

EXECUTIVE SUMMARY OF SAFETY AND TOXICITY INFORMATION

BIS (TRI-N-BUTYLTIN) OXIDE

CAS Number 56-35-9

September 30, 1991

Submitted to:

NATIONAL TOXICOLOGY PROGRAM

Submitted by:

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Chemical Evaluation Committee Draft Report

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OVERVIEW¹

Nomination History: Bis(tri-n-butyltin) oxide (TBTO) was nominated by NCI in 1988 for carcinogenicity testing with a high priority. The request was based on its high and increasing production and its widespread use, the potential for human exposure, the lack of adequate chronic and carcinogenic testing, potential for genotoxicity, and its association with adrenal and pituitary tumors in rats. In addition, small amounts have been shown to be highly toxic to aquatic organisms.

Chemical and Physical Properties: TBTO is a clear, colorless to slightly yellow liquid with a strong odor. The compound has a melting point of -45°C (-49°F) and a boiling point of 180°C (356°F) at 2 mm Hg. TBTO is insoluble in water, soluble in most organic solvents (alcohol, chloroform, and benzene), and incompatible with oxidizers and acids.

Production/Uses/Exposure: The total production volume of TBTO was reported in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory in 1983 by 4 manufacturers to range from 11,000-111,000 pounds. In addition, between the years 1965 and 1976, the annual production of TBTO was estimated to range from 860,000-4,600,000 pounds. No production data on TBTO were available from the United States International Trade Commission's Publication Synthetic Organic Chemicals or from SRI's Chemical Economics Handbook. TBTO is widely used as a biocide (in antifouling paints), a fungicide (for the preservation of wood, cotton, textiles, and leather), a mildewcide (in polyvinylchloride systems), a bactericide (in urethane foams and secondary oil recovery injection water), and a slimicide (in the paper industry). Due to the extensive use of TBTO in industry, the potential for worker exposure exists. Data from the National Occupational Exposure Survey (NOES) conducted during 1981-1983, estimated that 36,413 employees, including 7,903 female employees, were potentially exposed to TBTO. OSHA has not established a permissible exposure limit (PEL) for TBTO. However, the current OSHA PEL for organic tin compounds (as Sn) is 0.1 mg/m³ (skin) averaged over an 8-hour work shift. The (skin) notation indicates that transdermal exposure may contribute significantly to the overall exposure burden. The ACGIH has not recommended a threshold limit value (TLV), and NIOSH has not established a recommended exposure limit (REL) for TBTO. However, the ACGIH TLV and the NIOSH REL for organic tin compounds (as Sn) is 0.1 mg/m³ (skin) averaged an 8-hour work shift and a 10-hour work shift, respectively. TBTO is regulated by the FDA and the EPA. On October 4, 1988, due to the inadequacy of the data submitted by the registrants, the EPA announced that it would cancel all TBT antifouling paint registrations that did not comply with the release rate established by the Organotin Antifouling Paint Control Act (1988), and that are used on non-aluminum vessels under 82 feet. The announcement also designated all TBT-formulated antifouling paints as restricted-use pesticides as of March 1, 1990. This restriction excludes products packaged in aerosol containers 16 ounces or less and labeled for use on only outboard motors, propellers and other non-hull underwater aluminum components. In addition, all registrants were ordered to develop training programs for the certification of applicators. After March 1, 1990, only certified applicators will be able to purchase TBT antifoulants, and only certified applicators and those under their direct supervision will be able to apply them.

Toxicological Effects:

Human: TBTO has been shown to be a severe skin irritant causing erythema, vesiculation, and dermatitis in workers handling the compound, or formulations containing the compound. In addition, female workers exposed to spray paint preparations containing TBTO suffered nasal and respiratory irritation. Patch tests conducted with TBTO indicate that the compound does not appear to be an allergen. There were no data found on the chemical disposition, or on the prechronic, chronic, carcinogenic, teratogenic, or reproductive effects of TBTO in humans.

Animal: Following oral administration of TBTO to rats and mice, tissue accumulation of tin was shown to be directly proportional to the administered dose. The highest levels of tin were found in the kidneys and liver. In a study that exposed female mice to ¹⁴C-labelled TBTO in drinking water, fat tissue was also seen to accumulate high levels of TBTO. This study showed that the levels of TBTO seen in the excreta were greater than those found in the tissues, suggesting TBTO is rapidly excreted unchanged. In guinea pigs and mice exposed for one hour to an aerosol of TBTO, tissue tin concentrations were highest in the heart, liver, lung, and kidney. Finally, when TBTO was administered to rats orally or subcutaneously, tin levels in the liver and kidney were found to be significantly higher after oral treatment.

TBTO, administered orally, was found to be moderately to highly toxic to laboratory animals. The acute oral LD₅₀ ranged from 87-234 mg/kg for rats and 55-203 mg/kg for mice. When administered intraperitoneally, TBTO was more lethal; the LD₅₀ values for rats and mice ranged from 7.21-20 mg/kg and 12.5-16 mg/kg, respectively. In rats, signs of systemic toxicity observed following acute oral exposure included ataxia, labored respiration, diarrhea, closed eyes, bristled coat, and decreased body weight and food consumption. After dermal exposure of rats to TBTO, or of rabbits to formulations containing TBTO (Lastanox T and P), the skin of the test animals showed signs of severe irritation, including erythema, edema, and atonia. Gross signs of systemic toxicity observed in the rabbits included body weight loss, depression, labored respiration, and diarrhea. Autopsies of these animals revealed edema and congestion of the lungs, kidneys, and adrenals. Application of these Lastanox formulations to the rabbit eye were also shown to be severely irritating to the eyes of rabbits, causing erythema, edema, necroses of the eyelids, and corneal transparency.

In two 4-week prechronic toxicity study with rats, dietary TBTO, at a concentration of 320 mg/kg, caused significant body and organ weight loss, a reduction in feed and water consumption, and weakness and emaciation. These animals also exhibited signs of general toxicity (roughened fur, hairless and pale skin, discharge around eyes and nose). In one study, necropsy of the rats revealed atrophy of the thymus, spleen, and mesenteric lymph nodes, and multifocal necrosis of the liver. Exposure to this concentration of TBTO for six weeks resulted in similar hepatic lesions, defects in iron uptake (or an iron loss), and possible hemolytic effects in the rats. Similar results were seen in rats exposed to an aerosol of TBTO (2.8 mg/m³) for 4 hours/day for 4-5 weeks. These animals also exhibited inflammation of the respiratory tract. TBTO fed to mature and young mice for 7 days caused retarded body and organ weight (spleen), and decreased leukocyte counts. In subchronic dermal studies, TBTO was shown to be irritating to the skin of guinea pigs and rabbits, but did not act as a skin sensitizer.

In a 106-week toxicity and carcinogenicity study with Wistar rats, dietary TBTO (50 mg/kg) caused general toxic effects (excess mortality, decreased body weight and increased organ weight, hematological changes) and an increase in non-neoplastic changes in the liver, spleen, kidney, and thyroid. In addition, there was an increased incidence of neoplastic lesions in the pituitary, adrenal, and parathyroid. The incidence of benign tumors of the pituitary was also elevated in rats exposed to 0.5 mg/kg TBTO, but not in rats fed 5.0 mg/kg TBTO. The authors of the study concluded that the significance of this increase was questionable due to the high incidence of spontaneous tumors in the strain of rats studied, the variable incidence in the treated groups, and the lack of a dose-effect relationship.

TBTO was shown to be embryotoxic in the rat and mouse after oral dosing of the mother. Some of the compound-related effects included reductions in litter size, decreases in pup viability and average pup weight, and increases in the number of resorptions. The primary malformation noted in rat and mouse fetuses was cleft palate, but this occurred only at dosages overtly toxic to the mothers and was not considered to be indicative of a teratogenic effect of TBTO. In a study examining the toxicity and teratogenicity of TBTO in rabbits, the compound was determined not to be teratogenic, and the no observable effect level (NOEL) for maternal and fetal toxicity was determined to be 1 mg/kg/day. *In vitro* studies examining the toxic effects of TBTO on mouse limb bud development found that the compound interfered with the differentiation of the paw skeleton, scapula, ulna, and radius, and caused malformations of the skeletal elements of the limb (fusion of carpals and metacarpals).

Several studies have reported toxic effects of dietary TBTO on the immune system of rats. Some of the general effects included: decreased weight of the lymphoid organs (thymus, spleen, lymph nodes); lymphocyte depletion of the spleen and lymph nodes; and changes in serum antibody titers (increased IgM, decreased IgG). Specific immune function tests have shown that exposure to TBTO affects the thymus-dependent immunity and non-specific resistance of the rat. The effects on thymus dependent immunity included: suppression of thymus-

dependent antibody synthesis (to SRBC, ovalbumin, and tetanus toxoid); suppression of delayed-type hypersensitivity reactions; reduced resistance to *Trichinella spiralis* infection; changes in the mitogenic responses of spleen cells; increased B-cell counts and decreased T-cell counts; and decreased cell viability in the spleen and thymus. Effects indicating an impairment in the rats' non-specific resistance included decreased clearance of *Listeria monocytogenes*, and suppression of the natural killer activity in spleen cells. Two studies compared the effects of TBTO on the immune system of adult and young rats; the results from both studies indicated that the developing rat is more sensitive to the immunotoxic effects of TBTO than the adult rat.

TBTO has also been shown to effect the nervous system (decreased brain weights, decreased motor activity, and nerve fiber damage), and the endocrine system (increased adrenal and pituitary weights, decreased thyroid weight, and decreased serum hormone levels) in rats. In addition, TBTO has been found to have biochemical effects on the rat, including changes in enzyme activity (cytochrome P-450 and heme oxygenase).

Genetic Toxicology: TBTO was nonmutagenic in *Salmonella* strains TA97, TA98, TA100, TA1530, TA1535, and TA1538, and in *Bacillus subtilis* and *Klebsiella pneumoniae* with and without metabolic activation. TBTO was also nonmutagenic to several eukaryotic organisms (*Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, Chinese hamster cells, mouse lymphoma cells, human lymphocytes, *Drosophila melanogaster*). TBTO caused a significant increase in the number of chromosomal aberrations, endoreduplications, and polyploid cells in Chinese hamster ovary cells at the highest test concentration (8.4 mM) and in the presence of metabolic activation. The compound did not, however, induce sister chromatid exchange in these cells.

Structure Activity Relationships: TBTO is structurally similar to triphenyltin acetate, tributyltin fluoride and triphenyltin hydroxide. Of the three, only triphenyltin hydroxide has been shown to induce a significant increase in tumor formation. In an 8-week study using Strain A mice, male and female mice were administered intraperitoneal injections of 0.5-2.5 mg/kg of the compound 3 times/week for 8 weeks. Forty percent of the females treated with 2.5 mg/kg developed tumors. This was significantly higher ($P \leq 0.05$) than the vehicle control group, in which only 11% of the females developed tumors after 8 weeks of treatment with tricaprylin. However, it should be noted that triphenyltin hydroxide was negative in NCI/NTP feeding carcinogenicity studies in F344 rats and B6C3F1 mice.

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.

I. NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Source: National Cancer Institute [NCI, 1988a,b]
2. Date: September, 1988
3. Recommendations: Carcinogenicity
4. Priority: High
5. Rationale/Remarks:
 - Used as an antifouling agent in marine paints and as a fungicide in the manufacture of textiles.
 - Lack of adequate chronic and carcinogenic testing; potential for genotoxicity.
 - Associated with adrenal and pituitary tumors in Wistar rats.
 - Increased potential for human exposure.
 - Increased production.
 - Ubiquitous in marine waters, especially in enclosed estuaries and harbors.

- Small amounts have been shown to kill fish and shellfish and cause deformities in the reproductive organs of snails and in the regenerated claws of fiddler crabs.

B. Chemical Evaluation Committee Review

1. Date of Review:
2. Recommendation:
3. Priority:
4. NTP Chemical Selection Principle(s):
5. Rationale/Remarks:

C. Board of Scientific Counselors Review

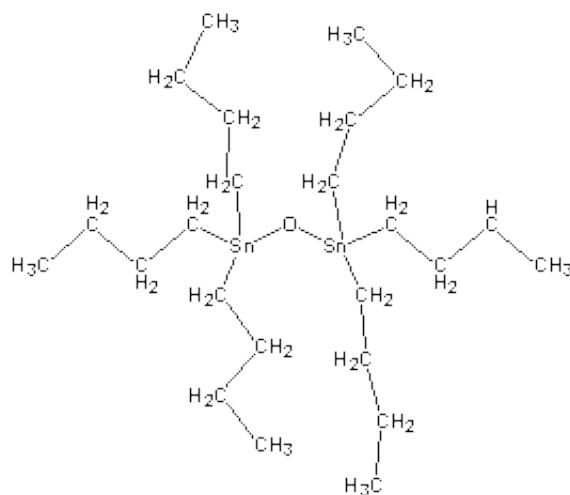
1. Date of Review:
2. Recommendations:
3. Priority:
4. Rationale/Remarks:

D. Executive Committee Review

1. Date of Review:
2. Decision:

II. CHEMICAL AND PHYSICAL DATA

A. Chemical Identifiers



BIS(TRI-N-BUTYL TIN) OXIDE

Cas No. 56-35-9

RTECS No. JN8750000

Molecular formula: $C_{24}H_{54}OSn_2$

Molecular weight: 596.08

B. Synonyms and Trade Names

Synonyms: distannoxane, hexabutyl- (8Cl)(9Cl); 6-oxa-5,7-distannaundecane, 5,5,7,7-tetrabutyl-; bis(tributyloxide) of tin; bis(tributylstannium) oxide; bis(tributylstannyl) oxide; bis(tributyltin) oxide; hexabutyl-distannoxane; hexabutyl-ditin; oxybis(tributyltin); BTO; TBTO; TBOT

Trade Names: BioMeT SRM; BioMeT TBTO; BioMeT 66; Butinox; C-Sn-9; ENT 24, 979; Lastanox F; Lastanox Q; Lastanox Q1; Lastanox T; Lastanox T 20; L.S. 3394; MT 1E; Mykolastanox F; NSC 22332; NSC 28132; Stannicide A; Stannicide O; Vikol AF-25; Vikol LO-25

C. Chemical and Physical Properties

Description: A clear, colorless to slightly yellow liquid with a strong odor [Pfaltz & Bauer, 1985].

