarks:
   - Potential for human exposure, particularly among pesticide workers and consumers
   - Potential for accumulation on crops
   - TCAB and TCAOB are unwanted contaminants formed during the synthesis of 3,4-dichloroaniline (DCA) or herbicides synthesized from DCA; they are also formed from the microbial transformation of several 3,4-dichloroacylanilide herbicides.
   - Preliminary reports indicate that TCAB and TCAOB are isosteric to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and bind to the same liver receptor site as TCDD. These compounds are potent inducers of hepatic aryl hydrocarbon hydroxylase activity.
   - The potential toxicity of TCAB and TCAOB are predicted to be similar to that of TCDD, a known teratogen, hepatoxin, and chloracnegen.

B. Chemical Evaluation Committee Review

1. Date of Review: March 13, 1991

2. Recommendation: Carcinogenicity

3. Priority: Moderate

4. NTP Chemical Selection Principles: 3, 8

5. Rationale/Remarks:
   - Potential for human exposure
   - TCAB and TCAOB are contaminants of dichloroaniline (DCA) and herbicides synthesized from DCA

- TCAB and TCAOB are microbial transformation products of several 3,4-dichloroacylanilide herbicides, such as Diuron, Linuron, and Propanil, which are still registered and commercially available
- EPA Office of Drinking Water (ODW) is concerned about exposures to TCAB and TCAOB from drinking water which is contaminated by these products from the use of 3,4-dichloroaniline-derived herbicides
- EPA ODW will use NTP data to determine need for regulation and, if necessary, to set appropriate regulatory levels

C. Board of Scientific Counselors Review

1. Date of Review:

2. Recommendations:

3. Priority:

4. Rationale/Remarks:

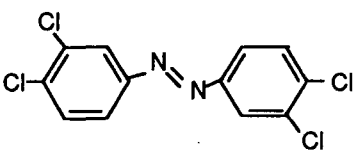
D. Executive Committee Review

1. Date of Review:

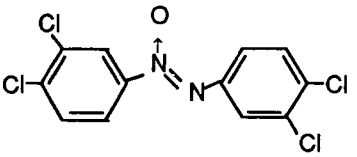
2. Decision:

II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers



3,3',4,4'-Tetrachloroazobenzene
(TCAB)



3,3',4,4'-Tetrachloroazoxybenzene
(TCAOB)

3,3',4,4'- TETRACHLOROAZOBENZENE (TCAB)
3,3',4,4'-TETRACHLOROAZOXYBENZENE (TCAOB)

Molecular formulas: $C_{12}H_6Cl_4N_2$ (TCAB) Molecular weights: **320.00**
$C_{12}H_6Cl_4N_2O$ (TCAOB) **336.00**

CAS Nos. 14047-09-7 (TCAB)
21232-47-3 (TCAOB)

RTECS Nos. CN2980000 (TCAB)
CO4150000 (TCAOB)

B. Synonyms and Trade Names

Synonyms: ***3,3',4,4'-tetrachloroazobenzene***
diazene, bis(3,4-dichlorophenyl)- (9CI); azobenzene, 3,3',4,4' -tetrachloro- (8CI); TCAB

3,3',4,4'-tetrachloroazoxybenzene
diazene, bis(3,4-dichlorophenyl)-, 1-oxide (9CI); azoxybenzene, 3,3',4,4'-tetrachloro- (8CI); TCAOB

Trade Names: No data were found.

C. Chemical and Physical Properties

Description: TCAB is a bright orange crystalline solid [Hsia *et al.*, 1977]. TCAOB is a yellowish-orange crystalline solid [Hsia and Burant, 1979].

Melting Point: 158.0-158.5 °C (316.4-317.3 °F) *(TCAB)*
142.5-143.0 °C (288.5-289.4 °F) *(TCAOB)*
[Beilstein, 1990]
139.0 °C (282.2 °F) (*TCAOB*) [Gaudry and Keirstead, 1949; Sundström, 1982]

Boiling Point: No data were found.

Density/Specific Gravity:	No data were found.
Refractive Index:	No data were found.
Solubility in Water:	1 µg/l {calculated} (*TCAB*) [Lyman *et al.*, 1982] (Based on the structural similarity to tetrachlorodioxins and tetrachlorodibenzofurans, it is speculated these compounds are practically insoluble in water, are lipophilic and have high organic carbon adsorption constants [Bellin, 1985].) No data were found for TCAOB.
Solubility in other Solvents:	May be soluble in ethanol (*TCAB*) [Hsia *et al.*, 1977]. No data were found (*TCAOB*).
Log Octanol/Water Partition Coefficient:	6.69 (*TCAB*) [USEPA, 1985] No data were found (*TCAOB*)
Reactive Chemical Hazards:	Emits toxic fumes of Cl^- and NO_x when heated to decomposition (*TCAB and TCAOB*)[Sax and Lewis, 1989].
Flammability Hazards:	No data were found.

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

- TCAB and TCAOB are not manufactured commercially [USEPA, 1985]. Both compounds are formed as trace contaminants during the synthesis of 3,4-dichloroaniline (DCA) and DCA-derived herbicides. Table 1 presents data on TCAB and TCAOB levels in DCA and several commercial herbicides derived from DCA including propanil, diuron, and linuron [Bellin, 1985].

- TCAB and TCAOB are synthetically produced for laboratory use via the reductive coupling of 3,4-dichloronitrobenzene or oxidative coupling of 3,4-dichloroaniline (DCA). 3,4-Dichloronitrobenzene is reduced to TCAB with ethanolamines and anhydrous sodium carbonate. The reduction of 3,4-dichloronitrobenzene is most commonly performed with lithium aluminum hydride. Other agents such as zinc metal in alkaline

solutions, sodium dihydrobis (2-methoxyethoxy) aluminate, and hydrazine-palladium have also been used. TCAB has also been synthesized from DCA by treatment with magnesium dioxide, silver oxide, or sodium perborate tetrahydrate. The synthetic routes to TCAOB are similar to those of TCAB but differ in reaction conditions, temperature, time, and reducing agent [Sundström, 1982].

Table 1. Occurrence of TCAB and TCAOB in Commercial Herbicides

Herbicide	TCAB (ppm)	TCAOB (ppm)
DCA	• 9 - 51	----
	• 13; 460;180	< 0.01
	• 60 - 8500	----
Propanil	• 2000 - 2900	----
	• 1000; 1400	----
Diuron	• 6 - 28 (avg. 13)	• 0.3 - 1.1 (avg. 0.8)
	• 28	
Linuron	• 7.5 - 8.8 (avg. 8.6)	< 0.05
	• 9.0	----

2. Producers and Importers:

No information was found on the producers of TCAB or TCAOB [USEPA, 1990; USITC, 1988; USITC, 1989; SRI, 1990]. However, the following manufacturers produce commercial herbicides which contain TCAB and TCAOB as impurities.

U.S. Producers:	Reference:
Diuron and 3,4-Dichloroaniline:	
Eagle River Chemical Company Phillips, Arizona	USEPA, 1990
E.I. du Pont de Nemours and Company, Incorporated Wilmington, Delaware	USITC, 1988, 1989
Monsanto Chemical Company St. Charles, Louisiana	USEPA, 1990
Velsicol Chemical Company Rosemont, Illinois	USITC, 1988
Propanil:	
Cedar Chemical Company Vicksburg, Missouri	USITC, 1989

Cumberland International Corporation Houston, Texas	USITC, 1989
Rohm & Haas Company Philadelphia, Pennsylvania	USITC, 1989

Importers:

No information was found on the importers of TCAB or TCAOB from the public file of the EPA Toxic Substances Control Act (TSCA) Inventory [USEPA, 1990].

3. Volume

TCAB and TCAOB were not included in the United States International Trade Commission's Publication Synthetic Organic Chemicals for the years 1986-1989 [USITC, 1987-1990]. However, it was reported that 13,038,000 pounds of propanil and 510,659,000 pounds of other cyclic herbicides were produced in the United States in 1988 [USITC, 1989].

There are no production data available on TCAB or TCAOB from the public file of the EPA Toxic Substances Control Act (TSCA) inventory. However, the public file of the EPA Toxic Substances Control Act (TSCA) inventory reported a production volume of 100,000-1,000,000 pounds/year of 3,4-dichloroaniline in 1977 by one manufacturer. It was estimated this volume will yield a production of 0.41-3900 kg/year of TCAB and 0.36-3.6 kg/year of TCAOB [USEPA, 1990].

4. Technical Product Composition

TCAB and TCAOB have been prepared by the lithium aluminum hydride reduction of 3,4-dichloronitrobenzene at a purity of greater than 99% [Hsia *et al.*, 1980, 1981; Bleavins *et al.*, 1985; Schrankel *et al.*, 1980].

B. Use

No information on commercial uses of TCAB and TCAOB was found. TCAB and TCAOB are contaminants found in dichloroaniline (DCA) and herbicides derived from DCA [Taylor and Lloyd, 1982]. DCA is used as an intermediate in the production of dyes and herbicides [Sax and Lewis, 1987].

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

No data were found on consumer exposure to TCAB or TCAOB. However, the EPA notes that TCAB and TCAOB persist in the environment and may accumulate on crops, possibly leading to dietary consumption [USEPA, 1988].

B. Occupational Exposure

TCAB and TCAOB are not listed in the National Occupational Exposure Survey (NOES) which was conducted between 1981 and 1983 by NIOSH. However, occupational exposure to TCAB and TCAOB among workers handling contaminated 3,4-dichloroaniline has been reported [Taylor *et al.*, 1977]. In cases concerning occupational exposure to TCAB and TCAOB, these compounds reportedly caused chloracne; however, exposure levels had not been determined [USEPA, 1985]. In addition, the EPA notes that pesticide workers may be exposed to TCAB and TCAOB during application of pesticides containing these compounds [USEPA, 1988].

C. Environmental Occurrence

3,4-Dichloroaniline (DCA) is present in the environment due to the hydrolysis of aniline herbicides including propanil, linuron, diuron, and neburon. In water and soil, TCAB and TCAOB are formed by photolysis of propanil to DCA which is followed by dimerization of DCA to TCAB [USEPA, 1985]. TCAB and TCAOB sorb very strongly to soils and are not likely to leach [USEPA, 1985]. Studies investigating the environmental presence of these compounds are summarized below. In addition, levels of TCAB and TCAOB in soils and water following the application of herbicides containing these compounds as contaminants are presented in Table 2.

- Thirty soybeans (*Glycine max. (L.) Merr.*) were harvested in soil of varying organic matter (0%, 1.7%, or 57%) containing 25 ppm TCAB for 12 days. TCAB levels in the roots and shoots were 58.4 ppm and 0.492 ppm in 1.7% organic matter, respectively, and were 14.4 and 0.178 ppm in 57% organic matter, respectively. Soil residues at the end of the experiment decreased as the percentage of organic matter increased. The author suggested that this may be a result of TCAB binding to the organic matter, or microbial degradation of TCAB via azo reduction of TCAB to other products. Since no other halogenated species were detected, the author suggested that the binding effect is a more likely explanation. TCAOB was detected at low levels in the roots harvested in 0% (0.317 ppm) and 1.7% (0.289 ppm) organic matter soil. TCAOB was also detected at low levels in the soil (0.02-0.043 ppm). The TCAOB detected was postulated to be a transformation product of TCAB [Worobey, 1984].

- Carrot seeds were added to 0.02 or 10 ppm carbon-14 labelled trans-TCAB treated soil. In the 0.02 ppm exposed carrots, mean levels of TCAB in the peels, pulp, and tops were 1.9, 1.1, and 0.1 ppb, respectively. In the 10 ppm exposed carrots, TCAB concentrations in the peel, pulp, and tops were 375, 20, and 30 ppb, respectively. As a recovery experiment, 2, 20, and 200 ppb carbon-14 labeled TCAB was added to carrot tissues. Recovery values of trans-TCAB were 78% (2 ppb), 76% (20 ppb), and 96% (200 ppb) [Worobey, 1988].