Melting Point: $-45^{\circ}C$ ($-49^{\circ}F$) [Pfaltz & Bauer, 1985].
 $<-45^{\circ}C$ ($<-49^{\circ}F$) [Kirk-Othmer, 1981b].

Boiling Point: $180^{\circ}C$ ($356^{\circ}F$) at 2 mm Hg [Aldrich, 1990; Dean, 1985; Pfaltz & Bauer, 1985; M&T Chemicals, 1979];
 $254^{\circ}C$ ($489^{\circ}F$) at 50 mm Hg [Mackison *et al.*, 1981; Clayton and Clayton, 1981];
 $179-180^{\circ}C$ ($355-356^{\circ}F$) at 2 mm Hg [Lancaster Synthesis, 1989-1990];
 $215^{\circ}C$ ($419^{\circ}F$) at 9.75 mm Hg [Kirk-Othmer, 1981b];
 $210-214^{\circ}C$ ($410-417.2^{\circ}F$) at 10 mm Hg [Yumoto, 1975].

Specific Gravity: 1.170 @ $25^{\circ}C$ [Aldrich, 1990; Mackison *et al.*, 1981; Dean, 1985; M&T Chemicals, 1979];
 1.17 ± 0.03 @ $20/4^{\circ}C$ [Pfaltz & Bauer, 1984];
 1.180 ± 0.010 @ $20/20^{\circ}C$ [Yumoto, 1975];
1.173 [Fluka, 1990].

Refractive Index: 1.4864 [Dean, 1985];
1.4860 [Aldrich, 1990];
 1.480 ± 0.010 [Yumoto, 1975];
 1.485 ± 0.01 [Pfaltz & Bauer, 1984];
1.489 [Fluka, 1990];
1.488 [Kirk-Othmer, 1981b].

Solubility in Water: Insoluble [Kirk-Othmer, 1981b; Yumoto, 1975; M&T Chemicals, 1979];
 < 20 ppm [PCOC, 1966; Pfaltz & Bauer, 1984];
10 ppm [Evans, 1990];
0.1% in hot water [Clayton and Clayton, 1981];
16, 19, and 21 ppm at $25^{\circ}C$, $35^{\circ}C$, and $45^{\circ}C$, respectively after 10-12 days [Sherman *et al.*, 1985].
The following are experimental values for the aqueous solubility of TBTO at varying pH values [Maguire *et al.*, 1983]:

solubility (mg/L) pH

60 ± 3	2.0
37 ± 1	2.4
30 ± 2	2.6
22 ± 2	2.8
12 ± 2	3.0
10 ± 1	4.0
7 ± 1	5.0
5 ± 2	5.6

0.75 ± 0.5	6.0
0.75 ± 0.5	6.6
4 ± 1	7.0
1 ± 0.5	7.6
1.5 ± 0.5	7.8
31 ± 1	8.1
29 ± 2	9.2
18 ± 6	10.0
14 ± 1	10.8

Solubility in other Solvents: Soluble in most organic solvents [PCOC, 1966; Pfaltz & Bauer, 1984] including alcohol, chloroform, and benzene [Yumoto, 1975].

Log Octanol/Water Partition Coefficient: 200 (estimated in deionized water); 5500 (experimental in 25% salinity); 6300 (experimental in 45% salinity); 7000 (experimental in deionized water) [Laughlin *et al.*, 1986]; 2,185 [M & T Chemicals, Inc., 1978].

Reactive Chemical Hazards: Incompatible with oxidizers [Lenga, 1988; Pfaltz & Bauer, 1985] and acids [Pfaltz & Bauer, 1985]. Irritating fumes or vapors may develop if compound is exposed to elevated temperatures or open flame [Pfaltz & Bauer, 1985]. Decomposition products include toxic fumes of carbon monoxide, carbon dioxide [Lenga, 1988], and tin/tin oxides [Lenga, 1988; Pfaltz & Bauer, 1985].

Flammability Hazards:

- Combustible
- Flash-point: >110°C (230°F) CC [Aldrich, 1990; Mackison *et al.*, 1981]; >112°C (233.6°F) CC [Dean, 1985]; >100°C (212°F) CC [Pfaltz & Bauer, 1984; M&T Chemicals, 1979].
- Vapor Pressure: 1.1 x 10⁻⁵ at 25°C [Pfaltz & Bauer, 1985]; much less than 1 mm Hg at 20°C [Mackison *et al.*, 1981]; 6.4 ± 1.2 x 10⁻⁷ mm Hg at 20 °C (estimated) [Maguire *et al.*, 1983].

III. PRODUCTION/USE

A. Production

1. Manufacturing Process

Commercial manufacture of organotins, a class that includes TBTO, involves the alkylation of tin tetrachloride or the direct reaction of tin with alkyl halides. The alkylation of tin tetrachloride can be accomplished using Grignard synthesis, the Wurtz method, or the aluminum alkyl method. With the Grignard method (the most versatile method), stannic chloride is reacted with n-butyl chloride in the presence of magnesium chips to produce a >95% yield of tetra-n-butyltin; this is the alkylating reaction. Then, tetrabutyltin undergoes redistribution with stannic chloride to produce a mixture of butyltin chlorides. Tri-n-butyltin is removed from the mixture and is alkali hydrolyzed to form the oxide. The Wurtz method is similar to the Grignard method, but uses sodium rather than magnesium in the alkylating reaction. In the aluminum alkyl method, stannic chloride reacts with tri-n-butylaluminum in the presence of sodium chloride and ether or amines to form stable complexes with aluminum chloride. This method permits full alkyl substitution of stannic chloride. The three alkylation routes usually involve excess alkylating agent to achieve complete conversion to the tetraalkyl compound [Kirk-Othmer, 1981b; Kirk-Othmer, 1979].

TBTO can be produced by a direct method in which tin is reacted with n-butyl iodide or n-butyl chloride to produce 90% yields of tri-n-butyltin halide. This organotin halide is hydrolyzed in base form to form the oxide [NCI, 1988b; Kirk-Othmer, 1981b].

TBTO has been produced by combining tetrabutyl tin and tin tetrabromide, and heating the mixture at 220°C. The reaction product was then cooled to room temperature, and mixed with alcoholic potassium hydroxide. Potassium bromide and undesirable by-products were filtered off at room temperature, while the alcohol and water were distilled off and evaporated. A final 59% yield of TBTO was obtained which showed no impurities [Brown *et al.*,

1977].

2. Producers and Importers

U.S. Producers	
Producers:	Reference
Akzo Chemicals, Incorporated Chicago, Illinois	Chemical Week Buyers' Guide, 1990
Atochem North America, Incorporated Buffalo, New York Axis, Alabama Carrollton, Kentucky	Chemical Week Buyers' Guide, 1990; OPD, 1990; SRI, 1990a
Cosan Chemical Corporation Clifton, New Jersey Carlstadt, New Jersey	USEPA, 1991
Hydron Laboratories New Brunswick, New Jersey	USEPA, 1991
M & T Chemicals, Incorporated Carrollton, Kentucky	USEPA, 1991; CSI, 1991
Organometallics, Incorporated East Hamstead, New Hampshire	CSI, 1991
Pfaltz & Bauer, Incorporated Waterbury, Connecticut	CSI, 1991
Troy Chemical Corporation Newark, New Jersey	ACS, 1987
Witco Chemical Corporation Taft, Louisiana Lynwood, California	USEPA, 1991
European Producers	
Commer SpA-Preparati Chimici Polivalenti Polivalenti Lodi, Italy	SRI, 1990b
Manchem Ltd. Clayton, UK	Chemical Industry Notes, 1991
M & T Chemicals BV Vlissingen The Netherlands	SRI, 1990b
Rhone-Poulenc Chimie Specialty Chemical Division Seine Maritime, France	SRI, 1990b
Schering AG Bergkamen, Germany	SRI, 1990b
Importers:	
Acetco Chemical Company, Incorporated Flushing, New York	USEPA, 1991
Danzas New York, New York	Piers Imports, 1991
Henley & Company, Incorporated New York, New York	USEPA, 1991

United Catalysts, Incorporated Louisville, Kentucky	USEPA, 1991
Thorson Chemical Corporation New York, New York	USEPA, 1991
Sakai Trading New York, Incorporated New York, New York	USEPA, 1991

3. Volume

The production volume of TBTO is reported in the public file of the EPA Toxic Substances Control Act (TSCA) Inventory. In 1983, 4 manufacturers were listed as producers of TBTO². Three manufacturers reported a total production volume ranging from 11,000-111,000 pounds. One manufacturer did not report a production volume [USEPA, 1991].

TBTO is listed in the United States International Trade Commission's publication Synthetic Organic Chemicals, but no specific production data were available for the years 1985-1988³. However, the USITC reported an annual production of 12,756,000-24,175,000 pounds for the years 1985-1988 for acyclic fungicides, a class which includes TBTO [USITC, 1986a-1989].

The annual production of TBTO between the years 1965 and 1976 was estimated to range from 860,000-4,600,000 pounds [Zuckerman *et al.*, 1978].

U.S. consumption of industrial biocides and marine antifoulants containing TBTO, tributyltin fluoride, or tributyltin copolymers was reported in the Chemical Economics Handbook to be 2.5 million pounds in 1984, with an average annual growth rate for 1984-1989 of 3-4%. U.S. consumption represents production plus imports minus exports. The average growth rate for production of triorganotins as marine antifoulants and industrial biocides was expected to be about 2.0% annually from 1984-1989. Higher growth is expected in the wood stain market where TBTO is used as a preservative at a level of 0.5% by weight per gallon. In July 1985, pentachlorophenol, creosote, and chromium and copper arsenates were banned from over-the-counter sales to the consumer wood preservative market, stimulating the market for TBTO [SRI, 1991]. In 1986, another source reported that the current U.S. production of TBT pesticides was about 400 tons per year [Goldberg, 1986].

Import Volume:⁴

Six companies were listed as importers of TBTO in the EPA Toxic Substances Control Act (TSCA) Inventory. In 1983, three importers reported a total import volume ranging from 100,000-1,002,000 pounds. One company did not report an import volume, and two reported a zero import volume of TBTO [USEPA, 1991].

The net quantity of tin compounds (a class which includes TBTO) exported to the United States between the years 1984 and 1988 was reported in the U.S. Department of Commerce's publication U.S. Imports for Consumption and General Imports to range from 1,437,520-1,409,149 pounds [USDC, 1985-1989].

4. Technical Product Composition

M&T Chemicals report their product to be >95% pure, with 38.8% tin content [M&T Chemicals, 1979]. Other producers report that TBTO is available at purities ranging from 96-97% [Aldrich, 1990; Lancaster Synthesis, 1989-1990]. Fluka Chemical Corporation markets a practical grade product [Fluka, 1990], and Troy Chemical Corporation produces this compound in liquid grade [ACS, 1987].

B. Uses

- Biocide in marine antifouling paints [Blunden *et al.*, 1982; Kirk-Othmer, 1979; SRI, 1991; M&T Chemicals, 1979] (concentrations of 15% TBTO have been recommended for effective biological activity [M&T Chemicals, 1979]).
- Fungicide to prevent slime and algae growth in closed-circuit cooling towers [SRI, 1991; Kirk-Othmer, 1981a]. For example, approximately 1.0-5.0 ppm of TBTO in combination with quaternary ammonium surfactants has proven effective against fungistatic growth in cooling towers, settling ponds, and related

installations [M&T Chemicals, 1979].

- Fungicide used to prevent the biodeterioration of wood (timber) [Ascher, 1985, Clayton and Clayton, 1981]; fungicide in water repellent formulations for wood preservation (at a concentration of 0.5% in mineral spirits) [M&T Chemicals, 1979; SRI, 1991].
- Component of textile finishes (0.025-0.050%, based on weight of fabric) to control bacteriostatic and fungistatic activity in textiles [M&T Chemicals, 1979]. Fungicide used to protect cotton textiles [Ascher, 1985; Clayton and Clayton, 1981], and leather [Clayton and Clayton, 1981].
- Mildewcide [M&T Chemicals, 1979; SRI, 1991] (0.25% in a polyvinylacetate emulsion); 1 part TBTO/100 parts resin controls mildew in polyvinylchloride system [M&T Chemicals, 1979].
- Bactericide and mildewcide in urethane foams; used in mattresses, furniture, and coat and shoe linings [M&T Chemicals, 1979].
- Bactericide against sulfate-reducing bacteria when used between 5-10 ppm for secondary oil recovery injection water [M&T Chemicals, 1979].
- Control of bacteria in hospitals; formulated for application on air filters, in mop water [M&T Chemicals, 1979] for use on floors [Ascher, 1985], in waxes and laundry washes, and for spot disinfection [M&T Chemicals, 1979].
- Slimicide for the paper industry [Ascher, 1985]. Approximately 0.2-1.0 ounces of TBTO per ton of finished paper used to control growth of most fungi, yeasts, and gram-positive bacteria in paper mills [M&T Chemicals, 1979]. Component of paper preservative [Clayton and Clayton, 1981; M&T Chemicals, 1979]; applied at levels of 500-1500 ppm directly into beater operations, size presses, or calendar stacks for the control of microorganisms [M&T Chemicals, 1979].
- Preservative for glass [Clayton and Clayton, 1981].
- Treatment of tapeworm infestations in fish [Kirk-Othmer, 1978].

It was reported in 1986 that annual usage of all tributyltin pesticides was estimated to be approximately 730,000-860,000 pounds, with antifouling paints containing TBTO accounting for 75,000-100,000 pounds. Approximately one third (250,000-300,000 pounds) of total annual tributyltin production is used in the manufacture of antifoulant paints [APCJ, 1986]. In 1978, 230 tons of TBTO were consumed in cooling-water applications; combined, 140 tons of TBTO and tributyltin fluoride (TBTF) were used in antifoulant paints [Kirk-Othmer, 1981a].