- In an exploratory experiment, TCAB at a concentration of 40 ppm was supplied for seven days to the roots of Nato rice plants grown in Hoagland's solution. This experiment indicated that the chemical was absorbed and translocated to the shoots. Therefore, a second study was conducted in order to determine the amount of TCAB absorbed and translocated by the rice plants. Thirty 20-day old rice plants were arranged in 4 trays that contained Hoagland's solution and one gram of celite on which was absorbed C^{14}-

TCAB at a concentration of 1.44 x 10^3 microCi/mg of celite. The plants were treated for 12 days. The concentration of radiolabelled TCAB in Hoagland's solution was considered to be the saturation concentration. Analysis of the plant tissues indicated that only 5.6% of the total C^{14}-TCAB administered to the plant was absorbed, and that 3.2% of the absorbed TCAB was translocated to the aerial portion of the plants [Still, 1969].

- Six, 50-gram Nixon loam soil samples were treated and incubated for 4 days with 0.25 grams glucose and 0.05 grams ammonium nitrate. They were then divided and treated with 10 milligrams 3,4-dichloroaniline (DCA), 10 milligrams DCA combined with 1 milliliter chloroform, or received no treatment.

  Increased peroxidase activity correlated with an increase in TCAB formation in the preincubated samples supplemented with glucose and ammonium nitrate. Preincubation appeared to increase DCA to TCAB formation. Chloroform decreased the peroxidase activity [Lay and Ilnicki, 1974].

- Fifty-gram soil samples were supplemented with 0.25 grams glucose, 0.05 grams ammonium nitrate, or both, and incubated with water. Twenty five milligrams of 3,4-dichloroaniline was added to one sample of each triplicate group and incubated for 10 days. TCAB levels were found to increase proportionally with peroxidase activity. Peroxidase activity was stimulated by the addition of a carbon and nitrogen source [Bordeleau and Bartha, 1972].

Table 2. Environmental Fate of TCAB and TCAOB in Soil and Water

Sample (Number)/Location	Pesticide Application Rate	Number of Samples Containing Residue Levels TCAB	Reference
Soil/(99)/Arkansas, California, Louisiana, Mississippi, Texas	1.2-118 kg/ha propanil	6/0.01-0.05 ppm	[Carey *et al.*, 1980]
Soil (47)/University of Arkansas[1]	6.7 kg/ha propanil	Unspecified/<6.01 ppm[2]	[Kearney *et al.*, 1970]
Soil (9)/Province of Ontario (7), Salt Marsh of Nova Scotia(2)	100 µg DCA or concentration propanil required to reach 100 µg DCA/g soil	7/0.08-22.5 µg/g[3] 5/3.0-9.5 µg/g	[Hughs and Corke, 1973]
Soil (5)/ Unspecified	100 parts propanil/10[6] soil	5/1-1-16% propanil[4]	[Burge, 1972]
Soil (rice paddy) (30)/Unspecified	10 ppmw carbon-14 labelled DCA[5]	Unspecified/6% C-14[6]	[Isensee *et al.*, 1982]
Soil (rice paddy) (10)/Unspecified	10 ppmw carbon-14 labelled DCA[5]	Unspecified/19% C-14[7]	[Isensee *et al.*, 1982]
Hagerstown Silty Clay[8] (Unspecified)/Not Applicable	1 µCi carbon-14 labelled DCA	Unspecified/increasing carbon-14 corresponding with increasing DCA	[Kearney and Plimmer, 1972]
Soil (Unspecified)[9]/Not Applicable	5 µCi carbon-14 labelled DCA	Not applicable/21% C-14 (Trans and cis TCAB)	[Kearney and Plimmer, 1972]
Water (Unspecified)/Lake Superior	Quantity propanil to reach 10 mg/L solution[10]	Not Applicable/0.67 mg/g	[Call *et al.*, 1983]

[1] Rice producing soil
[2] High concentrations of TCAB were found in the top layer of the soil sample. The concentration and occurrence of TCAB decreased with increasing time and depth of soil.
[3] TCAB concentrations were consistently higher in the DCA treated samples with a maximum yield within 6-30 days. TCAB production was highest in soils with pH 4.5-5.5.
[4] No TCAB was detected in the presence of high concentrations of *Arthrobactor* and *Nocardia* hydrolyzing microorganisms.
[5] In the presence of mosquito fish, B-g-algae, and several hundred daphnids submerged in water.
[6] On day 15.
[7] Up to day 45.
[8] One hundred grams of loam containing 1, 10, 100 and 1,000 ppm DCA.
[9] One kilogram soil sample containing 1 gram of DCA.
[10] A 500 milliliter water sample.

D. Regulatory Status

- OSHA has not established a permissible exposure limit (PEL) for TCAB or TCAOB.

E. Exposure Recommendations

- ACGIH has not recommended a threshold limit value (TLV) for TCAB or TCAOB.
- NIOSH has not recommended an exposure limit (REL) for TCAB or

TCAOB.

V. TOXICOLOGICAL EFFECTS

A. Chemical Disposition

1. Human Data

No data were found on the chemical disposition of TCAB or TCAOB in humans.

2. Animal Data

oral, rat (TCAB/ TCAOB)

- An unspecified number of male Sprague-Dawley outbred albino rats were administered 10 milligrams of carbon-14 labelled TCAB or TCAOB (249.3 μCi/mmol, 10 mg/ml corn oil) by stomach intubation. Urine and fecal samples were collected every 24 hours. Rats were sacrificed on day 5. Based on liquid scintillation spectrometer counts, TCAB was found to clear from the body more rapidly than TCAOB in 24-hour urine and feces samples (66% and 37% clearance, respectively). Based on this rapid elimination phase, the half-lives for the elimination of TCAB and TCAOB were determined to be 18 and 34 hours, respectively. The terminal phase half-lives for both compounds were determined to be greater than 20 days. The authors suggest that this data demonstrate the potential for these compounds to bioaccumulate under chronic exposure conditions. For both compounds, the feces was the major route of excretion. After 48 hours, TCAB and TCAOB levels (% of radioactive dose administered) in the feces were 55.1±4.9% (TCAB) and 50.0±14.3% (TCAOB). Levels of TCAB and TCAOB in the urine were 27.1±3.8% (TCAB) and 20.1±1.8% (TCAOB) after 48 hours. For both compounds the highest levels of radioactivity were found in the fat (2.70±1.17% for TCAB and 4.97±2.83% for TCAOB). High concentrations of radioactive TCAB and TCAOB were also found in the pancreas, lymph nodes, kidney, liver, and bladder. The lowest concentrations of radioactivity were found in the brain (0.12±0.01% for TCAB and 0.09±0.02% for TCAOB) [Burant and Hsia, 1984].

intraperitoneal, rat microsomes, TCAB

- Rat liver microsomes were prepared from male Sprague-Dawley rats given an intraperitoneal injection of TCAB at a concentration of 25 mg/kg/day mixed in corn oil for five days. Control rats received 5 ml/kg/day corn oil only. A mixture containing carbon-14 labelled TCAB (112-138 μg, 128 μCi/mmol), bovine serum albumin (2 mg), and dimethyl sulfoxide (DMSO) (1% v/v) was added to an NADPH-generating system. Samples designated as controls used heat-deactivated microsomes. Nearly all the radioactivity added to the incubation system was recovered. The rate of TCAB metabolism was determined to be 381 ± 59 pmol/min/mg microsomal protein. The major metabolite detected was a TCAB phenol. Other metabolites detected included N-hydroxy-3,3',4,4'-tetrachlorohydrazobenzene and 3,3'4,4'-tetrachlorohydrazo-benzene. In order to gain insight into the underlying biochemistry of the formation of the TCAB phenol, and the concurrent binding with the macromolecule pellet, the monooxygenase activity in the incubation system was modulated. Monooxygenase inhibitors, carbon monoxide and 2-diethylaminoethyl 2, 2-diphenyl-valerate hydrochloride were added to the system and the system was deprived of NADPH. Under these conditions, a significant reduction in TCAB phenol formation and covalent binding was observed [Hsia and Kreamer, 1981].

B. Acute

1. Human Data

No data were found on the acute toxicity of TCAB or TCAOB in humans.

2. Animal Data

oral, dermal, inhalation, rat (TCAB)

- An acute oral rat LD_{50} of > 5000 mg/kg (0/10 deaths), a skin approximate lethal dose (ALD) in rabbits of > 1000 mg/kg (0/6 deaths), and an inhalation (4-hour) acute lethal concentration (ALC) in rats of 0.92 mg/l have been reported for TCAB [Taylor and Lloyd, 1982]. No other data were provided.

E.I. du Pont de Nemours & Company (Haskell Laboratories) conducted the following acute toxicity test on laboratory animals:

dermal, rabbit (TCAB)

- Pairs of rabbits of unspecified sex and strain received an application of either TCAB, 3,4-dichloroaniline (DCA) containing 5% tar, DCA containing 8% TCAB, chloroform (negative control), dimethyl sulfoxide (negative control) in 5% chloroform solutions, or 0.1 milliliters of a Dow positive control on the left external ear canal. Right ears of each rabbit were treated with 0.1 milliliters of chloroform only. Two days after the test application, rabbits were sacrificed. Upon pathological analysis, rabbits that received TCAB, DCA containing 5% tar, and DCA containing 8% TCAB had marked epidermal hyperplasia, sebaceous gland hyperplasia, squamous metaplasia, and dilation of hair follicles (with an increase in keratin material, fibrosis of dermis, and some inflammatory infiltrate). Thickening of the skin and possible systemic effects were also noted. Chloroform produced similar but less pronounced changes. The TCAB-containing compounds were determined to be acnegenic [E.I. du Pont de Nemours & Company, Inc., 1982b].

dermal, rabbit (TCAB)

- Table 3 summarizes results of rabbit ear bioassays following the application of TCAB at various concentrations [Hill *et al.*, 1981].

Table 3. The Concentration of TCAB in Different Herbicides and Their Precursors, and the Results of Rabbit Ear Bioassays

Compound Tested	Formulation for Ear Test mg/ml (MIBK)[a]	TCAB Content of Sample (ug/g)	Total dose TCAB Applied (ug)	Microscopic Evaluation of Rabbit Ear Test[b]
TCAB	0.001	--	0.5	-,+
TCAB	0.01	--	5	+,++
TCAB	0.02	--	8	+,++
TCAB	0.04	--	16	++,++
TCAB	0.08	--	32	++,++

NOTES: - = no hyperkeratosis
+ = mild hyperkeratosis
++ = moderate hyperkeratosis
a) MIBK = methyl isobutylketone
b) Two rabbits per test were evaluated. If both "treated" ears reacted the same, only one rating was given; if they reacted differently, both ratings were given.

Reference: Hill *et al.*, 1981

C. Prechronic

1. Epidemiological Evidence/Case Reports

A number of cases of chloracne have been reported following occupational exposure to herbicides. In these cases, the observed chloracne was attributed to TCAB and/or TCAOB. In general, chloracne is characterized by comedone formation, straw-colored cysts, and

inflammatory papules. The most sensitive area of the skin is around the eyes and ears. Chloracne may be associated with systemic toxicity. Direct skin contact is expected to be the primary route of exposure; however, inhalation and ingestion are speculative routes.

occupational, human (TCAOB/ methazole)

- One of the first reports of TCAOB-induced chloracne involved 41 production workers (average age 29) in a chemical manufacturing plant. Workers developed bumps within 1-2 months after they began manufacturing 2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione (methazole) which contains unspecified concentrations of TCAOB as a contaminant. Ninety percent of the workers developed chloracne. Of the 41 workers who developed chloracne lesions, 38 had lesions on the face, 33 on the neck, 31 on the arms, 31 on the legs, 27 on the trunk, and 15 on the genitals. No significant differences were found in serum glutamic oxaloacetic transaminase or porphyrin levels. Four family members of four workers who had never been inside the plant developed chloracne. This may have resulted from direct contact with contaminated clothing or tools [Taylor *et al.*, 1977].

  Seven to eight years later, a long-term follow-up study was conducted on 5 of the workers and 2 children who had developed chloracne. Three of the workers still had evidence of chloracne. Four of the five workers were sensitive to sunlight. The 2 children had mild scarring, and one of the children (a 15-year-old girl) had *acne vulgaris* [Taylor and Lloyd, 1982].

occupational, human (TCAB/ methomyl, propanil)

- In a methomyl (1-(lamda-methylthio)ethylideneamino methyl carbamate)/propanil (pesticides contaminated with TCAB) pesticide manufacturing plant, 102 of 111 employees (plant workers and office workers) participated in a medical survey. Ninety six percent of the workers were male (88% white). The average employee age and length of employment were 28.7 years and 24 months, respectively. Among the participating employees, 6.9% had chronic health problems. Workers involved in the production of propanil had the highest rate of chloracne with an average of 1.39 symptoms per worker. Symptoms reported among the 28 production workers included acne (78%), rash/skin irritation (46%), eye irritation (25%), and cyanosis (21%). In 101 workers, symptoms included acne (39%), rash/irritation (34%), eye irritation (26%), and cyanosis (68%). Other symptoms observed included small pupils, nausea/vomiting, blurred vision, muscle weakness, coughing, headache, fatigue, confusion, increased salivation, and asthma. Seventeen (61%) of the 28 production workers were hospitalized due to chloracne. No significant hematological abnormalities were noted. In addition, cholinesterase activity was not affected by propanil exposure [Morse and Baker, 1979].

occupational, human (TCAB)/ 2,4,5-T

- In an epidemiological case control study, 54 pesticide applicators who sprayed 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) (a herbicide contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or TCAB for 63 weeks were compared with 54 workers who did not spray 2,4,5-T. No significant differences in liver function, porphyrin excretion, or prevalence of acne were found in the exposed compared to the non-exposed group [Houdt *et al.*, 1983].

occupational, human (TCAB/ TCAOB)

- Table 4 presents data on the occurrence of chloracne outbreaks from 3,4-dichloroaniline derived herbicides containing TCAB and/or TCAOB [Taylor and Lloyd, 1982].

Table 4. Chloracne Outbreaks From 3,4-Dichloroaniline Derived Herbicides

Location	Year	Herbicide	Source	Number of Workers (%)	Probable Chloracnegenic Chemical
Illinois	1960s and/or 1970s	Methazole	Chemical Plant	<10	TCAOB
England	1970s	Methazole	Chemical Plant	U	TCAOB
Ohio*1	1972-73	Methazole	Chemical Plant	41 (90%)	TCAOB
Michigan	1970s	Methazole	Chemical Plant	U	TCAOB
New Jersey	1960s and/or 1970s	Propanil	Chemical Plant	U	TCAB
Southern U.S.A.	1960s and/or 1970s	Propanil	Railroad cars delivering herbicide	U	TCAB
Arkansas**1	1974-77	Propanil	Chemical Plant	17 (61%)	TCAB
Europe	1970s	U	Chemical Plant	U	TCAB

U--unknown; *Taylor and co-workers, 1974; **Morse and co-workers, 1977, 1979.
1--Previously described.

Reference: Taylor and Lloyd, 1982

2. Animal Data

intraperitoneal, rat, mouse (TCAB/ TCAOB)

- Three groups of male Sprague Dawley rats (n=4) were injected intraperitoneally with 25 mg/kg TCAB 4 times per day, or with TCAOB 2 times per day in corn oil on days 1 and 5. Animals that received TCAB and TCAOB were sacrificed on days 5 and 10, respectively. Control rats received 2.5 ml/kg corn oil only. In addition, a group of female ICR outbred Swiss albino mice of unspecified number were injected intraperitoneally with 20 mg/kg TCAB, 5 times per day following lactation. On day 6, the mice were sacrificed.

  In mice and rats, both compounds caused histopathological changes of the liver, including hypertrophy and hyperplasia (greater than 50% compared to controls). Liver cells of the TCAB and TCAOB treated rats had granular cytoplasm containing vacuoles; some hepatocytes contained mitotic figures ($P < 0.001$). In addition, TCAB and TCAOB treatment induced proliferation of the smooth endoplasmic reticulum of rat liver cells. Mice livers treated with TCAB developed mitotic figures in which chromosomes appeared in a tripolar arrangement [Schrankel *et al.*, 1980].

dermal, mouse, rabbit (TCAOB)

- The chloracnegenic potential of TCAOB was investigated in five strains of mice including the hairless rhino (develops spontaneous follicular hyperkeratosis), rhino+ (does not develop spontaneous follicular hyperkeratosis), DBA/2J (an aryl hydrocarbon hydroxylase (AHH) enzyme induction insensitive strain), and C57BL/6 (an AHH enzyme induction sensitive strain). Male New Zealand white rabbits served as the positive control as this strain appears to be the most sensitive and reliable animal model for chloracne. Three studies were conducted using 3-5 mice of each strain per dose. In studies 1, 2, and 3, (see below) mice received test doses 5 days per week for 3 to 9 weeks (depending on when chloracne developed) on the hair-clipped dorsa. TCAB was applied to the inner surface of the right ear and control doses were applied to the left ear in all animals.

  In study 1, male mice from each strain were tested with 0.001% TCAOB in acetone 5 days per week for 9 weeks. Termination of the experiment was based on mortality. Three positive control rabbits were treated with 0.001% TCAB as follows: 1 per week for 17.5 weeks; 3 times per week for 17.5 weeks; or 5 times per week for 6 weeks.

  In study 2, female rhino and hairless mice received daily applications of 0.001%, 0.01%, and 0.1% TCAOB five days per week until fatalities occurred. Two positive control male rabbits received 0.001% TCAOB daily and 100 microliters of acetone.

  In study 3, male hairless mice and rhino mice received doses of 0.01 and 0.1% TCAOB. Rhino mice received 18 treatments (3.5 weeks) at both dose levels and hairless mice received 12 treatments of 0.1% TCAOB or 18 treatments of 0.01% TCAOB.

  Based on gross and histologic examination of the skin, no abnormalities occurred in treated mice from study 1. However, signs of chloracne, including hyperplasia and hypertrophy of the right ear, were seen in rabbits within 2.5 weeks. A dose-dependent follicular hyperkeratosis and epithelial hyperplasia (characteristic of a chloracnegenic response) were seen in mice in studies 2 and 3. Mice receiving 0.01% and 0.1% TCAOB experienced more severe effects including erythema, skin-fold thickening, discoloration, and weight loss. In studies 2 and 3, eyes of the rhino mice treated with TCAOB were swollen shut with keratinaceous ocular discharge. Rabbits also developed chloracne. One male hairless mouse in the 0.1% TCAOB dose group and one rhino mouse in the 0.01% TCAOB dose group died at 3 and 4 weeks, respectively. Rhino mice in the 0.1% TCAOB dose group developed necrotic foci of the liver [Horton and Yeary, 1985].

dermal, rabbit (TCAB)

- Albino rabbits of unspecified sex and number received applications of 0.0001%, 0.001%, or 0.01% TCAB dissolved in acetone solutions to the ear five days per week for 4 weeks. Fifty percent Halowax 1041 in mineral oil and acetone served as positive and negative controls, respectively. Comedone formation was observed in all TCAB-treated rabbits. Comedone response in the 0.0001% TCAB-dosed rabbits closely resembled Halowax-treated comedone formation. No comedones were seen in the acetone treated rabbits [Taylor *et al.*, 1977].

E.I. du Pont de Nemours & Company, Inc., (Haskell Laboratories), conducted the following prechronic skin absorption studies in albino rabbits of unspecified strain:

dermal, rabbit (TCAB)

- In the first part of the study, groups of male rabbits (n=10) received an application of 20 mg/kg TCAB in a 10% solution of acetone or 5 milliliters of acetone as a control. Control and test materials were applied by wrapping to the clipped shoulders and backs of the rabbits for 6 hours per day for 10 days. After 6-8 days of administration, 3/10 deaths had occurred in the test group. Animals appeared lethargic and had cold extremities prior to death. The surviving animals were necropsied on day 15. Blood was taken from the marginal ear vein of each rabbit prior to the application of test material and after the fifth and tenth applications. All rabbits developed moderate to severe skin irritation with fissuring and thick, crusty, and necrotic skin. The authors concluded that this effect was due to acetone. Clinical observations included decreased hematocrit values, hemoglobin levels, and erythrocyte counts compared to controls on days 5 and 8. Test group rabbits had an increased level of glutamic pyruvic transaminase activity. All animals had an increased methemoglobin level by day 10. From gross pathologic examination it was determined that death occurred due to liver toxicity. The livers appeared swollen and soft with fatty infiltration. The test group had slightly enlarged livers.

- In the second part of the study, 40 male albino rabbits were divided into groups in order to establish a no-effect level for "Still Bottom Tars." Groups 1 and 2 were divided into 2 dose groups. Group 1a (n=15) (control) received 3.5 milliliters of acetone for 10 days (wrapped). Group 1b (n=10) received 0.35 milliliters of acetone for 20 days (not wrapped). Group 2 (n=10) received 0.35 milliliters TCAB in 0.1% acetone solution (unwrapped) for 20 days. The day after the 10th treatment, five animals from each group were sacrificed. The remaining rabbits were sacrificed on day 14 or 30. Blood was taken from the marginal ear vein before the test began, after the 10th treatment, after the 30th treatment (group 1 and 2), and 14 days after the last treatment. By day 6, severe skin irritation had developed in all test groups with characteristics similar to those described for the first part of the study. [E.I. du Pont de Nemours & Company, Inc., 1982f].

dermal, rabbit (TCAB)

- Eight groups of rabbits (n=2) of unspecified strain or sex received approximately 0.1 milliliters of TCAB at a concentration of 0.002%, 0.02%, 0.2%, or 2.0% in 2,3-dichloroaniline (2,3-DCA); a solution of 3,4-dichloroaniline (DCA) 50% by weight in chloroform; a solution of DCA 50% by weight in 2,3-DCA; pure 2,3-DCA (99.25%); or chloroform. The solution of DCA 50% by weight in chloroform and 2,3-DCA both contained 23 ppm TCAB. TCAB in 2,3-DCA served as the positive control. All solutions were applied to the distal inner right ear of each rabbit, 5 days per week for 4 weeks. One animal per group was sacrificed after a 2-day rest period.