² Two of the four manufacturers, Witco Chemical Corporation and Cosan Chemical Corporation, were listed at two locations. Cosan did not report a production volume for TBTO at either location, and Witco did not report a production volume for its Lynwood, California location [USEPA, 1991].

³ Production statistics for an individual chemical are given only when there are three or more producers, no one or two of which may be predominant. Moreover, even when there are three or more producers, statistics are not given if there is any possibility that the publications would violate the statutory provisions relating to unlawful disclosure of information accepted in confidence by the Commission. Data are reported by producers for only those items where the volume of production or sales or value of sales exceeds certain minimums. Those minimums for all sections are 5,000 pounds of production or sales, or \$5,000 value of sales with the following exceptions: plastics and resin materials - 50,000 pounds or \$50,000; pigments, medicinal chemicals, flavor and perfume materials, and rubber processing chemicals - 1,000 pounds or \$1,000.

⁴ Imports for consumption is a measure of the total volume of merchandise that has cleared through Customs, whether such merchandise enters consumption channels immediately, is withdrawn for consumption from warehouses under Customs custody, or is entered into U.S. Customs territory from Foreign Trade Zones.

IV. EXPOSURE/REGULATORY STATUS

A. Consumer Exposure

No quantitative data were found on consumer exposure to TBTO.

B. Occupational Exposure

Data from the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) during the years 1981 to 1983, estimated that 36,413 employees, including 7,903 female employees, were potentially exposed to TBTO. Some of the industries or occupations that had over 2,000 employees potentially exposed included the following: chemical and allied products; transportation equipment; communications; water transportation; health services; maids and housemen; janitors and cleaners; telephone installers and repairers; other mechanics and repairers; painting and paint spraying machine operators; and marine engineers. The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemicals listed therein [NIOSH, 1991].

Between the years 1960 and 1981, approximately 182 wood-treating workers in Hawaii had been exposed to TBTO for a period of 3 or more months [Gilbert *et al.*, 1990].

In occupational exposure studies conducted by the Finnish Institute of Occupational Health, the concentration of TBTO was measured in the workroom air at 19 plywood plants between 1975 and 1984. A total of 33 measurements were taken. In each case, the levels were below the detection limit of the measuring method (40 mg m⁻³) [Kauppinen, 1986].

C. Environmental Occurrence

TBTO reportedly does not occur naturally; however, several studies have been conducted that measure the concentrations of tributyltin (TBT) (a class which includes TBTO) in marine and fresh water environments (water, sediment, and organisms). Generally, the highest concentrations of tributyltin were found in areas of heavy boating or shipping traffic (harbors and marinas), which is consistent with the use of these chemicals in antifouling paints for pleasure and commercial boats [Maguire *et al.*, 1986; Cleary and Stebbing, 1985; Waldock *et al.*, 1988; Langston *et al.*, 1987; Grovhoug *et al.*, 1986; Valkirs *et al.*, 1986; Stallard *et al.*, 1987]. One study conducted in the United Kingdom, however, found that tributyltin concentrations were independent of boating activity, with significant levels seen only in harbor waters and not in coastal areas and rivers, despite heavy boating. The authors of this study concluded that tributyltins are most likely removed from coastal water by a combination of dilution and exchange with the open sea, degradation to inorganic tin, and sedimentation after binding to particulate matter [Cleary and Stebbing, 1985]. This conclusion is further supported by findings that show higher concentrations of tributyltin compounds in harbors that have small entrance channels, which limit flushing, than in harbors that do not have restricted water circulation [Valkirs *et al.*, 1986; Langston *et al.*, 1987; Cleary and Stebbing, 1987]. In addition, levels of tributyltin measured in water samples were often found to be greater in the surface microlayer than in subsurface samples [Maguire *et al.*, 1982; Maguire and Tkacz, 1987; Waldock *et al.*, 1987]. For example, water samples from Ontario lakes and rivers had surface levels of tributyltin ranging from 0.15-60.7 mg/L, compared to subsurface levels between 0.01 and 2.91 mg/L [Maguire *et al.*, 1982]. Tables 1-2 present the ranges of tributyltin levels found in sea water, estuarine water, fresh water, and sediment samples from several locations throughout the United States.

Table 1: Concentrations of Tributyltin in Estuarine, Sea, and Fresh Water from Locations in the United States^a

Location	Year	Sample depth (meters) ^b	Concentration (ug/liter)	Detection Limit (ug/liter)	Reference
Chesapeake Bay	1985	surface	ND-1.171	0.008-0.01	Hallet al, 1986
	1986	microlayer			
Chesapeake Bay	1985	~ 1.0	ND-0.464	0.008-0.01	Hallet al, 1986
	1986				
Chesapeake Bay (South)	1986	0.15	ND-0.1	0.001	Huggette et al, 1986 (as reported in WHO, 1990)
San Diego Bay	1986	>0.5	0.005-0.25	0.005	Seligman et al, 1986
California Marinas	1986	NR	ND-0.59	0.001-0.00	Stallard et al, 1987
Californian Coast	1986	NR	ND-0.004	0.001-0.00	Stallard et al, 1987
San Diego Bay	1983	0.3-0.6	<0.01-0.95	0.01	Valkirs et al, 1986
	1985				
San Diego Bay	1983	(0.1)	<0.01-0.55	0.01	Valkirs et al, 1986
	1985				
USA harbors and estuaries		(0.5)	<0.005-0.5	0.005	Grothougt et al, 1986
New York State waterways (fresh)		surface microlayer	2.0-23.8	1.0	Maguire and Tkacz, 1986

^a Table based on data summarized in Table 3, "Concentrations of Tributyltin in Estuarine and Sea Water" and Table 4, "Concentrations of Tributyltin in Fresh Water," from World Health Organization (WHO), 1990.

^b Figures in parentheses indicate distance from water bottom.

Table 2: Concentrations of Tributyltin in Sediment from Locations in the United States^a

Location	Year	Sample depth (meters)	Concentration (ug/kg)	Detection Limit (ug/kg dry weight)	Reference
California marinas	1986	0.1	ND-23.0	1.0-2.0	Stallard et al, 1987
California coast	1986	0.1	ND-1.5	1.0-2.0	Stallard et al, 1987
Californian coast	NR	NR	15-527	NR	Stephenson et al, 1987 (as reported in WHO, 1990)
Virginian coast	NR	0.02	23-290	NR	Rice et al., 1987
San Diego Bay	1985	0.35	<2.0-300	NR	Stang and Seligman, 1986 (as reported in WHO, 1990)
Great Bay Estuary, NH	NR	0.02	12-44	NR	Weber et al., 1986

^a Table based on data summarized in Table 5, "Concentrations of tributyltin in sediment," from WHO, 1990.

Many species of marine organisms sampled from coastal waters and harbors have been found to bioaccumulate TBT. For instance, Wade *et al.* sampled bivalves from several coastal bays and estuaries in the United States. The concentrations of TBT found in oysters ranged from <5-790 ng/g; the concentrations in mussels ranged from 140-1540 ng/g [Wade *et al.*, 1988]. Humphrey and Hope sampled shellfish and finfish from British Columbia and Canada and found that the concentrations of TBT in tissue reached 1,800 ng/g and 11,000 ng/g, respectively [Humphrey and Hope, 1987].

The data presented above are clear evidence that tributyltins, a class which includes TBTO, are present in the aquatic environment. Once introduced into the environment, TBTO is subject to transformation resulting from the splitting of the carbon-tin bond. This splitting can result from several mechanisms occurring simultaneously in the environment, including physico-chemical mechanisms (hydrolysis and photodegradation) and biological mechanisms (degradation by microorganisms and metabolism by higher organisms) [WHO, 1990]. In most cases, degradation appears to involve successive debutylation to inorganic tin, with the degradation products including dibutyltin and monobutyltin [Maguire *et al.*, 1983; Bressa *et al.*, 1990; Shizong *et al.*, 1989]. Since these

dealkylated tin derivatives have a lower toxicity, the reactions may be considered as detoxification [Barug, 1981].

The following are representative studies that examine the degradation of TBTO. The three bullets concern its hydrolysis, photodegradation, and biodegradation, respectively.

Studies carried out in the darkness and in a sterile medium to assess the importance of hydrolysis in the degradation of TBTO, show that degradation occurs either not at all or only slowly under normal environmental conditions of pH and temperature. Maguire *et al.*, found that tributyltin was stable (no cleavage of butyl groups) over the course of 63 days at 20°C, in the dark at pH values between 2.9 and 10.3 [Maguire *et al.*, 1983]. In a study conducted for M & T Chemical, it was found that 0.5 ppm of TBTO undergoes minimal degradation in seawater in the absence of light; after 35 days, 95.5% of the parent compound was still present [M&T Chemicals, Inc., 1978]. In another study, when TBTO was added to seawater, 50% degraded during the first 15 days. After this time, however, the degradation rate was much slower, with complete degradation occurring only after 60 days [Bressa *et al.*, 1990].

Results of laboratory studies examining the photodegradation of TBTO vary considerably and depend on whether experiments are conducted under natural sunlight or ultraviolet (UV) light of known wavelength. According to a study conducted by M&T Chemicals, the half-life of 0.22 ppm TBTO in sea water irradiated with UV lights is 18.5 days. In the presence of a photosensitizing substance, such as acetone, the half-life is reduced to 3.5 days [M&T Chemicals, Inc., 1978]. In another study, photolysis of tributyltin (from either TBTO or tributyltin chloride) under natural light conditions in distilled or natural water was limited; the tributyltin half-life was in excess of 89 days. When the samples were irradiated with UV light at 300 and 350 nm, the half-lives were 1.1 days and >18 days, respectively. This study also reported that, under natural conditions in the port of Toronto, Canada, the half-life of TBTO was greater than 89 days [Maguire *et al.*, 1983]. Seligman *et al.*, found that, at high concentrations of tributyltin (744 mg/L), photodegradation was less important than biological action (see below), with natural sunlight causing no degradation over 144 days [Seligman *et al.*, 1986].

Studies conducted in the laboratory show that TBTO undergoes microbial degradation under anaerobic and aerobic conditions. In one study, TBTO was biodegraded by a mixed bacterial culture from activated sludge under aerobic and anaerobic conditions with half-lives of 5 and 3 days, respectively [Shizong *et al.*, 1989]. A study conducted by M&T Chemicals showed that microflora derived from activated sludge and soil were capable of partially degrading TBTO; the half-life was 70 days under aerobic conditions, and 200 days under anaerobic conditions [M&T Chemicals, Inc., 1978]. Another study examining the microbial degradation of TBTO found that, of the many bacterial species investigated that are common to both soil and water, only two, *Pseudomonas aeruginosa* and *Alcaligenes faecalis*, degraded sublethal amounts of the compound during aerobic growth in the presence of suitable carbon sources. All the fungal species investigated were able to degrade TBTO indicating that fungi may significantly contribute to the degradation of this compound [Barug, 1981]. In a study in which the fate of ¹⁴C-labelled TBTO in soil was investigated, the half-lives of TBTO in silt loam and sandy loam were 15 and 20 weeks, respectively [Barug and Vonk, 1980]. Finally, in a study conducted by Seligman *et al.* to investigate the degradation rate of tributyltin in harbor waters, unlabeled or ¹⁴C-labeled tributyltin was added to water samples collected in San Diego Bay, California. In yacht basin water polluted with tributyltin, the tributyltin (0.5 mg/L) half-life was 6 and 7 days in the light and dark, respectively; the half-lives from a clean-water site (<0.03 mg tributyltin) were 9 and 19 days for light and dark treatment, respectively. When the formation of ¹⁴C-labelled carbon dioxide from the degradation of ¹⁴C-labelled tributyltin was used to measure complete mineralization, the calculated half-life was 50 and 75 days in the light and dark, respectively. The authors concluded that microbial degradation appeared to be the primary process accounting for tributyltin degradation in seawater [Seligman *et al.*, 1986].

D. Regulatory Status

Domestic

OSHA has not recommended a permissible exposure limit (PEL) for TBTO. However, the current OSHA permissible exposure limit (PEL) for organic tin compounds (as Sn) is 0.1 mg/m³ (skin) averaged over an eight-hour work shift. The (skin) notation indicates that transdermal exposure may contribute significantly to the overall exposure burden. A short-term exposure limit (STEL) has not been determined {29 CFR 1910.1000} [Office of the Federal Register, 1990a].

The Food and Drug Administration has approved the use of TBTO for the following applications:

- as a preservative in adhesives for food packaging when the adhesive is used in accordance with the prescribed conditions {21 CFR 175.105} [Office of the Federal Register, 1990b].
- as stabilizers in vinyl chloride plastics, alone or in combination, at levels not to exceed a total of 3 parts per hundred of resin {21 CFR 178.2650}[Office of the Federal Register, 1991].