  TCAB (concentrations ranging from 0.002%-2.0% TCAB in 2,3-DCA) and DCA were acnegenic. DCA 50% by weight in chloroform and DCA

50% by weight in 2,3-DCA produced only mild acnegenic effects accompanied by pore enlargement. Gross examination of the skin revealed erythema, sloughing, and ear thickening. Pathological findings included hair follicles filled with yellow plugs and keratinous material on the skin [E.I. du Pont de Nemours & Company, Inc, 1982a].

dermal, rabbit (TCAB)

- Eight groups of rabbits of unspecified strain and sex received applications of 3,4-dichloroaniline (DCA) "Incident Tars" at concentrations of 0.01%, 0.1%, 1.0%, and 10.0% in chloroform, and TCAB at concentrations of 0.002%, 0.02%, 0.2%, and 2.0% in chloroform on the distal half of the inner left ear five days per week for 4 weeks. The lowest concentrations of both compounds were continued for 2 additional weeks. Both compounds were found to induce strong dose-dependent acnegenic responses including skin sloughing and thickening and plug formation of the skin. However, "Incident Tars" were 1/5 as active as pure TCAB. The NOEL for incident tars and TCAB was determined to approach 0.01% and 0.002%, respectively [E.I. du Pont de Nemours & Company, Inc., 1982d].

dermal, rabbit (TCAB)

- Five groups of rabbits (n=2) of unspecified strain and sex received an application of approximately 0.1 milliliters of 3,4-dichloronitrobenzene, 3,4-dichloroaniline (DCA), DCA in 10% chloroform solution, a 10% solution of TCAB in chloroform, a positive control Halowax 50% suspension in chloroform, or a negative (chloroform) control to the left ear, 5 days per week for 4 weeks. DCA was found to be acnegenic, producing skin sloughing, thickening and plug formation. TCAB was found to be a strong acnegen, causing sloughing, crusty skin, erythema, thickening, plug formation, and hair loss. Symptoms worsened with time. Slight sloughing and erythema was observed in the negative control group [E.I. du Pont de Nemours & Company, Inc., 1982c].

D. Chronic/Carcinogenicity

1. Human Data

No data were found on the chronic/carcinogenic effects of TCAB or TCAOB in humans.

2. Animal Data

oral, rat (TCAB/ TCAOB)

- Groups of 10 male Sprague Dawley rats were fed a diet containing 100 ppm TCAB or TCAOB in corn oil (control) for 120 days. On the last day of the experiment, animals were anesthetized and blood was collected. Food consumption and body weights were measured 2 and 3 times weekly, respectively. TCAB and TCAOB intake over the entire study was 25.2 ± 2.4 milligrams and 24.0 ± 2.3 milligrams, respectively.

  A significant decrease in body weight was seen in the TCAB (9.4%) and TCAOB (16.9%) treated rats compared to controls. Hematocrit values and hemoglobin levels decreased in test groups. This decrease was more significant in TCAOB-treated rats ($P < 0.001$) than TCAB treated rats ($P < 0.05$). The white blood cell count was insignificantly decreased in both treated groups. The red cell count was significantly decreased in the

TCAOB group ($P < 0.001$) and insignificantly decreased in the TCAB treated group. Liver, spleen, and testicular weights increased significantly ($P < 0.05$, $P < 0.05$, and $P < 0.005$, respectively) in TCAOB treated rats compared to controls. These organ weights were insignificantly increased in TCAB treated rats. Biochemical measurements conducted in this study are reported in section VG.3 [Hsia *et al.*, 1980].

E. Reproductive Effects and Teratogenicity

1. Human Data

No data were found on the reproductive or teratogenic effects of TCAB or TCAOB in humans.

2. Animal Data

oral, mice (TCAOB)

- Bleavins *et al.* (1985a) conducted a reproductive/immunocompetence study in order to evaluate the effects of *in utero* and early postnatal exposure to TCAOB on pup survival and immune function, and on the reproductive efficiency of their dams. The doses were selected so that no overt indications of toxicity were likely to be observed in the adult females. These doses were based on the results of a previous immunotoxicity study of TCAOB in young female mice that was conducted by Bleavins *et al.* (1985b). Four groups of 13 adult virgin female Swiss-Webster mice were administered for 14 days, 0 (control), 0.1 ppm, 1 ppm, or 10 ppm TCAOB dissolved in corn oil and mixed in powdered feed. The females were mated with untreated males on day 14, and pregnant females were continued on the test diet until delivery. On day 28 postpartum, females and pups were sacrificed in order to measure immune function in the pups, and to obtain organ weights in the mothers and offspring. Immune parameters including thymus weight and plaque forming cells (PFCs) per leukocyte and per spleen were measured. Immunocompetence among the offspring was determined by injecting pups with sheep red blood cells (SRBCs) on day 23 post partum and measuring immune parameters.

  Adverse effects in the treated groups were compared to controls. The only maternal toxic effect observed was a significant ($P < 0.05$) decrease in the thymus weight in the 10 ppm TCAOB treated group. A significant ($P < 0.01$) decrease in the number of pups per female whelping at birth and at weaning was observed in the 10 ppm TCAOB treated mice. A significant ($P < 0.01$) increase in pup weight at birth compared to controls was observed in the 1 ppm TCAOB treated group. The sum of the individual pup weights (litter mass) was significantly decreased at birth and on days 7, 14, 21, and 28 post-partum ($P < 0.01$, $P < 0.05$, $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively) in the 10 ppm TCAOB treated group. A significant ($P < 0.05$) decrease in the litter mass was also observed in the 1 ppm TCAOB treated group on day 21.

  In the 28-day-old pups used to assess immune function, thymus weights were insignificantly less than controls. However, the thymus weights of the 10 ppm TCAOB treated pups not immunized with SRBCs were significantly ($P< 0.01$) lower than control pups. In the immunized mice,

no significant difference in liver and spleen weights was observed compared to control mice. However, plaque forming cells were significantly decreased ($P < 0.01$) compared to control mice [Bleavins *et al.*, 1985a].

intraperitoneal, mice (TCAOB)

- The teratogenicity of TCAOB was studied in *Ah*-responsive and non-responsive mice. Three-month-old *Ah*-responsive (C57BL and NMRI) and *Ah*-nonresponsive (AKR/NBom and DBA/2J) mice were mated. Pregnant females were injected intraperitoneally with 6, 8, or 16 mg/kg TCAOB dissolved in dioxane on days 10-13 of gestation. The control group received 320 µl/kg dioxane only. On day 17, animals were sacrificed and their uteri were examined for number of implantations, dead, resorbed or alive fetuses.

  In addition, pregnant C57BL mice were treated with dioxane or TCAOB at 8 mg/kg on day 12, or cortisone acetate at 2.5 mg/animal on days 11-14 and sacrificed on day 15. The embryos were removed and heads were prepared for electron microscope viewing in order to examine palate cells.

  The specific dosing scheme and results from the experiments outlined above are presented in Table 5.

  As part of the same study, the effect of TCAOB in the offspring of the above matings (AKR X C57BL; C57BL X AKR; NMRI X DBA) was compared to the effect of this compound on inbred parental strains. Backcrosses between the F_1 generation of NMRF and DBW with inbred NMRI was also tested. The specific treatment regimen and experimental results are described in Table 6.

Table 5. Treatment Regimen (TCAOB) of Pregnant Mice (C57BL, DBA, and AKR) and Outcome of Teratology Study

Strain	Day Treated (3 p.m.)	Dosage	Dams with malformed fetuses %, (no. of affected[b]/treated)	No. of implan-tations	Resorption + dead fetuses % (early/late)[c]	Cleft Palate %[d]	Hydro-nephrosis %	Hydrops %
C57BL	10	TCAOB 6 mg/kg	100 (11/11)	77	31.2 (15/9)	33.6	63	0
	10	Dioxane[a]	0 (0/7)	46	19.6 (9/0)	0	0	0
	11	TCAOB 6 mg/kg	100 (10/10)	77	28.6 (15/7)	64.7	79.3	5.1
	11	Dioxane[a]	28.6 (2/7)	52	15.4 (7/1)	4.5	0	0
	12	TCAOB 6 mg/kg	100 (11/11)	80	21.3 (9/8)	56.6	37.3	8.1
	12	TCAOB 16 mg/kg	66.7 (6/9)	50	60 (29/1)	95	—	2
	12	Dioxane[a]	40 (2/5)	35	14.3 (5/0)	0	12.5	0
	13	TCAOB 6 mg/kg	90.9 (10/11)	82	19.5 (8/8)	23	26.9	7.9
	13	Dioxane[a]	0 (0/5)	33	18.2 (6/0)	0	0	0
DBA	10	TCAOB 8 mg/kg	28.6 (2/7)	54	5.6 (2/1)	1.9	2	0
	11	TCAOB 8 mg/kg	28.6 (2/7)	50	20 (10/0)	5	0	0
	12	TCAOB 8 mg/kg	14.3 (1/7)	48	8.3 (4/0)	2.3	0	0
	12	TCAOB 16 mg/kg	11.1 (1/9)	52	38.4 (14/6)	3.1	—	0
AKR	10	TCAOB 8 mg/kg	12.5 (1/8)	38	10.5 (4/0)	0	2.9	0
	11	TCAOB 8 mg/kg	25 (2/8)	34	14.7 (5/0)	0	10.3	0
	12	TCAOB	28.6 (2/7)	40	7.5 (3/0)	2.7	2.7	0

[a] Dioxane used as solvent for the TCAOB
[b] Malformations only
[c] Dead Fetuses less than about 6 mm of length have been assigned to the group of early dead
[d] Based on fetuses being alive or dead in late stage and possible to investigate
—Not investigated

Reference: Hassoun *et al.*, 1984

TABLE 6. Treatment Regimen (TCAOB) of Inbred Strains (C57BL, AKR, NMRI, and DBA) and Some Crosses and Backcrosses Between These Strains, and the Outcome of Teratology Data

Strain	Dose mg/kg	No. of dams	No. of implantations	Resorptions + dead fetuses %	No. of live fetuses		Cleft palate % of live fetuses[a]	
					Non-pigm.	Pigm.	Non-pigm.	Pigm.
1) C57BL	6	11	80	21.3	—	63	—	50.8
2) AKR	8	7	40	7.5	37	—	2.7	—
3) C57BL f x AKR m	6	12	84	11.9	—	74	—	1.4
4) C57BL f x AKR m	10	10	73	16.4	—	61	—	1.6
5) AKR f x C57BL m	6	9	19	10.5	—	17	—	0
6) (AKR x C57BL) f x AKR m	10	10	95	4.2	47	44	2.2	0
7) AKR f x (AKR x C57BL) m	10	10	55	18.2	21	24	4.8	8.3
8) NMRI	8	16	147	8.8	134	—	90.3	—
9) DBA	8	7	48	8.3	—	44	—	2.3
10) NMRI f x DBA m	8	12	102	9.8	—	92	—	6.5
11) NMRI f x (NMRI x DBA) m	8	16	148	9.5	66	68	48.5[b]	51.5[b]
12) (NMRI x DBA) f x NMRI m	8	17	150	5.3	61	80	18[b]	23.8[b]

f = female
m = male
[a] See Table 5
[b] Percent malformations among combined black and white offspring of NMRI f x (NMRI x BDA) m singificantly different from that of (NMRI x DBA) f x NMRI m
$P < 0.01$

Reference: Hassoun *et al.*, 1984

No evidence of maternal toxicity was observed in either the *Ah*-responsive or *Ah*-nonresponsive mice. C57BL mice treated with 6 mg/kg TCAOB on days 10, 11, 12, or 13 of gestation had an increased percentage of malformed fetuses (90.9-100%). Malformations observed included cleft palate, hydronophrosis, and hydrops in several cases (see Table 5). The percentage of hydrops increased in C57BL mice following treatment with 6 mg/kg TCAOB on days 11, 12, and 13 of gestation. Treatment of C57BL mice with 16 mg/kg TCAOB on day 12 of gestation resulted in a 60% occurrence of resorbed and dead fetuses, and a 95% occurrence of cleft palate. The frequency of the four parameters of fetal toxicity presented in Table 5 changed with time of administration in the C57BL dams. Generally, TCAOB treatment resulted in high frequency of late fetal death. An exception to this was observed in the group treated with 16 mg/kg TCAOB, where the frequency of hydrops showed a tendency to increase (not significantly) when the TCAOB was given late. Treatment of DBA mice with 16 mg/kg TCAOB caused an increase in resorption and fetal death (38%) compared to controls; however, no increase in cleft palate was noted. No evidence of toxicity was seen in the AKR mice after treatment with 8 mg/kg TCAOB on days 10, 11, or 12 of gestation. The authors suggested that this increase in sensitivity to TCAOB treatment by the *Ah*-responsive mice demonstrates an involvement of the *Ah*-locus in cleft palate formation.