Because of increased evidence of their aquatic toxicity, antifouling paints containing tributyltins have been the focus of a series of regulatory actions taken by the Environmental Protection Agency (EPA). The chronology of these actions is as follows:

- On January 8, 1986, the EPA issued a "Notice of Special Review" on certain pesticide products containing any of the nine tributyltin (TBT) compounds that were registered as antifoulants (including TBTO). This Special Review was initiated on the basis of bioassay and laboratory studies indicating that TBT compounds are highly toxic to nontarget marine and fresh water aquatic organisms. The Special Review process is currently governed by 40 CFR Part 154 [Office of the Federal Register, 1988]. In addition, the U.S. Senate, in 1986, banned the use of TBT paints by the Navy until the EPA certified that such use would not pose an unacceptable hazard to the marine environment [APCJ, 1986].
- Using its authority under section 3(c)(2)(B) of the Federal Fungicide, Insecticide, and Rodenticide Act (FIFRA), the EPA issued a data call-in (DCI) notice on July 29, 1986 to all registrants of TBT antifouling paints and the producers of TBT active ingredients. The DCI required data on product chemistry, ecological effects, environmental fate, release rates, worker exposure, quantitative use, application, and efficacy. According to FIFRA, failure to comply to the DCI notice can result in cancellation of registration.
- On October 7, 1987, the EPA made a preliminary determination to:
 - (1) cancel the registrations of TBT antifouling paint products with short-term cumulative release greater than 168 mg of organotin/cm² or average release rates >4 mg/cm²/day;
 - (2) prohibit the use of TBT antifouling paints on non-aluminum hulled vessels less than 65 feet in length;
 - (3) classify TBT antifouling paints as restricted use pesticide and restrict their sale to certified commercial applicators and their use by persons under the direct supervision of an on-site certified commercial applicator; and
 - (4) require compliance with requirements pertaining to removal and disposal of old paint prior to application of new paints, and/or application of new TBT paints.
- On June 16, 1988, President Reagan signed the Organotin Antifouling Paint Control Act of 1988 (OAPCA), which contained both interim and permanent TBT use restrictions, as well as provisions regarding sale and use of existing stocks, environmental monitoring research on alternatives, reports to Congress, and penalties for non-compliance. Some of the restrictions of the OAPCA include the following:
 - (1) Only products that do not exceed a release rate of 4 mg organotin/cm²/day can be sold and used. Paint manufacturers must certify that a paint's TBT release rate does not exceed the limit. This release rate was set as the interim rate until EPA could accumulate sufficient data to set a final release rate figure.
 - (2) All TBT products are prohibited from use on non-aluminum vessels that are less than 25 meters (82 feet). Outboard motors and lower drive units are exempt from the prohibition.
 - (3) Subject to existing stock provisions, all retail sale, distribution, purchase, and receipt for TBT additives used in antifouling paints is prohibited.

- On October 4, 1988, due to the inadequacy of the data submitted by the registrants, the EPA announced that it would cancel all TBT antifouling paint registrations that did not comply with the established release rate and are used on non-aluminum vessels under 82 feet. The announcement also designated all TBT-formulated antifouling paints as restricted-use pesticides as of March 1, 1990. This restriction excludes products packaged in aerosol containers 16 ounces or less and labeled for use on only outboard motors, propellers and other non-hull underwater aluminum components.
- All registrants were ordered to develop training programs for the certification of applicators. After March 1, 1990, only certified applicators will be able to purchase TBT antifoulants, and only certified applicators and those under their direct supervision will be able to apply them [Office of the Federal Register, 1988].

Foreign

Current legislation varies among countries but, in general, TBT antifoulants have been banned, or a ban is imminent, on small vessels (< than 25 meters in length) in all major yachting areas (Western Europe, Scandinavia, North America, Australia, and New Zealand). TBT antifouling is still permitted on vessels of more than 25 meters in length throughout the world, although certain countries have restrictions on the types of coatings allowed [Trade and Industry ASAP, 1991].

E. Exposure Recommendations

The ACGIH has not recommended a threshold limit value (TLV) for TBTO. However, the current ACGIH threshold limit value-time weighted average (TLV-TWA) for organic compounds of tin (as Sn) is 0.1 mg/m³ (skin) averaged over an eight-hour work shift. In its Notice of Intended Changes list, ACGIH has proposed a short-term exposure limit for organic tin compounds (as Sn) of 0.2 mg/m³ (skin) [ACGIH, 1990].

NIOSH has not recommended an exposure limit for TBTO. However, the NIOSH-recommended exposure limit (REL) of organic tin compounds (as Sn), averaged over a ten-hour work shift is 0.1 mg/mg (skin) [NIOSH, 1990].

V. TOXICOLOGICAL EFFECTS

A. Chemical Disposition

1. Human Data

No data were found.

2. Animal Data

oral, rat

In a long-term toxicity and carcinogenicity study (see section VD.2), total tin content was determined in the liver and kidney of SPF-derived Riv:TOX Wistar rats following a one- or two-year exposure to TBTO. The test chemical was mixed with olive oil and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 mixture of premix and untreated feed. The animals were fed experimental diets containing 0 (negative control), 0.5, 5.0, or 50 mg TBTO/kg diet *ad libitum* for one or two years. After sacrifice at 1 and 2 years, the concentration of tin was determined in liver and kidney tissues sampled from five rats/sex/group.

A dose-dependent increase in total tin was found in all treatment groups, and no differences were noted between males and females. The data are presented below in [Table 3](#). In general, the mass concentrations were similar after 12 and 24 months, except for kidney concentrations in the high-dose females, which showed higher values after 24 months. The authors did not report any other conclusions [Wester *et al.*, 1990a].

Table 3: Total Tin Content in Kidneys and Liver of Rats Fed Diets Containing TBTO for 12 or 24 Months

Total Tin Content (mg/kg wet weight) of:

Dietary conc. of TBTO (mg/kg)	Kidney		Liver	
	Males	Females	Males	Females
12 months				
0 (control)	<0.05	<0.5	<0.1	<0.1
0.5	1.66 ± 0.41*	2.28 ± 0.57	0.22 ± 0.08	0.23 ± 0.08
5	2.75 ± 0.35	3.09 ± 0.45	0.91 ± 0.12	1.07 ± 0.12
50	7.27 ± 0.83	6.06 ± 1.13	5.55 ± 0.51	6.63 ± 0.32
24 months				
0 (control)	<0.5	<0.5	<0.1	<0.1
0.5	1.73 ± 0.23	1.79 ± 0.48	0.12 ± 0.02	0.22 ± 0.06
5	2.22 ± 0.39	2.09 ± 0.17	1.00 ± 0.12	1.09 ± 0.15
50	7.90 ± 2.48	10.9 ± 1.74	5.79 ± 0.81	7.6 ± 0.87

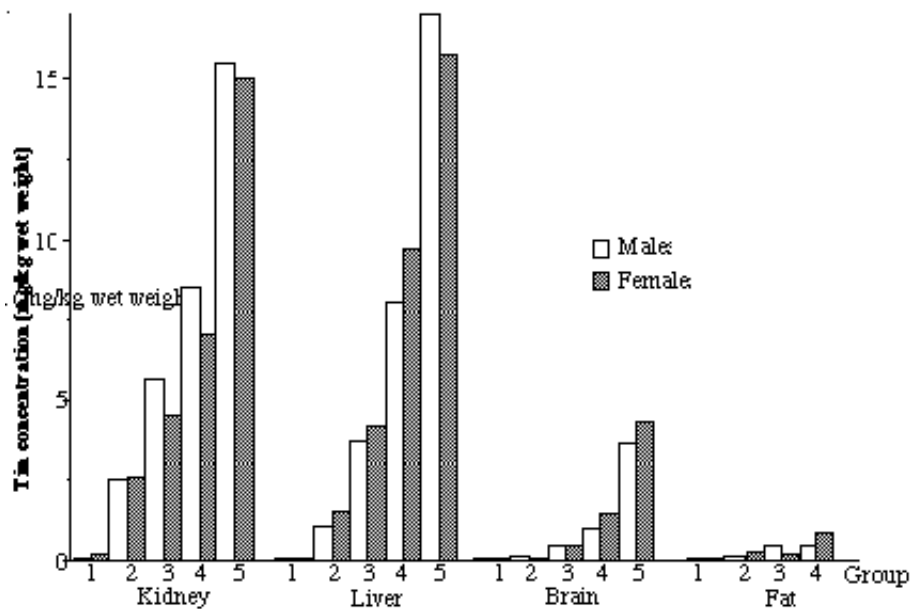
*Values are means ± SD for four to five rats/group.

Reference: Wester *et al.*, 1990a

oral, rat

In a short-term toxicity study (see section VC.2), total tin levels were determined in organs from male and female SPF-derived Wistar rats (Riv: TOX[M]) following 4 weeks of dietary TBTO exposure. Technical grade TBTO was mixed with olive oil, and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 dilution. Groups of 10 rats/sex were fed final dietary concentrations of 0 (group 1-control), 5 (group 2), 20 (group 3), 80 (group 4), or 320 mg TBTO/kg feed (group 5). Control animals received feed containing equivalent amounts of olive oil. Animals were sacrificed after 4-weeks, and the total tin was determined in weighed samples of liver, brain, kidneys, and perianal adipose tissue, which were pooled from each sex and dose group. The results are presented below in [Figure 1 5](#). For both sexes, dose-related increases in tin concentrations were observed in all organs. The kidney and the liver showed the highest levels, with residues in the brain and adipose tissue being 5-10 times lower. The authors concluded that a steady state was most likely not reached [Krajnc *et al.*, 1984].

Figure 1: Tin Concentrations in Organs from Rats Treated with Dietary TBTO^a



^aTotal tin concentrations in pooled samples, obtained from male and female rats after 4 weeks of dietary exposure to TBTO. In group 5 (320 mg/kg), insufficient adipose tissue was present for analysis.

reference: Krajnc *et al.*, 1984

oral, mouse

Female albino mice of the COBS strain were used to examine the accumulation, clearance, and excretion of TBTO, following continuous exposure to low concentrations. Carbon-14 labelled TBTO formulations [6](#) were used in drinking water at concentrations (determined by liquid scintillation counting) of 0.86, 6.20, and 31.0 nmol/mg (0.51, 3.75, and 18.5 ppm, respectively). Water containing [¹⁴C] TBTO was the only available drinking water. To assess the accumulation of TBTO by selected tissues, 5 mice per concentration were orally administered the [¹⁴C] TBTO solutions for 5, 10, 15, 20, 25, or 30 days. At the end of each test interval, the mice were sacrificed and the lungs, liver, brain, kidney, spleen, fatty tissue (abdominal area), and muscle tissue (upper thigh) were removed and homogenized. Blood samples were also taken from each animal. In a parallel experiment, to determine the persistence of the test compound in the selected tissues, groups of 5 mice were administered the test substance at the above concentrations for a period of 31 days. After 31 days, half of the animals in each group were sacrificed and the remainder were placed on TBTO-free water for 15 days and then sacrificed. At the time of sacrifice, the selected tissues were removed and solubilized. For both experiments, the concentrations of labelled-TBTO were determined in each tissue and blood sample using standard liquid scintillation methods. Finally, excretion of TBTO was studied by collecting daily urine and fecal samples from mice (4/group) which were administered a 100 nmol TBTO solution (per 100 mg wet sample) via drinking water for 5, 10, 15, 20, 25, or 30 days.

Tissue accumulation of TBTO in the kidney, liver, spleen, and fat tissues was directly proportional to the administered dose, with the highest concentrations found in tissues from the 31.0 nmol/mg dose group ([Table 4](#)). In all dose groups, the highest mean concentrations of test compound were found in the liver, kidney, spleen, and fat tissue. Lower levels of [¹⁴C]TBTO were seen in the brain, lung, and muscle tissues of each dose group, but were only detectable in the blood from the highest-dose group. After a 31-day exposure period and a 15-day clearance period, tissue TBTO levels showed a marked decrease (compared to tissue levels in the animals that were exposed for 31 days and not subjected to the clearance period) in almost all tissues examined. The liver and kidney showed 97% and 73% clearance of the test compound, respectively; the blood showed apparently complete clearance. Fat tissue, however, had a relatively high retention, with clearance of only 30%; lung tissue showed complete retention. Data obtained from feces and urine samples of the rats exposed to 100 nmol/100 mg wet sample ([Table 5](#)) indicate that more of the labeled test compound was excreted in the feces than in the urine,

and that the levels of TBTO found in the excreta were greater than those found in the tissues. Also, the total excreted [¹⁴C] increased over time while the tissue level remained "reasonably" constant. When total excreta level was compared to the total tissue level per 5-day period, it was found that the test compound was excreted at a much higher rate than it was accumulated.

Of the organs sampled, the organs with high metabolic functions had the highest accumulation levels and clearance rates. From these results, the authors concluded that TBTO may be rapidly excreted unchanged. Also, the high levels of TBTO seen in the fat coupled with the low blood levels suggest, to the authors, that the compound may be transported from the intestine to the adipose tissue via the chylomicron system. The high levels of TBTO found in the excreta, especially the feces, indicate low absorption by the body and direct removal via the feces. The fact that the total tissue level remained constant, while the excreta level tended to increase over time, suggests that the concentration of the compound is regulated by the body's excretory and metabolic functions. The authors noted that since only [¹⁴C] was monitored, it is not certain whether the actual species detected was TBTO, or a metabolite or degradation product [Evans and Cardarelli, 1979].

Table 4: Tissue Accumulation of TBTO in Mice as a Function of TBTO Concentration in Drinking Water

Tissue	[TBTO] in tissue at concentration in water of (nmol/mg) ^a		
	31.0	6.20	0.86
Liver	0.20	0.043	0.013
Spleen	0.11	0.036	0.013
Kidney	0.35	0.13	0.25
Brain	0.061	0.018	0.0051
Muscle	0.056	0.025	0.0076
Fat	0.27	0.069	0.02
Lung	0.089	0.020	0.01
Blood	0.0051	ND	ND

^a Nanomoles per 100 mg wet tissue obtained from 5 mice per test concentration, averaged over a 30-day period.
ND = none detected

reference: Evans and Cardarelli, 1979

Table 5: Excreta and Totaled Sampled Tissue Concentration in Mice Exposed to TBTO

	TBTO (nmol/100 mg) ^a for test period (days)					
	5	10	15	20	25	30
Feces	12.0	22.0	29.0	45.0	62.0	77.0
Urine ^b	1.5	4.0	7.0	9.5	11.0	13.0
Tissue	1.1	1.3	0.65	0.70	1.4	1.4
Excretion/accumulation ratio ^c	12.1	19.2	55.7	78.7	53.8	64.7

^a Values are per 100-mg wet sample for a 100 ppm test on 4 mice in metabolism cages

^b Sum of tissue concentrations (all sampled tissue from Table 6)

^c Sum of excretion concentrations divided by sum of tissue conc.