The cross breeding study indicated that "the nonresponsiveness of DBA and AKR mice segregates as a dominant trait" in the crosses with C57BL and NMRI, respectively. The authors suggest that the percentage of

malformations was significantly higher among the backcross fetuses where the mother was an inbred NMRI (father NMRI x DBA) compared to the situation where the mother was NMRI x DBA (father inbred NMRI). Provided that the sensitivity is not linked to the sex chromosomes, the authors suggest that the host maternal factor is involved in the teratogenic mechanism. NMRI mice had a high incidence of cleft palate formation in the 8.0 mg/kg TCAOB dosed group on day 12 of gestation. The authors also assert that because approximately 20% of the offspring had cleft palate after TCAOB treatment, the fetal genotype may be determined by sensitivity to the teratogenic action of TCAOB.

Examination by scanning electron microscopy of the palate cells from embryos of pregnant C57BL mice treated on day 14 with TCAOB did not reveal degeneration [Hassoun *et al.*, 1984].

intraperitoneal, mice (TCAOB)

- Eight NMRI (*Ah*-responsive) and DBA2J (*Ah*-nonresponsive) 3-month-old pregnant mice were sacrificed on day 3 of gestation, and their uteri were excised. A reciprocal blastocyte transfer was performed on day 2 of the host gestation. On day 12 of gestation, mice were injected intraperitoneally with either 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or TCAOB dissolved in dioxane at doses of 30 µg/kg and 8 mg/kg, respectively. On day 16 or 17 of gestation, animals were sacrificed and their uteri were examined for the number of implantations and dead, resorbed or live fetuses, and the occurrence of cleft palate.

  None of the DBA fetuses (including those transferred into NMRI uteri) developed cleft palates. Of the NMRI fetuses that remained in the NMRI uteri following treatment with TCDD, 85% (29/34) had cleft palate while 100% (11/11) of the NMRI fetuses in the DBA dams had cleft palate. In the TCAOB treatment group, 90% (56/62) of the NMRI fetuses that remained in the NMRI uteri developed cleft palate and 93% (13/14) developed cleft palate following transfer to a DBA uterus [D'Argy *et al.*, 1984].

intraperitoneal, mice (TCAOB)

- Three groups of 3-month-old NMRI pregnant mice were injected intraperitoneally with 3 doses of D,L-alpha-difluoromethyl ornithine (DFMO) dissolved in saline at concentrations of 100, 200, or 300 mg/kg at 12-hour intervals, or 3 doses of 5000 µg/kg saline on days 11 or 12 of gestation. The control group received no treatment (n=10). A dose of 4 mg/kg TCAOB dissolved in dioxane was injected into the saline (n=14) and DFMO (n=18) treated mice on days 11 or 12 of gestation. In addition, some DFMO treated animals (n=10) were injected intraperitoneally with dioxane (300 µl/kg). Animals were weighed and sacrificed on day 17 of gestation and their uteri were examined for the number of implantations as well as dead, reabsorbed, and live fetuses.

  Maternal toxicity from TCAOB or DFMO treatment was not observed. The effects of TCAOB treatment on NMRI pregnant mice on day 11 and DFMO on days 11 through 12 of gestation are presented in Table 7. The effects of the treatment of pregnant NMRI mice with TCAOB on day 12 of gestation and DFMO on days 12 through 13 of gestation are presented in Table 8. Administration of DMFO at any dose produced no cleft palates. At a dose of 300 mg/kg, DMFO increased the rate of fetal death

compared to that observed in the vehicle control group ($P < 0.02$). DFMO decreased the frequency of cleft palate when co-administered with TCAOB. TCAOB-induced fetal death was not affected by DFMO administration. The authors concluded that the possible mechanism of cleft palate formation involves TCAOB stimulation of the polysubstrate monooxygenase system and other enzymes of the *Ah*-locus system, thus keeping epithelial cells alive leading to the formation of cleft palate. DFMO inhibits the activity of this enzyme and thereby decreases cleft palate formation [Hassoun and Arif, 1988].

Table 7. Effects of the Treatment of Pregnant NMRI Mice with TCAOB Given at 12-hour Intervals, On Days 11 Through 12 of Gestation

Dosage	Dams with Fetuses Having Cleft Palate% and (Number of Affected/Treated)	Mean of Implantation Number/Dam ± S.E.M. and (Total Number)	Percent of Fetuses/Dam Being Resorbed or Dead ± S.E.M. and (Number Early/Number Late)[a]	Percent of Fetuses/Dam Having Cleft Palate ± S.E.M. and (Number Among Investigated)
No Treatment	0 (0/11)	6.1 ± 0.73 (67)	11.9 ± 4.3 (7/1)	0 (0/60)
DFMO + dioxane	0 (0/10)	8.8 ± 0.56 (88)	13.6 ± 3.4 (10/0)	0 (0/78)
TCAOB + saline	42.9 (6/14)	7.6 ± 0.62 (106)	33.9 ± 8.1* (25/11)	37.0 ± 7.4 (30/81)
TCAOB + DFMO	33.3 (6/18)	8.9 ± 0.43 (160)	36.3 ± 10.7 (38/20)	17.2 ± 3.2** (21/122)

a Resorbed plus dead fetuses (<6 mm of length) have been assigned to the group of early dead, while dead fetuses more than 6 mm of length have been assigned to the group of late dead.

* Significantly different from control (DMFO-plus dioxane-treated), by Student's *t*-test, $P = 0.025$.

** Significantly different from control (saline-plus TCAOB-treated), by Students's *t*-test, $P < 0.01$.

Table 8. Effect of the Treatment of Pregnant NMRI Mice with TCAOB on Day 12 of Gestation, and DFMO Given at 12-hour Intervals, on Days 12 Through 13 of Gestation

Dosage	Dams with Fetuses Having Cleft Palate% and (Number of Affected/Treated)	Mean of Implantation Number/ Dam ± S.E.M. and (Total Number)	Percent of Fetuses/Dam Being Resorbed or Dead ± S.E.M. and (Number Early/Number Late)[a]	Percent of Fetuses/ Dam Having Cleft Palate ± S.E.M. and (Number Among Investigated)
DFMO + dioxane	0 (0/7)	9.3 ± 0.58 (65)	9.2 ± 3.1 (6/0)	0 (0/59)
TCAOB + saline	100 (14/14)	7.6 ± 0.68 (106)	11.3 ± 4.3 (7/5)	77.8 ± 8.2 (76/99)
TCAOB + DFMO	75 (12/16)	7.5 ± 0.60 (120)	13.3 ± 3.4 (16.0)	42.3 ± 7.5* (44/104)

[a] Resorbed plus dead fetuses (<6 mm of length) have been assigned to the group of early dead, while dead fetuses more than 6 mm of length have been assigned to the group of late dead.

* Significantly different from control (saline-plus TCAOB-treated), by Students's *t*-test, $P < 0.005$.

Reference: Hassoun and Arif, 1988

in ovo, chick (TCAOB)

- Fertile eggs from a crossbred F1(NH X SCWL) were injected with TCAB or TCAOB in corn oil at doses ranging from 0.1 ng to 100 µg/egg on day 4 or days 11-13 of incubation (volume of 20 µl per egg). Control eggs were injected with corn oil only. Surviving embryos were incubated until hatching occurred.

  An insignificant decrease in hatchability was observed in eggs injected with TCAB and TCAOB on day 4 compared to controls. The majority of deaths occurred before day 13 of incubation. Few deaths were observed in eggs injected on days 11-13. A 100% mortality was observed in eggs treated with 1.0 µg TCAB and 0.1 µg TCAOB on day 4 of incubation. An LD_{50} of 44 ng and 12 ng was estimated for TCAB and TCAOB, respectively.

  Numerous malformations were detected in both hatched chicks and embryos that died prior to hatching. A direct causal relationship between TCAB and TCAOB exposure and rump edema was observed. Edema was detected in embryos and hatched chicks from eggs treated with 0.01 µg (3.4%) - 1.0 µg (4.2%) of TCAB and 0.005 µg (2.9%) - 0.05 µg (7.3%) of TCAOB. The highest incidence of rump edema in treated embryos occurred in the 0.0075 µg TCAOB/egg (22.5%) and 0.05 µg TCAB/egg (5.1%) treated fetuses. The percent of embryos with rump edema ranged from 2.5-5.1% (TCAB) and 2.1-22.5% (TCAOB). Rump edema was not observed in the control group. All embryos (except 2) with rump edema died before hatching. The authors concluded that TCAB and TCAOB are "extremely toxic and potentially teratogenic" in the chick. However, these compounds appear to be less toxic than TCDD [Schrankel *et al.*, 1982].

F. Genetic Toxicology

1. Human Data

No data were found on the genetoxic effects of TCAB or TCAOB in humans.

2. Prokaryotic Data

Salmonella typhimurium (TCAB)

- In the standard Ames test, TCAB was found to be weakly mutagenic in *Salmonella typhimurium* in the presence and absence of metabolic activation. A dose response was seen at concentrations greater than 100 µg/per plate TCAB. In the TA98 strain, 0.5 revertants/µg were observed. No other information was provided [Hsia *et al.*, 1977].

Salmonella typhimurium (TCAB)

- Standard Ames mutagenicity tests of TCAB were conducted on *Salmonella typhimurium strains* TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, TA1532, TA1950, TA1975, and TA1978 under both aerobic and anaerobic conditions, and in the presence and absence of metabolic activation. TCAB had a positive but not significant mutagenic effect toward TA1532 at concentrations greater than 0.05 mg/plate under aerobic conditions in the presence of metabolic activation. TCAB was non-mutagenic in the strains tested [Mercier *et al.*, 1978].

Salmonella typhimurium (TCAB/ TCAOB)

- In the Ames test, the mutagenic effects of TCAB and TCAOB were tested in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA104 in the presence and absence of metabolic activation at concentrations ranging from 0-250 µg/plate. Both compounds were non-mutagenic in all tested strains in the presence and absence of metabolic activation [McMillan *et al.*, 1988].

Salmonella typhimurium (TCAB/ TCAOB)

- The mutagenic effects of 19 herbicide-derived chlorinated azobenzenes, including TCAB and TCAOB, were tested in *Salmonella typhimurium* using both the classical Ames (plate incorporation method) and the fluctuation tests. Assays were performed under both aerobic and anaerobic conditions in the presence and absence of liver S9 metabolic activation at concentrations ranging from 1-2000 µg/plate. TCAB and TCAOB were found to be non-mutagenic to all strains of *Salmonella* tested (TA98, TA100, TA1530, TA1535, TA1537, TA1538, TA1532, TA1950, TA1975, TA1978, G46) using the Ames assay. In the fluctuation test, very weak mutagenic activity was observed with TCAB (1.25 and 250 µg/ml) in the presence of metabolic activation in *Salmonella* strains TA1538 and TA1532. However, the authors indicated that the statistical significance of these studies was debatable. TCAOB was found to be non-mutagenic in all *Salmonella* strains tested [Gilbert *et al.*, 1980].