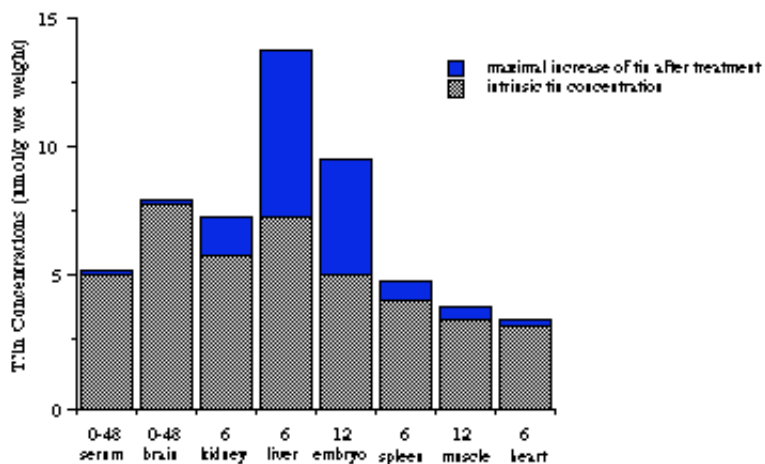
reference: Evans and Cardarelli, 1979

oral, mouse

Pregnant Long-Evans mice were used to examine the accumulation of TBTO in maternal tissue and whole embryo following a single oral dose. On day 11 of gestation, an unspecified number of pregnant mice were orally administered 110 mg TBTO/kg body weight (vehicle unreported). The mice were sacrificed from 2 to 48 hours after dosing (exact times unreported); the liver, brain, spleen, kidney, skeletal muscle, heart, and fetus were removed and frozen in liquid nitrogen. Fetal and maternal serum samples were also obtained. The concentration of tin in each sample was measured in an atomic absorption spectrophotometer. Because tin is a normal constituent of all tissues, intrinsic tin concentrations were determined for the fetus and maternal serum, liver, brain, spleen, kidney, skeletal muscle, and heart in mice not exposed to TBTO. Intrinsic tin concentrations were compared to concentrations following TBTO administration.

Peak intrinsic tin concentrations and peak tin concentrations following TBTO administration are presented below in [Figure 2](#)⁷. After a single oral dose of TBTO, the tin content of all tissues increased, but to different degrees. Maximal increases were found in the embryo and in the maternal liver (70% and 60%, respectively). The tin content in the liver peaked 6 hours after treatment and remained at this high level for at least 26 hours, while the tin level in embryos increased over a period of 12 hours and then dropped. Serum tin content was elevated by approximately 20% over a period of 24 hours, while tin concentration in the brain was found to be constant. The authors of this study point out that total tin, not TBTO, was measured, and that therefore, they can't establish whether the original compound or some metabolite is responsible for the increased tin levels. They calculated that a tin increase of 4.5 nmoles tin per gram embryonic tissue following a maternal treatment of 110 mg/kg corresponds to about 1.31 mg TBTO/g embryonic tissue of unmetabolized TBTO or active metabolites. According to *in vitro* studies by Krowke *et al.*, 1986 (see section VE.2), this concentration is highly toxic to the embryonic tissue. However, since the authors of this study also showed that TBTO had no effect on the embryo *in vivo* (see section VE.2), they suggested that TBTO is not transferred through the placenta to an extent which exhibits embryotoxic activity, or it is converted within the maternal organism to biologically less active compounds [Davis *et al.*, 1987].

Figure 2: Tin Concentrations in Embryos, Serum, and Organs of Mice Following Oral Treatment with TBTO



reference: Davis *et al.*, 1987

inhalation, mouse

In an acute toxicity study (see section VB.2), groups of 12 male Swiss mice were exposed to an aerosol of pure TBTO, or an aerosol of a 50% mixture of TBTO in toluene for one hour at an aerosol dispersion rate of 10 liters/minute. The aerosols were administered at ambient temperature and heated to 200-600°C. At the time of death or after sacrifice, the animals were necropsied, and the concentration of tin in the heart, brain, liver, lung, spleen, and kidney were determined. [Table 6](#) below presents the mean values of organotin concentrations for 6 animals [Truhaut *et al.*, 1981].

inhalation, guinea pig

In an acute toxicity study (see section VB.2), groups of 6 male Hartley guinea pigs were exposed to an aerosol of pure TBTO or an aerosol of a 50% mixture of TBTO in toluene for one hour at an aerosol dispersion rate of 10 liters/minute. The aerosols were administered at ambient temperature and heated to 200-600°C. At the time of death or after sacrifice, the animals were necropsied, and the concentration of tin in the heart, brain, liver, lung, spleen and kidney were determined. [Table 7](#) presents the mean values of 6 animals [Truhaut *et al.*, 1981].

inhalation, guinea pig

In an acute toxicity study (see section V.B.2), male and female Hartley albino guinea pigs (n=105) were exposed to aerosols of TBTO in olive oil at concentrations ranging from 0.1-1 mg TBTO/liter air for one hour. Mean concentrations of tin were determined in the major organs of each animal and are presented in [Table 8](#). [Anger *et al.*, 1976].

Table 6: Organ Tin Concentrations in Mice Exposed to Aerosols of TBTO

Organ tin concentrations (mg/100 g of fresh after inhalation of aerosols of:						
pure TBTO						
Temp.°C	hear	brain	liver	lung	spleen	kidney
amb	4.2 [±]	1.3 [±]	2.8 [±]	8.60	1.8 [±]	0.8 [±]
200	1.7 [±]	0.9 [±]	2.2 [±]	5.84	1.5 [±]	0.8 [±]
300	5.5 [±]	1.8 [±]	2.0 [±]	29.8 [±]	3.3 [±]	0.7 [±]
400	2.8 [±]	2.0 [±]	1.5 [±]	38.2 [±]	3.2 [±]	0.6 [±]
500	3.2 [±]	0.8 [±]	0.5 [±]	20.8 [±]	4.3 [±]	0.5 [±]
600	3.0 [±]	0.5 [±]	0.7 [±]	16.8 [±]	4.8 [±]	0.6 [±]
TBTO in toluen						
amb	1.6 [±]	1.1 [±]	1.0 [±]	2.35	4.2 [±]	1.3 [±]
200	2.6 [±]	1.1 [±]	1.0 [±]	2.94	2.5 [±]	1.0 [±]
300	2.9 [±]	1.6 [±]	0.9 [±]	5.51	6.4 [±]	1.2 [±]
400	2.7 [±]	0.8 [±]	0.7 [±]	13.5 [±]	2.0 [±]	0.5 [±]
500	3.1 [±]	0.5 [±]	1.1 [±]	28.0 [±]	2.0 [±]	0.8 [±]
600	1.7 [±]	1.2 [±]	0.5 [±]	18.5 [±]	4.8 [±]	1.0 [±]

amb.= ambient

reference: Truhaut *et al.*, 1981

Table 7: Organ Tin Concentrations in Guinea Pigs Exposed to Aerosols of TBTO

Organ tin concentrations (mg/100 g of fresh tissue)
after inhalation of aerosols of:

Temp. °C	pure TBTO					
	heart	brain	liver	lung	spleen	kidney
amb.	0.96	0.61	0.41	2.34	1.75	2.37
200	2.81	0.71	0.89	2.83	5.25	0.71
300	1.20	0.76	0.49	1.74	2.17	0.44
400	1.31	1.46	0.50	3.73	3.54	1.52
500	1.00	0.98	1.28	8.11	6.95	0.72
600	1.45	0.83	1.36	16.85	3.95	0.80
	TBTO in toluene					
amb.	0.87	0.22	0.55	2.42	1.04	1.12
200	1.26	0.78	0.65	2.80	1.56	0.69
300	1.15	0.52	0.69	4.57	1.43	0.74
400	0.82	0.50	0.67	9.54	3.08	0.43
500	0.89	1.61	0.79	18.43	3.30	0.32
600	1.70	1.01	0.84	18.10	3.68	0.90

reference: Truhaut *et al.*, 1981

Table 8: Organ Tin Concentrations in Guinea Pigs Exposed to Aerosols of TBTO

TBTO concb	Organ tin concentrations (mg/100 g of fresh tissue) ^a						
	liver	kidney	brain	heart	lung	spleen	testes
1.0	0.703	0.862	0.547	0.875	0.305	0.751	0.375
0.4	0.940	0.780	0.721	0.535	0.650	0.652	0.425
0.33	0.925	0.570	0.346	0.880	0.790	0.550	0.307
0.2	0.634	0.577	0.302	0.645	0.850	0.642	0.963
0.15	0.434	0.514	0.392	0.742	1.380	0.625	0.481
0.17 ^c	0.562	0.368	0.742	0.660	0.418	1.660	NA

^a Values are mean concentrations for 5 males, except for the 0.17 mg/kg values, which are the means of 5 females.

^b Mean concentration of TBTO in air (mg/L)

^c After 3 hours of TBTO exposure

reference: Anger *et al.*, 1976

intraperitoneal, mouse

In a study comparing the half-life of organic and inorganic tin in the mouse, groups of 5 Swiss mice were given an intraperitoneal injection of ¹¹³Sn-labelled TBTO (0.1 ml of a solution of 1.2 mg/ml of TBTO and 0.04 mCi/ml). Radioactivity was measured using a whole-body counter instrument with four NaI crystals. The data from four mice (one mouse died during the first 24 hours) demonstrated a rapid decrease in whole-body radioactivity that became progressively slower with increasing time. Initially TBTO was cleared from the body of the mice rapidly, but the rate decreased with time. The authors stated that this trend may be due to the conversion of the organotin to inorganic tin in the body, or that fractions of TBTO may have become lodged in organs where it would not turn over rapidly. They also concluded that the problem of long-term accumulation of organotin probably does not exist in the mammal [Brown *et al.*, 1977].

oral/subcutaneous, rat

In a biochemical study described in section VG.3, tissue (brain, kidney, and liver) tin levels were determined in male Sprague-Dawley rats following administration of TBTO. Groups of 3 rats were given either a single subcutaneous dose of TBTO dissolved in ethanol (30 mg/kg) or a single oral dose of TBTO suspended in corn oil (60 mg/kg). Control animals received an equivalent volume of corn oil or ethanol. Forty-eight hours after dosing, the animals were sacrificed and tissue tin levels were measured.

The levels of tin measured in the kidney, liver, and brain at 48 hours show that after oral administration, the liver and the kidney achieve similar levels (28.68 ± 6.09 and 26.09 ± 3.39 mg Sn/g dry weight, respectively). However, when TBTO was administered parenterally, the tissue tin levels were found to be considerably lower in the liver and the kidney (5.26 ± 0.96 and 9.29 ± 0.22 mg Sn/g dry weight, respectively). In the brain, tissue tin levels were less than 10% of those found in the liver after oral treatment, and could not be detected after parenteral treatment. The lack of correlation between tissue tin levels in the target organ and ultimate biological response strongly suggests, according to the authors, the presence or route of administration-dependent formation of biologically active metabolites [Rosenberg and Kappas, 1989b].

oral/subcutaneous, rat

In a biochemical study described in section VG.3, tin levels were determined in liver tissue obtained from male Sprague-Dawley rats following administration of TBTO. TBTO was either dissolved in ethanol and administered subcutaneously in a single dose, or suspended in corn oil and administered as a single dose by gavage. In either case, the test animals received 50 mmol of test chemical/kg body weight. Control animals received an equivalent volume (1.0 ml/kg) of either corn oil or ethanol. Each test and control group consisted of at least three animals (exact numbers not reported). Forty-eight hours after dosing, all animals were sacrificed, and tin concentrations in the liver were measured. Total tin content of the liver was lower in rats exposed to the test compound subcutaneously, than in rats exposed to the chemical orally (2.29 ± 0.36 and 20.18 ± 4.79 mg Sn/g dry weight, respectively). Tin was undetectable in the livers of control animals. The author concluded that the toxicity produced in the liver can be circumvented when the compound is given by gavage, suggesting that intestinal "first pass" metabolism of TBTO produces a metabolite that is less toxic to the liver [Rosenberg, 1990].

⁵ Because the authors did not report the actual values for tin concentrations, Figure 1 of the study was reconstructed using our estimated values.

⁶ The radiolabelled test solution was prepared using an elastomeric controlled release formulation (BioMet) SRM. This molluscicide is composed of 6% TBTO in a natural rubber matrix.

⁷ Because the authors did not report the actual values for tin concentrations, Figure 6 of the study was reconstructed using our estimated values.

B. Acute

1. Human Data/Case Reports

dermal, human

A case report concerning dermatitis caused by the use of TBTO for sock disinfection has been described. Seventy men in a military regiment developed an acute foot dermatitis after marching on several hot days in July, 1973. The dermatitis was localized to the dorsal and lateral parts of the feet and ankles, and was characterized by itching and sometimes painful erythematous, vesiculous to bullous and hemorrhagic lesions. Approximately 50 additional soldiers of the same regiment developed marked itching in the same region, but did not exhibit visible skin eruptions. The lesions disappeared, in most cases, within one to two weeks after treatment with saline compresses and topical corticosteroid creams. Two cases, however, exhibited red to violet, slightly tender skin more than six months later. Routinely, the socks of military personnel are laundered and then disinfected with a solution added to the rinse cycle that contains 0.5% TBTO and 2.5% alkyl dimethyl benzyl ammonium chloride. After the socks are spin-dried, the content of TBTO in the remaining fluid within the socks is approximately

0.001%. However, in the actual batch of socks received by the affected men, the disinfectant had been mistakenly added in about seven times the normal concentration, and the final concentration of TBTO was calculated to be nearly 0.01%.

Patch tests were first performed on eczema patients who had not had previous exposure to TBTO. The tests were conducted using occluded patches containing 0.001, 0.01, 0.1, and 1.0% TBTO in distilled water. The patches were removed, and the sites were scored 1 and 24 hours later. At 1.0%, TBTO caused strong necrotic reactions in each of the five patients tested. Sixty-two percent of the eczema patients tested had a positive reaction to patches containing 0.1% TBTO, while only 3% reacted to patches containing 0.01% of the test compound. No positive reactions were seen using 0.001% of the test compound. From these results, the primary irritant concentration was estimated to be between 0.1 and 0.01%. Additional patch tests using 0.01% and 0.001% TBTO (non-irritating concentrations), and the quaternary ammonium compound found in the disinfectant, were done on 14 soldiers who had exhibited severe dermatitis 2 months before the test. All tests were negative for both concentrations of the test substance and for the quaternary ammonium compound. The authors concluded that the observed dermatitis was caused by the presence of TBTO in the socks in excess of the primary irritant concentration, and was not allergic contact dermatitis. The boots functioned as an occlusive bandage and TBTO was dissolved from the socks by sweat. Due to the narrow span between the optimal concentration for disinfection and the primary irritant concentration, this compound, according to the authors, should not be used to launder textiles [Molin and Wahlberg, 1975].

dermal, human

After using a new brand of undercoat and top coat, two male painters noted an itchy rash, characterized by redness, swelling and vesiculation, on skin sites exposed to the new paint. The new paint system contained 0.6% TBTO, which is a "slightly high" concentration (another non-marine paint described in the report contained only 0.06%). The rash appeared 8-10 hours after contact and cleared within a few days. Also, when the men painted ceilings, the paints dripped onto their faces, necks, and trunks; and severe itching, redness, swelling, and blistering developed over these areas after only one day. Hospital examinations revealed vesiculo-bullous lesions with redness and edema on the face, neck, trunk, arms, and thighs of both men. The lesions cleared after 10 days of treatment. Although specifications were not reported, many of the 36 other painters who also worked with these new paints developed similar symptoms. Two weeks after being discharged from the hospital, the two patients were patch tested with 0.01% and 0.05% w/w aqueous TBTO, as well as all other components of the paint. Two control groups (5 patients each) were also tested with 0.01% and 0.001% w/w aqueous TBTO. Both patients exhibited erosive changes at both concentrations of TBTO after 48 and 96 hours. Neither man exhibited positive responses to any other constituent of the paint. The 5 control patients patch tested with 0.001% w/w aqueous test compound also had similar erosive reactions. The authors of this study concluded that TBTO was most likely the responsible irritant present in the new paint formulation. They caution that although the compound does not appear to be an allergen, it is a strong irritant and workers should use precaution when working with items containing TBTO [Goh, 1985].

dermal, human

A 39-year-old shipwright developed pruritus, erythema, and vesiculation on both wrists and forearms and a few lesions on the abdomen after using an organotin-containing antifouling paint for wooden blocks. During the painting operation, his skin contacted overspray which contained 10-11.7% TBTO and unspecified concentrations of xylene, cuprous oxide, and copper thiocyanate. Also, the patient and 4 other workers had developed dermatitis 2 months earlier when using a TBTO-containing paint. Within an hour after using the paint, the patient developed some irritation. By the second day, erythema and ulceration were noted, and the patient had pustular lesions on the mucous membranes of his lips. This last symptom was apparently related to his habit of wiping his lips with a paint-contaminated arm. The patient was treated with Valisone® spray and cream, Halog and Eucerin cream, and prednisone, and the symptoms cleared within three weeks. One week later, however, the dermatitis recurred on previously affected sites. The reaction was considered to be a result of an allergic reaction to the TBTO-containing paint, or an allergic reaction to a medicament. Standard patch testing was done over the next several weeks with each topical medication, and serial dilutions of TBTO (0.1%, 0.01%, and 0.0015% aqueous). A second series of patch tests was done with the components of the Valisone cream.

Results from the patch test indicate that the patient developed allergic contact dermatitis from Valisone® cream, or more specifically, from the preservative, p-chloro-m-cresol, present in the cream. The reaction to TBTO was

considered to be an irritant reaction, not an allergic response, based on its morphology. Forty-eight hours after patch testing with 0.1% TBTO, a large bulla was observed and at 96 hours, crusting was noted. No reaction was seen using 0.01% or 0.0015% of the compound. The authors concluded that TBTO is a strong irritant, and great care should be taken to protect the skin against products containing this chemical [Lewis and Emmett, 1987].

dermal, human

In a study by Baaijens, two workers who were accidentally exposed to TBTO during the manufacture of organotin compounds developed severe dermatitis where splashes of the material had been left on the skin for extended periods of time. One worker, who complained of the intense smell of TBTO, suffered nausea and vomiting after ten minutes of exposure. Urine tin levels in this worker were elevated for several days. In all three cases, the symptoms disappeared within a few days [Baaijens, 1987, as reported in WHO, 1990].

dermal, human

Fifteen workers involved in the production of dibutyl and tributyl tin compounds were accidentally exposed to unspecified concentrations of TBTO. The workers suffered severe burns of the skin of the face, limbs, and eyelids [Lyle, 1958].

inhalation, human

Female workers exposed for 14 days to a commercial spray paint preparation (Lastanox T) containing 20% TBTO exhibited signs of nasal and respiratory irritation. Symptoms included nasal discharge and bleeding, inflamed nasal mucosae, and hemorrhagic areas on the nasal septum, which disappeared upon discontinuation of exposure [Landa *et al.*, 1973, as reported in NCI, 1988b].

2. Animal Data

Data on the acute toxicity of TBTO in animals are presented in Table 9. Additional data are described below.

Table 9: Acute Toxicity of TBTO

Route	species/strain	number/sex	LD ₅₀ ^a (range)	Reference
oral	rat/NR	NR/NR	148-234	Cardarelli and Evans, 1980
oral	rat/albino-Holtzman	7/male	194 (165-22)	Elsea and Paynter, 1958
oral	rat/albino-Holtzman	7/male	148 (113-19)	Elsea and Paynter, 1958
oral	rat/Sprague-Dawley	NR/male	197 (197-27)	Funahashi et al, 1980
oral	rat/NR	NR/NR	87	RTECS, 1991
oral	rat/NR	NR/NR	127	Schweinfurth, 1985
oral	rat/NR	NR/NR	234	Sheldon, 1975
oral	rat/Wistar	10/NR	180 ± 48	Truhautet al, 1976
oral	mouse/NR	NR/NR	122-203	Cardarelli and Evans, 1980
oral	mouse/Swiss OF	10/male	152 (140-24)	Poitouet al, 1978
oral	mouse/Swiss OF	10/female	92 (44-130)	Poitouet al, 1978
oral	mouse/NMRI	12/female	74 (66-82)	Davis et al, 1987
oral	mouse/NR	NR/NR	85 (52-130)	Polster and Halacka, 1971
oral	mouse/NR	NR/NR	55	RTECS, 1991
oral	chicken/Studler-Corn	NR/NR	380-400 ppm	Guta-Socaciu, 1986
inhal	rat/NR	NR/NR	56 (LG) ^b	Cardarelli and Evans, 1980
inhal	rat/NR	NR/NR	77 (LG) ^c	Schweinfurth, 1985
i.p.	rat/Osborne-Mendel	18/female	7.210	Robinson, 1969
i. p.	rat/OFA Sprague-Dawley	20/male and female	20 (18-21)	Poitouet al, 1978
i. p.	mouse/non-line albino	NR/male and female	12.5	Voronkovet al, 1979
i. p.	mouse/Swiss OF	20/male and female	16 (15-17)	Poitouet al, 1978
s. c.	rat/NR	NR/NR	11700	PCOC, 1966
s. c.	mouse/NR	NR/NR	200 (140-27)	Polster and Halacka, 1971
s. c.	mouse/Swiss	10/NR	6.0 ± 0.5	Truhautet al, 1976
derma	rabbit/Fauve de Bourgogne	6/male and female	9000	Truhautet al, 1976
derma	rabbit/NR	4/NR	11,700	Cardarelli and Evans, 1980 Elsea and Paynter, 1958

^a Unless otherwise stated, LD values are in mg/kg, and LG values are in mg/m³. The values in parenthesis are the confidence limits.

^b Duration of exposure not reported; value expressed in mg/kg.

^c This value was estimated by measurement of the total airborne droplets following a four-hour exposure. When only particles less than 10 microns were considered, the value decreased to 65 mg/m³.

NR = Not reported
i.p. = intraperitoneal
s.c. = subcutaneous

oral, rat

In conjunction with a teratogenicity study described in section VE.2, nonpregnant female rats were used to determine a maximum tolerated dose (MTD) for TBTO over a 14-day exposure period. Sprague-Dawley rats (n=5/dose group) were intubated for 14 consecutive days with TBTO in corn oil at concentrations of either 0, 18.75, 37.5, 75.0, or 150.0 mg/kg/day. A vehicle control was also used. This experiment was terminated after 12 days of dosing as a result of significant weight loss and mortality at all dose levels. The 14-day LD₅₀ was estimated to range from 18-37 mg TBTO/kg/day. Animals in dose groups that received 37 mg/kg or greater exhibited bloody mouth and nose, salivation, and inflammation of the oral and anal mucosa.

In a follow-up study, Sprague-Dawley rats (n=5/dose group) were intubated for 14 consecutive days with TBTO in corn oil at concentrations of either 0, 2, 4, 8, or 16 mg TBTO/kg/day. A vehicle control was also used. No dosage dependent mortality was observed. The greatest body weight loss (7.6% decrease compared to controls) was seen following the second dose of 16 mg/kg TBTO. Based on body weight data, the MTD of TBTO for nonpregnant rats was estimated to be approximately 16 mg/kg/day [Crofton *et al.*, 1989].

oral, rat

In conjunction with the study referred to above and the teratogenicity study described in section VE.2, Long-Evans rat pups were used to evaluate the acute oral toxicity of a single postnatal dose of TBTO. Groups of 10 dams, which had not been previously exposed to TBTO, were allowed to litter; and on postnatal day (PND) 1, pups were randomized and 4 males and 4 females were assigned to each dam. On PND 5, groups of two pups (1

male and 1 female) from each litter were administered a single oral dose of TBTO in corn oil at concentrations of 0, 40, 50, or 60 mg TBTO/kg. The control group was administered an equivalent volume (10 ml/g of body weight) of corn oil. Pups were examined for signs of toxicity and for mortality daily until PND 64. Also, each pup was weighed on PND 5, 10, 15, 20, 30, 48, and 64. Motor activity of individual rats was monitored in figure-8 mazes on PND 13-21, 47, and 62; and the acoustic startle response was tested on PND 22, 47, and 62. On PND 64, rats were decapitated and their brains were removed. Wet weights of the whole brain, cerebellum, and hippocampus were recorded.

By PND 5, postnatal exposure of rat pups to 50 or 60 mg TBTO/kg resulted in a mortality of 32%. A single postnatal dose of TBTO also caused a decrease in body weight. By PND 10, the mean body weight in all dose groups was decreased by 25% compared to controls. These weights remained decreased through PND 30. By PND 62, however, body weight recovered for the 40 and 50 mg/kg dose groups, but was still decreased for the 60 mg/kg dose group. The effects of TBTO on the acoustic startle response were transient; on PND 22, there was a decrease in the amplitude of the response in all dose groups, but there were no significant differences on PND 47 and 62. Postnatal TBTO treatment had no effect on preweaning (prior to PND 21) or postweaning motor activity at any dosage. TBTO also reduced adult brain weight in pups following postnatal exposure. In the highest dose group (60 mg/kg), whole brain and cerebellum weights were significantly decreased ($P < 0.05$) compared to controls. The authors of this study concluded that postnatal exposure of rat pups to TBTO produces transient alterations in growth, viability, and postnatal behavior [Crofton *et al.*, 1989].

oral, rat

Male albino Holtzman rats were used to study the acute oral toxicity of TBTO when administered orally by stomach tube as a single dose. Groups of 7 rats were administered the test chemical as a 10% volume/volume aqueous suspension or a 10% solution in corn oil at final concentrations of 91, 117, 151, 172, 195, 251, 369, or 542 mg/kg. The animals were observed for seven days for mortality and signs of systemic toxicity. Animals that died during the study were necropsied at the time of death, and survivors were sacrificed (by cerebral concussion) and necropsied after the 7-day observation period.

From the mortality data, the LD₅₀ values for TBTO when administered as an aqueous suspension and in a corn oil solution were calculated to be 194 and 148 mg/kg, respectively. The gross signs of systemic toxicity exhibited by the two groups did not show any essential differences. In general, the animals showed preening, depression, squinting eyes, labored respiration, diarrhea, and ataxia for 4-5 days following dosing. Some animals in the higher dose groups exhibited debilitation throughout the observation period. The majority of deaths, for both groups of animals, occurred between the second and fourth days following dosing and were preceded by a bloody nasal discharge, bloating, and depressed reflexes. Gross autopsies performed on animals that died showed hyperemia of the lungs, irritation of the gastrointestinal tract and peritoneum, and congestion of the kidneys and adrenals (frequencies not reported). Gross autopsies of survivors revealed mottled and granular livers, and a thickening of the wall of the cardiac portion of the stomach (frequencies not reported). The authors concluded that TBTO is moderately hazardous and care should be taken to avoid accidental ingestion [Elsea and Paynter, 1958].

oral, rat

In a study that examined the effects of TBTO on the lymphoid and endocrine system (see sections VG.1 and VG.4), the acute oral toxicity of TBTO was also determined. Six groups of Male Sprague-Dawley rats were administered, via a stomach tube, a single dose of TBTO dissolved in olive oil at concentrations ranging from 89-336 mg/kg. Although the specific dose levels and numbers of animals used per dose level were not reported, it can be inferred from the results reported that one group was dosed with 100 mg/kg TBTO. "At least" 4 animals/group were examined at 3, 6, and 12 hours, and at 1, 2, 3, 4, 5, 6, 8, 10, 14, and 21 days.