3. Eukaryotic Data

Chinese hamster ovary cells (TCAB/ TCAOB)

- TCAB and TCAOB were tested for mutation induction at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus using Chinese hamster ovary (CHO-K2-BH_4) cells in the presence and absence of S9 metabolic activation. Neither TCAB (0-14.4 µg/ml) nor TCAOB (0-15.1 µg/ml) caused mutation induction at the HGPRT locus in Chinese hamster ovary cells in the presence or absence of metabolic activation [McMillan *et al.*, 1988].

rat hepatocytes (TCAB, TCAOB)

- The ability of TCAB (1.6-14.4 µg/ml), TCAOB (1.7-15.1 µg/ml) and other derivatives of propanil to induce DNA damage and effect lactate dehydrogenase release was investigated in rat hepatocytes in an unscheduled DNA synthesis assay. Hepatocytes were treated in culture with the test compounds and incubated overnight in the presence of [^{3}H] thymidine. Autoradiographic detection of unscheduled DNA synthesis and the percentage of hepatocytes in repair was determined. TCAB (6.4 µg/ml) was the only compound found to induce DNA repair (20.3%) (insignificant). However, a concentration-dependent increase in unscheduled DNA synthesis was not observed. A concentration-dependent increase in lactate dehydrogenase release was induced by all test compounds [McMillan *et al.*, 1988].

oral, mouse (TCAOB)

- The genotoxicity of TCAOB has been tested in Swiss Webster mice as part of a reproductive toxicity study (see section V.E.2). The cytogenic effects of TCAOB following oral exposure were measured on the splenic lymphocytes of pups born to, and raised by, dams consuming 10 ppm TCAOB, and in weanling pups consuming 40 ppm TCAOB. The average chromosome breakage per cell and the mitotic index of splenic lymphocytes of the mouse pups were determined. A slightly increased level of isochromatid breakage in the animals born to mothers exposed to 10 ppm TCAOB was observed. Other types of chromosomal abberations including translocation and fragments were not observed.

  There was no significant effect on the mitotic index in weanling mice that consumed 40 ppm TCAOB in their diet for 28 days. However, a significant increase in chromatid breaks ($P < 0.01$) and exchanges ($P < 0.01$) in cells from TCAOB-treated mice compared to the negative controls was observed. Although TCAOB was clastogenic, it did not cause an elevation in the number of sister chromatid exchanges compared to control values [Bleavins *et al.*, 1985b].

Aspergillus nidulans (TCAB)

- 3',4'-Dichloropropionanilide, 3,4-dichloroaniline (DCA), and TCAB were tested for their effect on the back-mutation frequency of the *meth*$_3$ locus in *Aspergillus nidulans* at concentrations ranging from 5-200 µg/ml for 5 days. TCAB was reported to increase the back mutation frequency of the *meth*$_3$ locus. The following mutagenic potential of TCAB in *Aspergillus nidulans* in relation to DCA and 3',4'-dichloropropionanilide was determined: DCA > TCAB > 3',4'-dichloropropionanilide [Prasad, 1969].

4. Other

Mouse embryo fivbroblast cells (TCAB)

- The cytotoxicity of TCAB to mouse embryo fibroblasts was determined using the C3H/10T1/2 cell line. TCAB was found to cause an inhibition of cell growth in mouse fibroblast cells after 3 days of treatment at concentrations greater than 2 μg/ml, and after 7 days of treatment at a concentration of 0.78 μg/ml. Cultures exposed to 1 μg/ml TCAB for 10 days showed focal morphologic alterations characteristic of transformed cells. The authors reported that the foci consisted of thickened spindle cells arranged in random or "swirls". Contact inhibition was lost and cell growth continued after a monolayer was established [Hsia *et al.*, 1977].

Allium cepa L. roots (TCAB)

- The cytogenic effects of propanil and its derivatives have been tested in *Allium cepa* L. roots. Roots (2 to 4 cm long) were placed in solutions of 0 to 10 ppm TCAB, 3,4-dichloroaniline (DCA), and propanil for 3 to 5 hours. Control roots were untreated. Inhibition of cell division and deformed nuclei were observed in all test groups (propanil > DCA >TCAB). In the TCAB treated group, 0.05% of the cells appeared abnormal in the metaphase and anaphase division following exposure to 5 ppm TCAB for 3 hours. Chromosomal aberrations were not observed in cells of untreated roots [Prasad and Pramer, 1969].

G. Other Toxicological Effects

1. Immunologic Toxicology

oral, mice (TCAOB)

- Four-week-old Swiss Webster mice were given 100 ppm (positive control) cyclophosphamide in rat chow, 40 ppm TCAOB treated rat chow, or untreated rodent chow for 28 days. Body weight, food intake, and clinical symptomology were monitored. Mice not examined for blastogenic response were injected intraperitoneally four days prior to sacrifice with 0.2 milliliters of a 10% sheep red blood cell suspension. Animals were sacrificed according to a staggered schedule such that 18 animals were killed on 4 different termination dates. Lymphocyte blastogenesis was performed on splenic cells of mice killed on the first 2 termination dates. Lymphocyte T-induction and T-suppressor markers were also measured.

  No differences regarding food intake and behavior were observed between control and test groups. Two TCAOB treated mice had mild alopecia around the eyes. On day 28, the TCAOB treated unimmunized mice showed a significant decrease ($P < 0.05$) in body weight compared to controls. Thymus weights were significantly lower than control values in both cyclophosphamide and TCAOB treated groups ($P < 0.05$ and $P < 0.01$, respectively) in both immunized and unimmunized mice. The liver weights were not significantly altered in any of the treatment groups and no porphyria was observed in livers. The total white blood cell count was significantly lower than control values in both immunized and unimmunized cyclophosphamide treated groups ($P < 0.01$) and immunized TCAOB ($P < 0.05$) mice. The number of esonophils were significantly less ($P < 0.05$) in the unimmunized cyclophosphamide group and immunized TCAOB mice ($P < 0.01$) compared to controls. No

other significant effects on leukocytes were noted. A significant increase in segmented neutrophils ($P < 0.01$) was seen in the unimmunized cyclophosphamide and TCAOB groups and the immunized cyclophosphamide group ($P < 0.01$). A significant decrease in lymphocyte number was seen in cyclophosphamide immunized mice ($P < 0.01$). The number of cells recovered from the spleen was significantly less than controls in the TCAOB and cyclophosphamide immunized and unimmunized groups ($P < 0.05$ and $P < 0.01$, respectively). T-lymphocyte suppressor and inducer markers were significantly decreased in unimmunized TCAOB mice ($P < 0.05$ and $P < 0.01$, respectively). In addition, the T-inducer marker was decreased in immunized cyclophosphamide and TCAOB treated mice ($P < 0.01$). Cyclophosphamide treated mice had a significantly decreased ($P < 0.05$) blastogenic response compared to controls. This effect was not observed in TCAOB treated mice. Plaque forming cells were reduced below control values for the TCAOB and cyclophosphamide groups ($P < 0.01$). No changes were observed in plasma protein; however, a significant decrease in hemolysin titer ($P < 0.01$) was observed in both the TCAOB and cyclophosphamide test groups. The authors concluded that TCAOB compromised the ability of mice to immunorespond [Bleavins *et al.*, 1985b].

in vitro mice (TCAOB)

- Fetal thymus cultures from C57BL/6 (B6) and DBA/2J (D2) mice taken on days 14 and 15 of gestation, respectively were incubated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCBDF), or TCAOB in 1,4-dioxane at unspecified concentrations. Controls received 0.6 microliters of 1,4-dioxane/ml of medium. A dose-dependent decrease in the number of lymphocytes per test group was observed. TCAOB was an order of magnitude less potent than TCDBF. After 6 days in culture, TCDD and TCDBF were equally potent ($EC_{50} \approx 10^{-10}$ M) and TCAOB was slightly less active ($EC_{50} \approx 2 \times 10^{-10}$ M) [Denker *et al.*, 1985].

intraperitoneal, mice (TCAOB)

- As part of the study described above, B6 mice were injected intraperitoneally with TCDD, TCDBF, and TCAOB in 1,4-dioxane at doses of 20 µg, 200 µg, and 6 mg/kg, respectively on days 12 or 13 of gestation. Control animals received 320 µl/kg dioxane only. On day 14 of gestation, fetal thymuses were removed and cultured. Two days after treatment, lymphoid development was maximally inhibited in all 3 groups. On day 6, the number of lymphocytes from the TCDBF and TCAOB treated groups were comparable to control levels. The authors suggest that TCDD and its congeners act directly on the thymus and not secondarily via nutritional and hormonal disturbances. They also determined that 3 ligands of the *Ah* receptor (TCDD, TCDBF, and TCAOB, which have approximately the same affinities for the receptor) show an *in vitro* toxicity that corresponds to their affinity [Dencker *et al.*, 1985].

The following four experiments were conducted in male Sprague Dawley outbred albino rats to investigate the thymic atrophy induced by TCAB and TCAOB:

intraperitoneal, rat (TCAB/ TCAOB)

- In experiment 1, 3 groups (n=8) of 8-week-old rats were injected intraperitoneally with TCAB or TCAOB at a concentration of 25 mg/kg in corn oil or corn oil only (controls) on days 1 and 5. Animals were sacrificed on day 11, and organs were weighed and examined.

  Rats treated with TCAOB exhibited more severe toxic effects than TCAB treated rats. Livers from the TCAOB treated rats weighed significantly ($P < 0.01$) greater than control weight. TCAOB treated organs weighing significantly less than controls, included the thymus (51% of control weight), and fat (unspecified % of control weight) ($P < 0.01$ and $P < 0.05$, respectively). In the TCAB group, organs weighing significantly greater ($P < 0.01$) than controls included the liver (33% greater than control weight) and spleen (unknown % greater than control weight). The thymus of the TCAB treated rats weighed significantly ($P < 0.01$) less (31% of control) than controls [Hsia *et al.*, 1982].

- In experiment 2, the influence of age on TCAOB-induced thymic atrophy was investigated. Twenty-three, 24-day-old weanling rats were administered the same TCAOB test diet as experiment 1. Two groups with significant differences in body weight at the start of the experiment (group 2a, 64 ± 2 grams, n=12; group 2b, 51 ± 2 grams, n=6) were employed.

  Results of experiments 1 and 2 indicate that weanling rats are more sensitive to TCAOB than the 8 week-old rats. TCAOB treated rats had a significant ($P < 0.05$) decrease (1-4 grams) in food intake. A significant decrease in body weight (2a: $P < 0.01$ and 2b: $P < 0.05$) compared to controls was also noted. The thymus had the most significant ($P < 0.01$) decrease in weight compared to controls (2a: 68% of control weight and 2b: 76% of control weight). Thymus weights relative to body weight decreased 61% and 69% in groups 2a and 2b, respectively. Other organ weights reportedly decreased in treated groups; however, organ and body weight differences were insignificant [Hsia *et al.*, 1982].

- In experiment 3, the role of food intake in relation to decreased body weight observed in group 2 was investigated. One group of weanling rats (n=6) received the same TCAOB test diet as experiment 1. Another group of rats received a corn oil control diet. Food intake was controlled.

  A significant ($P < 0.01$) decrease in body weight, a 68% decrease in the thymus weight ($P < 0.01$) and the relative weight of thymus (61% of control weight) was observed in the TCAOB treated groups compared to controls [Hsia *et al.*, 1982].

- In experiment 4, the hypothesis that TCAOB toxicity in rats is due to a stress induced increase in glucocorticoid levels was investigated. Two groups of adrenalectomized rats and 2 groups of Sham operated rats (n=4 each) were fed either 25 mg/kg TCAOB or corn oil as outlined in experiment 1.

The 2 adrenalectomized rats experienced a progressive weight loss, and 2 rats died on day 10. Both adrenalectomized and Sham operated TCAOB treated rats experienced a 41% decrease ($P < 0.01$) in thymus weight compared with control groups. The authors suggest that this data indicates that thymic toxicity is not due to glucocorticoid induction.