The acute oral LD₅₀ was determined to be 197 mg/kg with 95% confidence limits of 137-273. At the six dose levels tested, the majority of deaths occurred between the first and third days. One day after a single dose of 100 mg/kg TBTO, the rats showed no apparent interest in food, remained in a hunched posture, had closed eyes, and a bristled coat. These signs of toxicity, however, disappeared by the fourth day. In addition, body weights in this dose group initially decreased following TBTO-exposure; the rats lost an average of 6 grams in 24 hours and more than 14 grams by the third day. They regained the weight between days 4 and 6 [Funahashi *et al.*, 1980].

oral, mouse

TBTO was tested for acute oral toxicity and repellency in deer and house mice of unspecified sex. For the first repellency test, 5 deer mice were offered 25 white wheat seeds treated with 2.0% (wt/wt) of TBTO for three days, followed by a 4 day observation period. The mice were also offered a less preferred food and water *ad libitum*. Mortality and the number of wheat seeds consumed were recorded daily. The percentage of seeds refused was determined and designated as the food reduction value (FR). The second repellency test (REP) was conducted using the same procedure, but in this test the food was offered to 10 house mice for a period of five days. The test results were summarized by the percentage of mice refusing to eat more than 50% of the 2.0%-treated wheat seeds. Acute oral toxicity was determined using deer mice and was derived from the FR value, the average weight of an individual wheat seed (50 mg), and the average weight of each individual test animal (200 mg). The calculated value (LD_{fr}) represents the amount of chemical ingested during the FR test which killed, or did not kill, more than 50% of the test mice.

The results of the tests show that in the first repellency test, the deer mice examined refused 84% of the seeds treated with 2.0% TBTO. In the second test, an REP value of 100 was obtained as 100% of the house mice refused to eat more than 50% of the treated seeds over the 5-day test period. Finally, the calculated LD_{fr} value was +200 mg/kg/day. The authors feel that these results are useful in predicting the potential for acute toxicity in wild mammals following exposure to TBTO. Also, the calculated LD_{fr} value, if used along with an acute lethal dose (ALD) and an LD₅₀ value, may be useful to obtain a rough estimate of the potential subacute toxicity of the test chemical [Schafer and Bowles, 1985].

inhalation, rat

The acute inhalation toxicity of TBTO (Bio-Met-TBTO) was investigated in Charles-River rats. Rats were exposed to concentrations of 100-140 g of the test chemical at room temperature, heated to 95-100°F, or to vapors of Bio-Met-TBTO heated to 250-400°F. Exposures were for 6 hours/day with a 14-day observation period. The abstract (original paper could not be obtained) was unclear regarding the effects of Bio-Met-TBTO at room temperature; two different observations were reported. The abstract states that rats treated with Bio-Met-TBTO at room temperature exhibited erythema and sneezing, followed by hypoactivity, dyspnea, ocular porphyrin, and slight cyanosis. Three rats died within 15 hours of exposure. The abstract also states that, at room temperature, Bio-Met-TBTO produced hypoactivity, erythema, dyspnea, salivation, nasal porphyrin, and nasal discharge. At 95-100°F, Bio-Met-TBTO produced similar signs of toxicity, with the addition of increased grooming of the head and nose areas. Escape behavior, excessive lacrimation, nasal discharge, and loose stools were also seen. At 250°F, these signs occurred earlier and were more marked. At this dosage, one animal died 3.5 hours after exposure, and rats that were not sacrificed died within 1 or 2 days. All rats exposed to Bio-Met-TBTO at 350°F died within 3 hours of exposure [Wazeter, 1967].

inhalation, rat

In acute inhalation studies, rats (unspecified strain, number, and sex) were exposed to an aerosol of TBTO (at unspecified concentrations) for 4 hours in "nose-only" chambers. These chambers minimize the possibility of dermal exposure and ingestion of the test compound. The LC₅₀ was estimated to be 77 mg/m³ by measurement of the total airborne droplets. This value decreased to 65 mg/m³ when only "inhalable" particles (diameters less than 10 mm) were considered. Examination of the animals revealed irritation of the contact surfaces, particularly edema of the lungs. In another study, rats were exposed for 7 hours to an atmosphere largely saturated with TBTO vapor. No deaths occurred and there were only minor clinical symptoms. The authors concluded that according to the guidelines of the Organization of Economical Cooperation and Development (O.E.C.D.) for testing of chemicals, TBTO can be regarded as harmless at a single exposure [Schweinfurth, 1985].

inhalation, mouse

The toxic effects caused by the inhalation of thermolysis products from TBTO were examined in male Swiss mice. Groups of 12 mice were exposed to aerosols of pure TBTO or a 50% mixture of TBTO in toluene, at ambient temperature or heated to 200-600°C⁸. Aerosols were delivered at a rate of 10 liters per minute. At low temperatures (ambient, 200°C, and 300°C), aerosols of pure TBTO and TBTO in toluene caused rapid death during an initial phase of irritation. Survival times ranged from 21-34 minutes. At ambient temperature, aerosols of pure TBTO and TBTO in toluene caused death in all 12 mice after only 22 and 21 minutes, respectively. The mortality of mice was less affected by the higher temperatures, with only 2/36 mice dying after exposure to pure

TBTO, and only 6/36 dying after an exposure to TBTO in toluene. These deaths occurred after one hour of exposure to the aerosols. Necropsies of animals (at time of death or after sacrifice) revealed that only tissues in the respiratory system showed significant lesions, and that the intensity of the lesions was greater in animals exposed to aerosols at lower temperatures. Abnormalities included diffuse congestion of the pulmonary blood vessels, inflammatory responses in the trachea and bronchi, mucous secretion in the bronchi and bronchioles, and distension and rupture of the alveoli. Mean concentrations of tin were determined in each organ and are presented in section VA.2. The authors of this study concluded that TBTO toxicity decreases as the temperature increases [Truhaut *et al.*, 1981].

inhalation, guinea pig

The toxic effects caused by the inhalation of thermolysis products from TBTO were examined in male Hartley guinea pigs. Groups of 6 guinea pigs were exposed to aerosols of pure TBTO or a 50% mixture of TBTO in toluene, at ambient temperature and heated to 200-600°C (see footnote 8). Aerosols were delivered at a rate of 10 liters per minute. At low temperatures (ambient, 200°C, and 300°C), aerosols of pure TBTO and TBTO in toluene caused rapid death during an initial phase of irritation. Survival times ranged from 9 (ambient temperature) to 10 minutes. At 400-600°C, an aerosol of pure TBTO caused death in almost all guinea pigs within 12 minutes. When TBTO was mixed with toluene, the aerosol was less deadly at the higher temperatures; 6/6 guinea pigs died after a 26-minute exposure at 400°C, one died after a one-hour exposure at 500°C, and none died after a one-hour exposure at 600°C. Necropsies of animals (at time of death or after sacrifice) revealed that only tissues in the respiratory system showed significant lesions, and that the intensity of the lesions was greater in animals exposed to aerosols at lower temperatures. Abnormalities included diffuse congestion of the pulmonary blood vessels, inflammatory responses in the trachea and bronchi, mucous secretion in the bronchi and bronchioles, and distension and rupture of the alveoli. Mean concentrations of tin were determined in each organ and are presented in section VA.2. The authors of this study concluded that the toxicity of TBTO decreases as the temperature increases [Truhaut *et al.*, 1981].

inhalation, guinea pig

Male and female Hartley albino guinea pigs (n=105) were exposed to aerosols of TBTO in olive oil at concentrations ranging from 0.1-1 mg TBTO/liter aerosol for one hour. A control group of males and females (n=12) were exposed to an oily aerosol that did not contain TBTO. All animals in the three highest dose groups (0.33, 0.4, and 1 mg/liter) died within one hour. Females were more resistant than males to TBTO; 12/15 females died after 47 minutes of exposure to 0.2 mg/liter, while 15/16 males died after 26 minutes of exposure to the same dose. Toxicity was characterized by ocular and nasal irritation followed by a short remission time. Death occurred after asphyxic convulsions. With doses less than 0.2 mg/liter, irritation occurred after one hour, but all the animals survived through another 7 days of observation. No lesions were observed upon histological examination of the brain, lung, trachea, liver, spleen, kidney, stomach, and testes. Mean concentrations of tin were determined in each organ and are presented in section VA.2. [Angers *et al.*, 1976].

dermal, rat

Male and female Wistar white rats were used to examine the toxic effects of TBTO, using two forms of the commercial product, Lastanox, applied as a single dose to the shaved backs of the animals. Lastanox 'T' is composed of 20% TBTO in lower alcohols, non-ionic surface active substances, and water. Lastanox 'P' is composed of 15% TBTO in the same solvent system as Lastanox 'T' with the addition of polyvalent chlorphenol. Both forms of the product were diluted to 0.25% and 0.5% in water, and 0.1 ml of the resulting solutions were applied, in drops, to the shaved backs of the test animals. The final doses of TBTO received by each animal in the test groups (5 males, 5 females/group) were as follows: 0.5% Lastanox 'T' = 0.95 mg/kg of body weight (group I); 0.5% Lastanox 'P' = 0.72 mg/kg (group II); 0.25% Lastanox 'T' = 0.42 mg/kg (group III); 0.25% Lastanox 'P' = 0.36 mg/kg (group IV). Three control groups (5 males, 5 females/group) received either 0.1 ml of the Lastanox 'T' or Lastanox 'P' solvent system, or water. Each animal was observed 4 times a day, for 35 days. On day 4, photographs of the animals were taken, and on day 7, 4 animals from each group were sacrificed and necropsied. The remaining animals were sacrificed and autopsied at the end of the study.

The first day after application, animals treated with both concentrations of Lastanox 'T' (0.25 and 0.5%) developed slight edema of the skin, erythema, and papules with inflamed borders. In the higher dose group, the edema and erythema were more pronounced and the papules were more numerous. By the second day, point-

like petechiae were observed in the animals treated with 0.5% Lastanox 'T', and the erythema seen in the 0.25% dose group was more pronounced. On day 3, petechiae were first observed in the 0.25% dose group, and the number of petechiae and hemorrhages were increased in the 0.5% dose group. These conditions did not change through days six and seven. After day seven, the hemorrhages in both Lastanox 'T' dose groups began to crust and the papules began to diminish, particularly in the 0.25% dose group. Also, some apical squamae were formed on the papules. The crusts and squamae were coming off up to day 10 in the 0.25% dose group and up to days 12-13 in the 0.5% dose group. All these changes disappeared by day 15 in the 0.25% dose group and by days 18-20 in the 0.5% dose group. The changes following the application of 0.25 and 0.5% Lastanox 'P' were similar to those in the corresponding groups treated with Lastanox 'T'. However, the erythema and edema were less pronounced, and the papules and hemorrhages were less numerous. Also, the damage caused by 0.25% and 0.5% Lastanox 'P' cleared by days 12-14 and 15-16, respectively.

Gross autopsy of the animals treated with Lastanox 'T' revealed hyperemia of the subcutaneous tissue under the exposed areas of skin. No other macroscopic changes were noted. Also, no macroscopic abnormalities were seen in animals treated with either dose of Lastanox 'P'. Histological examination of skin treated with 0.5% Lastanox 'T' revealed a few vacuolation foci in the cells of the stratum spinosum and stratum basale. Also, some of the papillae were edematous and unevenly infiltrated by lymphocytes, plasmocytes, and a few neutrophils. The endothelium of some capillaries was moderately hypertrophic. Histological observations in the 0.25% dose group were similar, only less pronounced. These microscopic changes were also seen in skin treated with Lastanox 'P'. In both the Lastanox 'P' dose groups, the corium displayed a greater number of regression segments and a greater number of mononuclear infiltrates invading the neighborhood of adnexa. In terms of vacuolar dystrophy, more cells were affected in the stratum spinosum than in the stratum basalis. Control animals showed no signs of skin damage, macroscopic changes, or microscopic abnormalities. The authors of this study concluded that the above effects were the result of exposure to TBTO [Pelik_n and Cerny, 1968].

dermal, rat

The dermal toxicity of TBTO was examined by spraying rats (unspecified strain, sex, and number) with a maximum dose of 300 mg/kg of TBTO as a 10% emulsion. The animals showed a marked apathy from a few hours up to four days after treatment. After 10 days of observation, autopsies performed on the animals revealed dry necrosis in the area of application, but no indication of systemic organ damage [Schweinfurth, 1985].

dermal, rabbit

To examine the acute dermal toxicity of TBTO, single graduated doses of the undiluted chemical (1.17, 2.51, 5.42, and 11.7 g/kg) were applied under a binder to the closely clipped abdominal skin of albino rabbits (n= 4/group). After a 24-hour exposure period, the binders and the unabsorbed material were removed. The animals were observed for seven days for mortality and signs of dermal irritation and systemic toxicity. Gross autopsies were performed on animals that died during the study at the time of death and on survivors after sacrifice (by air embolism) on day 7.

At dose levels of 1.17, 2.51, 5.42, and 11.7, mortality was 1/4, 1/4, 0/4, and 2/4 respectively. The LD₅₀ value calculated from the mortality data was approximately 11.7 g/kg (could not be statistically analyzed). Gross signs of systemic toxicity were observed in each dose group for 4-7 days following dosing. These signs included body weight loss, depression, labored respiration, weakness of the hind limbs, diarrhea, and unsteadiness (numbers not reported). The majority of deaths occurred on the fourth day following dermal application and were preceded by depressed reflexes, prostration, gasping, phonation, and mild clonic convulsions. A single application of TBTO produced moderate dermal irritation characterized by erythema, edema, atonia, blanching, and areas of brownish discoloration. These signs, except for erythema, persisted throughout the 7-day observation period.