Upon histological analysis of the thymus, all treated groups consistently exhibited severe atrophy of the cortex and the presence of macrophages (possibly due to lymphocytopoiesis). TCAOB at a concentration of 25 mg/kg caused a depletion of lymphocytes in the spleen and mesenteric lymph nodes. In addition, this concentration of TCAOB caused hypertrophy of the adrenal cortex and invasion of histocytes in several animals [Hsia *et al.*, 1982].

intraperitoneal, rat (TCAOB)

- Two groups each of weanling and adult male Sprague Dawley outbred albino rats (n=6 each) were administered intraperitoneal injections of 25 mg/kg TCAOB in corn oil on days 1, 6, 11, and 16. Control rats were injected intraperitoneally with 2.5 ml/kg of corn oil. Weanling and adult rats received their first injection of TCAOB 25 days after birth and on day 56, respectively. On day 13 of the study, all rats were injected intraperitoneally with 0.6 milliliters of a 10% suspension of sheep red blood cells (SRBCs) in saline. All animals were sacrificed on day 17. Organs were weighed and histopathologically examined. The spleen was assayed for plaque forming cells (PFC) to determine the number of lymphocytes per spleen. Bone marrow cellularity (BMNC) was determined and white blood cells and splenic antibody producing lymphocytes against SRBCs were counted. In addition, rat cell macrophage viability and cell ratio to peritoneal white blood cell count were determined.

  A 79% decrease in body weight ($P < 0.01$) was seen in TCAOB exposed weanling rats compared to controls. The only weanling rat organs weighing more than controls were the liver (40%) and lung (35%). Weanling rat organs weighing significantly less than controls included the spleen (13%), thymus (66%), heart (10-15%), and mesenteric lymph node (33%) ($P < 0.05$, $P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively). Adult rat organs weighing significantly greater than controls included the spleen (percent not specified), liver (40%), lungs (35%), and testes (% not specified) ($P < 0.05$, $P< 0.001$, $P < 0.01$, and $P < 0.05$, respectively). The thymus was the only organ weighing significantly ($P < 0.001$) less than controls. White blood cell counts were slightly less in weanling rats (97%) and higher in adults (115%) compared to controls. Weanling rats had a significant decrease in BMNC (74%), macrophage chemiluminescence (97%), PFC values for spleen lymphocytes (86%), spleen viable lymphocytes (91%) and hemolysin titers (93%) ($P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively). Adult parameters which were significantly less than controls included the macrophagechemiluminescence (95%), spleen viable lymphocytes (79%), PFC values for spleen lymphocytes (89%), hemolysin titers (69%), and BMNC (58%) ($P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively). From the results of this study, the authors concluded that severe depression of the T-cell dependent humoral

response (measured by PFC values and hemolysin titers), a decrease in peritoneal macrophage phagocytic capability (measured by chemiluminescence response to yeast), atrophy of thymus (the site of T-cell maturation), and depression of bone marrow cellularity resulted from exposure to TCAOB. Weanling rats were observed to be more sensitive to TCAOB than adults [Olson *et al.*, 1984].

in vivo, mice (TCAOB/ TCDBF)

- Cultures of thymus cells from 14-day-old C57BL mice embryos were treated with TCAOB and 2,3,7,8-tetrachlorodibenzofuran (TCDBF) dissolved in 1,4-dioxane (5×10^{-9} M or 5×10^{-10}M), or a combination of both compounds. Control cultures received 0.16 µl of 1,4-dioxane/ml medium. Both compounds at a concentration of 5×10^{-10}M or 5×10^{-9} M caused a significant (P values not reported) decrease in lymphocyte cell number compared to controls. Cultures treated with TCAOB and TCDBF had a significant decrease in lymphocytes compared to cultures treated with TCAOB or TCDBF alone ($2p < 0.001$). TCAOB and TCDBF administered together demonstrated an additive effect of 50% or 75%. Based on these results, the author suggests that TCAOB and TCDBF have common mechanisms of action [Hassoun, 1987].

in ovo, chick (TCAOB)

- The effects of TCAOB on lymphoid development in the bursa of fabricius of the chicken embryo were studied. Fertilized White Leghorn Shaver eggs were incubated for 13 days and injected with 50 µl of 1-30 µg TCAOB in peanut oil/kg egg. Controls received peanut oil only. Eggs were further incubated until day 19 of embryo development, and the bursae were removed and the number of viable lymphocytes and lymphocytes per bursae were determined. Histological and gross pathological examination of the bursae were conducted and aryl hydrocarbon hydroxylase (AHH) activity was determined.

  The number of lymphoid cells was dose-dependently decreased by TCAOB with an ED_{50} of 1.4 µg/kg egg. At 10 µg/kg TCAOB, an almost complete inhibition of lymphoid development was seen. Upon histological examination, the bursae were found to have a dose-related decrease in the number of follicles and cells per follicle compared to controls. Embryo weights were unaffected by TCAOB. However, bursal weights were significantly ($P < 0.001$) decreased to 75% and 65% of controls at 5 µg/kg and 30 µg/kg TCAOB, respectively. Hepatic lesions and death were observed in a few treated embryos. AHH enzyme activity was induced 10- and 50-fold by TCAOB at 1 µg/kg and 30 µg/kg, respectively, compared to controls. Based on the extent of enzyme induction an ED_{50} for TCAOB was estimated to be 4 µg/kg. The authors suggest that TCAOB AHH induction and lymphoid development are most likely due to a direct effect on the bursae and that lymphoid development is inhibited by TCAOB [Nikolaidis *et al.*, 1988].

in vitro, in ovo, chick (TCAOB)

- *In vitro*: Thymuses from 11-day-old White Leghorn chicken embryos were incubated with TCAOB (10^{-8} M), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (10^{-10}M), or 3,3',4,4'-tetrachlorobiphenyl (TCB) (10^{-8}M) for 5 days.

  In ovo: White Leghorn Shaver chicken embryos were injected with 40-200 µg TCB or 2-10 µg TCAOB mixed in peanut oil per kilogram egg

and incubated for 16 days. Controls received peanut oil only. Right thymuses were removed and the number of viable lymphocytes and lymphocytes per thymus were determined.

In vitro, the number of lymphocytes was decreased 60% by TCDD, TCAOB, and TCB treatment ($P < 0.05$) compared to controls. *In ovo*, the number of lymphocytes was significantly ($P < 0.002$) decreased. The ED_{50} values were estimated to be 3.6 and 60 µg/kg egg for TCAOB and TCB, respectively. An 86% reduction in lymphocytes occurred in thymuses obtained from the 10 µg/kg TCAOB exposed embryos.

Based on these results, the authors estimate that TCAOB is 100-fold less toxic than TCDD, and similar in toxicity to TCB in chicks [Nikolaidis *et al.*, 1988].

2. Neurotoxicity

No data were found on the neurotoxicity of TCAB or TCAOB in humans or animals.

3. Biochemical Toxicology

occupational, human (TCAOB)

- Eighty nine-workers (30 with chloracne) in a 3,4-dichloroaniline/Diuron manufacturing plant were tested for levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin, alkaline phosphatase, total protein, albumin, and gamma glutamyl transferase (GGT), as well as thymol turbidity. The exposed group was compared with a control group of men from an engineering plant that were not exposed to TCAOB. In addition to the above parameters, cholesterol, triglyceride, and high density lipoprotein (HDL) levels were measured. There were no significant differences in the levels of bilirubin, alkaline phosphatase, total protein, albumin, and thymol turbidity between the exposed and control groups. Significant results observed included an increase ($P < 0.01$) in triglyceride levels in the chloracne group and non-chloracne group ($P < 0.05$). The chloracne group also had higher levels of cholesterol ($P < 0.02$) and SGPT ($P < 0.01$) than controls. GGT levels were increased in the exposed group, although insignificantly. In two, 9-month interval follow-up studies, GGT levels in the exposed group had decreased; however, cholesterol and triglyceride levels remained elevated [Scarisbrick and Martin, 1981].

oral, mouse (TCAOB)

- Hepatic enzyme activity was assessed in rhino and hairless mice fed TCAOB as part of a subchronic study described in section V.C.3. In hairless mice, a dose-dependent significant ($P < 0.05$) induction of cytochrome P-450 and aniline hydroxylase was seen in the 0.01% TCAOB group. In addition, a significant induction ($P < 0.05$) of cytochrome C reductase and aniline hydroxylase in the 0.1% TCAOB group compared to the control group was observed. In the rhino mice, a significant ($P < 0.05$) induction of cytochrome C reductase in the 0.01% TCAOB group was observed. In addition, cytochrome C reductase, cytochrome P-450, and aniline hydroxylase induction was observed in the 0.1% TCAOB group [Horton and Yeary, 1985].

intraperitoneal, rat (TCAB)

- Two groups of male Wistar rats (n=8) were injected intraperitoneally with 25 mg/ml TCAB in corn oil or with corn oil only 2 times per week. Four animals per group were sacrificed on days 7 and 28, and liver enzyme activity was measured.

  On day 7, a significant increase in fructose-1,6-biphosphatase (F1,6BP) ($P < 0.001$) and pyruvate kinase (PK) ($P < 0.05$), and a significant decrease ($P< 0.05$) in phosphoenolpyruvate carboxykinase (PEPCK) compared to controls was observed. On day 28, a significant decrease in glucose-6-phosphatase (G6P) ($P< 0.01$) and PEPCK ($P < 0.05$), and an increase in F1, 6BP ($P < 0.001$) and PK ($P < 0.01$) compared to controls was observed. Non-gluconeogenic enzymes including cytochrome P-450 and malic enzyme (ME) had significantly ($P < 0.05$) increased by day 7 compared to controls. In addition, glutamic pyruvic transaminase (GPT) ($P < 0.01$) and glutamic oxaloacetic transaminase levels were decreased insignificantly compared to controls. On day 28, an increase in cytochrome P-450 ($P < 0.01$), and ME ($P < 0.01$), and a decrease in GPT ($P < 0.01$) was observed compared to controls [Hsia and Kreamer, 1985].

intraperitoneal, rat (TCAB/ TCAOB)

- To examine the effects of propanil and 3,4-dichloroaniline (DCA) on enzyme induction, liver microsomes were prepared from male Sprague Dawley rats injected intraperitoneally once daily for 3 days with corn oil (5 ml/kg), phenobarbital (80 mg/kg), propanil (100 mg/kg), or DCA (100 mg/kg). Other rats received single intraperitoneal injections of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (6.5 μg/kg), TCAB (100 mg/kg), or TCAOB (100 mg/kg). TCAB, TCAOB, and TCDD caused a significant reduction in acylimidase activity ($P < 0.05$). Hydroxylation of propanil at the 2'-position was induced 2.4-, 2.7- and 3.6-fold relative to controls by TCDD, TCAOB, and TCAB pre-treatments, respectively. Microsomal N-hydroxylation of DCA was induced 3-fold by TCDD, TCAB, and TCAOB. TCAB and TCAOB caused a significant induction ($P < 0.05$) of microsomal drug enzyme activities similar to TCDD including cytochrome P-450, cytochrome b_5, 7-ethoxyresorufin-O-deethylase, 7-pentoxyresorufin-O-dealkylase, and benzoxyresorufin-O-dealkylase compared to controls. A significant ($P < 0.05$) decrease in NADPH-cytochrome P-450 reductase was seen in the TCAOB group only [McMillan *et al.*, 1990].

intraperitoneal, rat (TCAB/ TCAOB)

- In the first part of the study, the effect of TCAB and TCAOB on zoxazolamine hydroxylase was determined. Male Sprague Dawley rats were injected intraperitoneally with 0.05-10 mg/kg TCAB and TCAOB in dimethyl sulfoxide (DMSO). An induction of zoxazolamine hydroxylase activity ($P < 0.01$) compared to controls receiving DMSO only was observed. In addition, TCAB and TCAOB caused a 2-fold increase (insignificant) in microsomal P-450 levels compared to controls.

  In the second part of the study, male Sprague Dawley rats were divided into 4 groups. Within each group, 3 rats received intraperitoneal injections of 10 mg/kg TCAB, TCAOB dissolved in DMSO, or 2.5 ml of DMSO/kg as a control. Injections were repeated at 5-day intervals; groups 1, 2, 3 and 4 received a total of 1, 2, 3, and 4 injections, respectively. Both TCAB and TCAOB caused a slight increase in liver weight. However, neither compound had a significant effect on arginase

activity [Saint-Ruf *et al.*, 1979].

oral, rat (TCAB)

- Microsomes were prepared from immature male Wistar rats injected with a single dose of 300 µmol/kg TCAB in corn oil. A significant ($P < 0.05$) increase in 7 alpha-hydroxylase activity, and a significant ($P < 0.01$) decrease in the activity of 3 testosterone hydroxylases were noted. In addition, a significant increase ($P < 0.01$) in aryl hydrocarbon hydroxylase and 7-ethoxyresofurin-O-deethylase activities, and a significant decrease in body weight ($P < 0.01$) was seen [Keys *et al.*, 1985].

intraperitoneal, rat (TCAOB)

- In Sprague Dawley rats, TCAOB caused a significant ($P < 0.025$) elevation of total lipid and glutamic oxaloacetic transaminase levels compared to control rats. Total serum lipids were 30% higher in treated rats compared to controls. In addition, TCAB and TCAOB were found to significantly ($P < 0.0005$) increase cytochrome P-448, aryl hydrocarbon hydroxylase ($P < 0.0005$), and the liver weight/body weights ($P < 0.005$) in test rats compared to controls [Hsia *et al.*, 1980].

intraperitoneal, mouse (TCAB/ TCAOB)

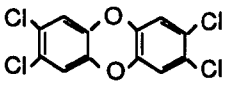
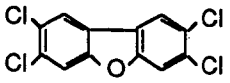
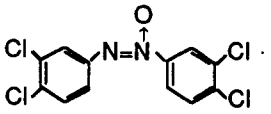
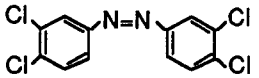
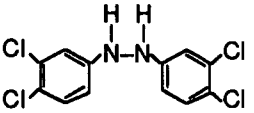
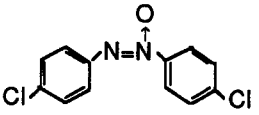
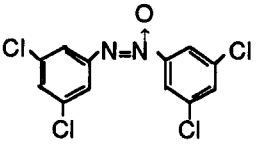
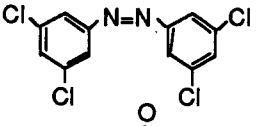
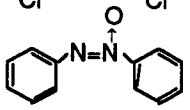
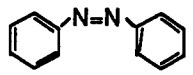
- Microsomes were prepared from male Sprague Dawley mice (n=15) injected intraperitoneally with TCAB and TCAOB in corn oil at 1, 10, 25, or 50 mg/kg/day for 5 days. Controls received 5 ml/kg/day corn oil only. Maximal induction of cytochrome P-450 occurred in the 25 mg/kg TCAB and TCAOB dosed groups with parallel increases in liver to body weight ratios. TCAB and TCAOB induced cytochrome P-450 to 2.7 times the control level. A single intraperitoneal injection of 10 mg/kg TCAOB was found to cause a 2-fold induction of cytochrome P-448 levels [Hsia and Kreamer, 1979a].

VI. STRUCTURE ACTIVITY CONSIDERATIONS

TCAB and TCAOB are isosteric to the highly toxic compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is known to cause chloracne and hyperkeratosis, involution in lymphoid organs, and has been shown to be teratogenic, embryotoxic, and hepatotoxic [Sundström, 1982]. Hassoun *et al.*, demonstrated that the teratogenic profile of TCAOB is similar to TCDD [Hassoun *et al.*, 1984]. TCDD and its congeners (including TCAOB) were demonstrated to have a common mechanism of action for cleft palate formation in rat embryos [Hassoun and Arif, 1987]. It is suggested that TCDD is more toxic and teratogenic than TCAOB, mainly because TCAOB is more rapidly metabolized and/or excreted than TCDD [Dencker *et al.*, 1985; Hsia and Burant, 1984]. Induction of aryl hydrocarbon hydroxylase (AHH) activity in chick embryos was used to determine the relative potencies of TCAB and TCAOB. Table 9 compares the AHH induction potencies and binding affinities of various azoxy-, azo-, and hydrazo- benzenes including TCAB and TCAOB. Two common properties of TCDD isomers which induce AHH activity include the presence of halogen atoms at the three and four positions of the lateral ring, at least one unhalogenated ring position, and planarity [Poland *et al.*, 1979]. TCAB and TCAOB in the *trans* configuration were found to assume a planar configuration [Hsia and Kreamer, 1981]. The toxicity of TCDD and its isomers is mediated by the stereospecific binding affinities for cystolic binding species. TCAB and TCAOB have been shown to bind with high affinity to TCDD

cystolic species and therefore have a similar toxic potential [Poland *et al.*, 1979]. The chloracnegenic properties of chlorinated azo-, and azoxy- compounds are strongly dependent on the number of chlorine atoms and their substitution patterns. Table 9 presents the binding affinities and thus the chloracnegenic properties, of azo- azoxy-, and hydrazobenzenes in relation to the number of chlorine atoms and their substitution patterns [Sundström, 1982].

Table 9. Induction of AHH Activity by Various azoxy, and azo-Compounds Compared to TCDD and TCDF

	Compound	Induction of AHH Activity in Chicken Embryos ED_{50} nmoles/kg	Binding Affinity to Mouse Liver Cytosol k_d nM
1		0.31	0.27
2		0.46	0.73
3		0.45	0.93
4		2.0	1.1
5		2.9	1.2
6		6000	23
7		inactive	inactive
8		inactive	inactive
9		inactive	inactive
10		inactive	inactive

1. 2.3.7.8-tetrachlorodibenzo-*p*-dioxin (TCDD, 1)
2. 2,3,7,8-tetrachlorodibenzofuran (TDCF, 2)
3. 3,3',4,4'-tetrachloroazoxybenzene (TCAOB, 3)
4. 3,3',4,4'-tetrachloroazobenzene (TCAB, 4)
5. 3,3',4,4'-tetrachlorohydrazobenzene (TCHB, 5)
6. Name not specified
7. Name not specified
8. Name not specified
9. Name not specified
10. Name not specified

a. Low values correspond to high activities or binding affinity which is correlated to chloroacne induction.

Reference: Sundström, 1982

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APPENDIX I. ON-LINE DATABASES SEARCHED

	DATE OF SEARCH	TIME PERIOD
BRS:		
HZDB	June, 1990	
DIALOG:		
Agricola	June, 1990	1970-1990
Agris International	June, 1990	1974-1990
Aquatic Sciences Abstracts	June, 1990	1974-1990
Biosis Previews	June, 1990	1969-1990
CAB Abstracts	June, 1990	1972-1990
Cancerlit	June, 1990	1963-1990
Chem Bus Newsbase	November, 1990	1984-1990
Chemical Exposure	June, 1990	1974-1987
Compendex Plus	June, 1990	1970-1990
CRIS USDA	November, 1990	
Embase	June, 1990	1974-1990
Enviroline	June, 1990	1970-1990
Environmental Bibliography	June, 1990	1974-1990
Federal Register	November, 1990	1977-1990
Foods Adlibra	June, 1990	1974-1990
FSTA	June, 1990	1969-1990
Life Sciences Collection	June, 1990	1978-1990
Medline	June, 1990	1966-1990
NTIS	June, 1990	1964-1990
Occupational Safety and Health	June, 1990	1973-1990
PTS Newsletter	June, 1990	1987-1990
PTS Prompt	November, 1990	1972-1990
Pollution Abstracts	June, 1990	1970-1990
Trade and Industry ASAP	June, 1990	1983-1990
MEAD:		
Nexis/Lexis-BNA ENV	May, 1990	
NLM:		
Chemline	May, 1990	
HSDB	June, 1990	
RTECS	May, 1990	
Toxline 65	June, 1990	1965-1980
Toxline	June, 1990	1981-1990
Toxlit	June, 1990	1981-1990
Toxlit 65	June, 1990	1965-1980
STN:		
CA	June, 1990	1967-1990
Chemlist	June, 1990	
Chem. Industry Note	November, 1990	

APPENDIX II. SAFETY INFORMATION

- **HANDLING AND STORAGE**

  TCAB and TCAOB are stable under normal laboratory conditions.

- **EMERGENCY FIRST AID PROCEDURES**

Eye: First check the victim for contact lenses and remove if present. Flush victim's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poison control center. Do not put any ointments, oils, or medication in the victim's eyes without specific instructions from a physician. Immediately transport the victim to a hospital even if no symptoms (such as redness or irritation) develop.

Skin: IMMEDIATELY flood affected skin with water while removing and isolating all contaminated clothing. Gently was affected skin areas thoroughly with soap and water. If symptoms such as inflammation or irritation develop, IMMEDIATELY call a physician or go to a hospital for treatment.

Inhalation: IMMEDIATELY leave the contaminated area and take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat, or chest) develop, call a physician and be prepared to transport the victim to a hospital.

Provide proper respiratory protection to rescuers entering an unknown atmosphere. Whenever possible, Self-Contained Breathing Apparatus (SCBA) should be used.

Ingestion: If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. Be prepared to transport the victim to a hospital if advised by a physician.

If the victim is convulsing or unconscious, do not give anything by mouth, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

- **PROTECTIVE EQUIPMENT**

  Eye: Safety glasses

  Gloves: Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical, otherwise one pair. When contact with this chemical has been known to occur, change gloves immediately.

  Clothing: Minimally, a disposable laboratory suit (e.g. Tyvek ®) shall be worn, as specified in the most current NTP Statement of Work or the NTP Health and Safety Minimum Requirements.

  Respiratory Protection: A NIOSH-approved chemical cartridge respirator with an organic vapor and high-efficiency particulate filter cartridge.

- **EXTINGUISHANT**

  Dry chemical, carbon dioxide or halon extinguisher

- **MONITORING PROCEDURES**

  There is no NIOSH analytical method reported in the NIOSH Manual of Analytical Methods for TCAB or TCAOB.

- **SPILLS AND LEAKAGE**

  Persons not wearing the appropriate protective equipment and clothing shall be restricted from areas of spills until cleanup has been completed. When exposure to unknown concentrations may occur, air-purifying respirators may not be used. Chemical cartridge respirators with organic vapor cartridges may not be used when airborne concentrations exceed 1000 ppm.

  If TCAB or TCAOB are spilled the following steps shall be taken:

  1. In order to prevent dust formation, use moistened paper towels to clean up a solid spill. Avoid dry sweeping.

  2. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.

  3. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.

  4. Dispose of all absorbed material as hazardous waste.

- **DECONTAMINATION OF LABORATORY EQUIPMENT**

  TDMS Terminal: Whenever feasible, a protective covering (e.g.,plastic wrap) shall be placed over the keyboard when in use.

General Equipment: Before removing general laboratory equipment (i.e., lab carts, portable hoods and balances) from animal dosing rooms and/or chemical preparation areas, a decontamination process shall be conducted in addition to routine housekeeping procedures.

- **WASTE MANAGEMENT AND DISPOSAL PROCEDURES**

Waste Management: If an inhalation study is to be conducted, all exhaust air from the inhalation chamber must be cleaned with appropriate air cleaning devices unless the laboratory has informed local and state air pollution regulatory agencies of both the laboratory's operating practices and the potential hazards of the chemical's in use. Compliance with all federal, state and local air pollution laws and regulations is required. A specific air cleaning system design must consider the specific conditions of the laboratory (eg., air flow rates and volumes, mixing of exhaust streams, size of inhalation chamber, etc.) and the dosing regimen selected. Air cleaning systems designs must be described by the laboratory and approved by the NTP Office of Laboratory Health and Safety.

Waste Disposal: Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, disposable cages, labware) shall be disposed of by incineration in a manner consistent with federal (EPA), state, and local regulations or disposed of in a licensed hazardous waste landfill.