Gross autopsies of animals that died during the study showed subcutaneous edema, congestion of the lungs, kidneys, and adrenals, and irritation of the gastrointestinal tract and peritoneum. Gross autopsies performed on survivors revealed subcutaneous edema, hyperemia of the lungs, excess quantities of clear fluid in the peritoneal cavity, irritation of the small intestine, and congestion of the kidneys. The authors concluded that TBTO is a primary skin irritant and can be absorbed through the skin [Elsea and Paynter, 1958].

ocular, rabbit

Male and female Albino rabbits were used to examine the toxic effects of TBTO when applied as a single dose to the eyes of the animals using two forms of the commercial product, Lastanox 'T' and Lastanox 'P' (see description of composition on p. 34). The rabbits were divided into 4 experimental groups (3 males, 3 females/group) and three control groups (2 males, 2 females/group). For test animals, 0.03 ml of Lastanox 'T' or Lastanox 'P' diluted to 10% or 1% in water, was applied, in drops, to the conjunctival sac of the left eye. The actual doses of TBTO (mg/kg body weight of rabbits) were: 10% Lastanox 'T' (group I) = 6.1 mg/kg; 10% Lastanox 'P' (group II) = 4.6 mg/kg; 1% Lastanox 'T' (group III) = 0.61 mg/kg; 1% Lastanox 'P' (group IV) = 0.46 mg/kg. The rabbits in the control groups received 0.03 ml of either the undiluted solvent components of Lastanox 'T' (group V) or Lastanox 'P' (group VI) without TBTO, or water (group VII). All rabbits were observed three times a day over a period of 100 days.

All animals in the four experimental groups were weak and showed hyperreflexia on the second to fifth day after dosage. These signs disappeared by day six. One to three minutes after application of the test chemical, rabbits in groups III and IV exhibited marked hyperemia of the bulbar and palpebral conjunctivae accompanied by violent watering, miosis, and distinct blepharospasm. Within 3 hours, these animals had erythema and mild edema of the eyelids, numerous large necroses, hemorrhages, early chemosis of the bulbar and palpebral conjunctivae, and a decrease in corneal transparency. Within 12 hours, the corneal transparency had decreased further, the aqueous humor was opalescent, and the iris was edematous and discolored. After 24 hours other abnormalities seen in the rabbits included edematous and erythematous eyelids, hemorrhages and necroses of the eyelids, a narrowing of the palpebral fissure, necrosis of the conjunctiva, and turbidity of the aqueous humor. Eschars and ulcerations formed on the eyelids and on the cornea within 2-5 days of dosing. Rabbits treated with 10% Lastanox 'T' or 'P' (groups I and II) showed similar abnormalities, but the changes were more numerous and severe. Control animals did not exhibit any deviations from the normal clinical picture.

One rabbit from group I and one rabbit from group II (sex unspecified) died on days 11 and 12, after exhibiting severe signs of eye irritation and extreme weakness. Post-mortem examinations of these animals revealed hyperemia of the brain, medulla oblongata, and all abdominal organs. Microscopic findings corroborate the clinical observations and included neutrophil infiltration of the cornea, an edematous sclera and endothelium, and necrosis of the skin adjoining the eye. With the exception of the spleen, which showed hyperplasia of the reticulo-endothelial cells, no histological changes were seen in any other organs [Pelikan, 1969].

⁸ Thermolysis of TBTO between 200 and 600°C gives saturated hydrocarbon and olefin gas, a condensate of tetrabutyltin and dibutyltin oxide, and a residue composed of stannous or stannic oxides.

C. Prechronic

1. Human Data

No data were found.

2. Animal Data

oral, rat

Fifty male and 50 female SPF-derived Wistar rats (Riv: TOX[M]) were used in a 4-week study to examine the toxicity of dietary TBTO and to establish dose and relevant toxicologic criteria for a long-term toxicity and carcinogenicity study. TBTO was mixed with olive oil, and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 dilution. Groups of 10 rats/sex were fed final dietary concentrations of 5, 20, 80, or 320 mg TBTO/kg feed. Control animals received feed containing only equivalent amounts of olive oil. Animals were weighed weekly, and feed and water consumption were estimated three times per week. In the fourth week, individual 16-hour urine samples were collected overnight. During this time, feed and water were not given to the animals. Urine volumes were calculated, and the samples were analyzed for protein and creatinine concentration, the presence of glucose, ketones, bilirubin, and blood, and for pH.

After 4 weeks of exposure, blood samples were taken from the orbital plexus of each animal and complete hematological analyses were performed (measurement of hemoglobin and hematocrit concentrations, blood cell

count, and red blood cell morphology). Immunological, biochemical, and endocrinological parameters were also examined in these samples and in samples taken after sacrifice, and are described in section VG.1, VG.2, and VG.3, respectively. Terminal body weight was determined and select organs (liver, heart, kidney, thymus, spleen, brain, and mesenteric lymph nodes) were grossly examined and weighed, and organ samples were processed for histological and immunocytochemical evaluation. Also, liver samples were homogenized for the determination of glycogen content. Finally, total tin content was determined in brain, liver, kidney, and perirenal adipose tissues; the results are reported in section VA.2.

Although all animals survived the feeding period, animals in the 320 mg/kg dose group appeared weak and emaciated from the first week to the end of the test period. These animals had roughened fur, hairless and pale skin, and discharge around the eyes and nose. By the fourth week, animals in the 20 and 80 mg/kg dose group also had roughened fur, animals in the 80 mg/kg dose group showed a decrease in activity, and those in the highest dose group exhibited ptosis or anophthalmia, and slight ataxia. In the first week of treatment, animals in the high dose group (320 mg/kg) exhibited a 50% decrease in feed and water consumption. These animals also had a significant loss ($P < 0.001$) in body weight: males and females lost an average of 16 and 13 grams, respectively. After this time, however, body weight loss in the high-dose group remained relatively constant: males and females lost an average of 21 and 13 grams, respectively, by week 4. After the first week, males in the 80 mg/kg dose group gained significantly less ($P < 0.05$) weight than controls, gaining a total of only 96 g compared to a control gain of 117 grams. Females in this dose group had weight gain comparable to controls. No significant differences were seen in weight gain in males or females from the two lower dose groups (5 and 20 mg/kg) at any time. In groups in which a significant decrease in body weight was present, a reduction in organ weights was also noted; the weight of the thymus, however, was also reduced at the next lower dose level. Relative organ weights ([Table 10](#)) showed an increase over control values in the 320 mg/kg dose group for most organs, with the exception of the thymus; the thymus decreased in males exposed to 20, 80, and 320 mg/kg, and in females exposed to 80 and 320 mg/kg.

Urinalyses conducted in the fourth week did not reveal any treatment-related effects. Analysis of blood samples showed that TBTO treatment resulted in a significant decrease in the hemoglobin concentrations and hematocrit values in males and females from the 320 mg/kg ($P < 0.001$) dose group. Hemoglobin concentrations were significantly decreased in males ($P < 0.01$) and females ($P < 0.05$) from the 80 mg/kg dose group, and hematocrit values were significantly decreased in females ($P < 0.05$), but not in males. Treatment with 320 mg/kg of TBTO significantly decreased the mean erythrocyte volume ($P < 0.05$ and 0.01 for males and females, respectively), while lower concentrations of TBTO had no effect. Treatment with 80 and 320 mg/kg of test chemical lowered the mean corpuscular volume ($P < 0.01$ and 0.001 for males and $P < 0.001$ and 0.01 for females, respectively), and the mean corpuscular hemoglobin mass ($P < 0.01$ and 0.001 for males and $P < 0.01$ and 0.05 for females, respectively). The mean corpuscular hemoglobin concentration remained unchanged. The leukocyte concentration was significantly decreased in the 80 mg/kg (males only, $P < 0.01$) and 320 mg/kg ($P < 0.001$) dose groups. Differential counts showed a marked decrease in lymphocyte concentrations ($P < 0.001$) and an increase in the concentrations of neutrophilic granulocytes ($P < 0.01$) in males and females of the 320 mg/kg dose group. The monocyte concentrations, however, were significantly increased in all but the lowest dose group for males only ($P < 0.05$). Finally, the concentration of eosinophils was significantly decreased in the 5 mg/kg (males only, $P < 0.05$), 80 mg/kg (males only, $P < 0.05$), and 320 mg/kg (males and females, $P < 0.01$ and 0.05 , respectively) dose groups.

Gross necropsy revealed that animals fed 320 mg/kg TBTO were undersized, emaciated, and showed atrophy of the thymus. A hemorrhagic aspect was noted in the mesenteric lymph nodes, which increased in incidence and extent in a dose-related manner in the 20, 80, and 320 mg/kg dose groups. The small intestine was slightly reddened in the high dose group, but this feature disappeared during exsanguination. Pale red foci were observed in the liver of three male rats in the 320 mg/kg dose group, and, except for the hemorrhagic mesenteric lymph nodes, no lesions were seen in the rats from the lower dose groups.

Histopathological examination of select organs revealed several treatment-related effects. Thymic atrophy, present in all animals in the high dose group, was caused by a lymphocyte depletion in the cortex. As a result of this cellular reduction, cortico-medullary junctions became indistinct and the density of ceroid/lipofuscin-loaded macrophages increased. No other changes were seen in thymus, except for slight cortex atrophy in two males from the 80 mg/kg dose group. Animals in the high dose group also exhibited diffuse atrophy of the white pulp of the spleen, affecting the follicles and the periarteriolar lymphocyte sheaths (PALS). In the 80 mg/kg dose group, one male and two females had atrophy of the spleen. Spleens obtained from all treatment groups showed some

depletion of iron (hemosiderin) pigment; and in all groups, spleens from male rats contained less iron than those from female rats. Extramedullary hematopoiesis was similar in control and treatment groups.

Immunocytochemical staining for T-lymphocytes in the spleen revealed the presence of these cells in the PALS and scattered within the red pulp. In the 320 mg/kg dose group, there was a "striking" decrease in the amount of staining in the PALS, which corresponded to the atrophy of the PALS seen in these animals histologically.

Slight atrophy of the mesenteric lymph nodes was seen in 2 males and 3 females from the 20 mg/kg dose group and 4 males and 2 females from the 80 mg/kg dose group. All rats in the 320 mg/kg dose group exhibited lymph node atrophy. Seven females and 9 males had moderate to marked deterioration. The size and cellularity of the medulla, the number and size of follicles, and the number of T-lymphocytes in the paracortex were also reduced in the lymph nodes of the high dose group; the germinal centers were inconspicuous. In the medullary sinuses of the lymph nodes, rosettes of erythrocytes were found around mononuclear cells and their occurrence increased with dose. In nearly half of the animals of the 5 mg/kg dose group (7 males, 2 females), a few to a moderate number of rosettes were observed, while the incidence increased to 100% in the 80 mg/kg dose group (15 with an abundant number, 5 with a few-moderate number). The 320 mg/kg dose group also had a high number of erythrocyte rosettes.

Marked (6 males, 10 females) or slight (3 males) atrophy of hepatocytes was seen in the centrilobular areas of the liver from animals in the 320 mg/kg dose group. Three males from this group exhibited multiple focal inflammatory processes, characterized by multifocal necrosis of hepatic parenchyma associated with mononuclear and polymorphonuclear infiltration, fibrosis, and intrahepatic bile duct hyperplasia. One other male and 1 female also showed slight intrahepatic bile duct hyperplasia. The affected areas in these animals were also characterized by dystrophic calcifications. In the lower dose groups, no hepatic lesions were found, except for slight atrophy of the hepatocytes in 3 animals from the 80 mg/kg dose group. No treatment-related lesions were observed in brain, kidney, or heart after 4 weeks of TBTO exposure.

The authors of this study concluded that the reduction in feed intake by animals in the 320 mg/kg dose group may be explained by the aversive taste or odor of the diet, and may be partly responsible for the initial weight loss in these animals. The clinical signs observed in the 320 mg/kg group may also be regarded as characteristic of emaciated animals. Also, since TBTO disturbed hemoglobin synthesis and caused microcytic anemia, a direct hemolytic action of TBTO can not be excluded. Krajnc *et al.*, reported that the hepatic lesions observed were located mainly around major portal tracts, were sharply demarcated, occurred at low incidence, and did not show lobular distribution. These observations suggest that direct hepatic TBTO-toxicity is not the likely cause of the lesions. The authors also concluded that a number of the parameters examined point towards a toxic effect of TBTO on the rat immune system (atrophy of the thymus and peripheral lymphoid organs, decrease in thymus weight, and decreases in lymphocyte populations). Subsequent studies (by these and other authors) that more closely examine immune function parameters are described in section VG.1. In addition, pronounced effects, as described in section VG.3, were found on the endocrine system [Krajnc *et al.*, 1984].

Table 10: Organ and Body Weights of Mice Following a 4-Week Exposure to Dietary TBTO^a

	Concnc		Dietary Concentrati							
			5 mg/kg		20 mg/kg		80 mg/kg		320 mg/kg	
	g	%	g	%	g	%	g	%	g	%
Male										
Body weight	245		240		241		226*		113***	
Heart	0.76	0.31	0.75	0.31	0.76	0.32	0.73	0.32	0.47***	0.43***
Brain	1.72	0.71	1.72	0.72	1.76	0.73	1.70	0.75	1.53***	1.37***
Liver	10.7	4.3	10.0	4.1	10.3	4.3	10.8	4.4	5.9***	4.7*
Kidneys	1.97	0.80	1.89	0.79	1.86	0.77	1.79*	0.80	1.11***	0.99***
Spleen	0.56	0.23	0.52	0.22	0.52	0.22	0.47**	0.21	0.38***	0.27*
Thymus	0.36	0.15	0.38	0.16	0.30*	0.13*	0.23***	0.18***	0.04***	0.04***
Mesenteric lymph nodes	1.0	0.04	0.10	0.04	0.09	0.04	0.08	0.04	0.04***	0.03
Female										
Body weight	162		162		167		161		100***	
Heart	0.60	0.37	0.59	0.37	0.58	0.35	0.57	0.35	0.48***	0.47***
Brain	1.64 (9)	1.00 (5)	1.65	1.01	1.62	0.97	1.61	1.00	1.47***	1.58***
Liver	6.3	3.9	6.0	3.7*	6.5	3.9	6.7	4.2*	5.0***	5.0***
Kidneys	1.58	0.97	1.42	0.87	1.43	0.85	1.40	0.87	1.03***	1.04***
Spleen	0.36	0.22	0.34	0.21	0.38	0.23	0.36	0.23	0.23***	0.25
Thymus	0.29	0.18	0.28	0.18	0.25	0.15	0.19***	0.13***	0.04***	0.04***
Mesenteric lymph nodes	0.10	0.06	0.09	0.05	0.07	0.04	0.08	0.05	0.03***	0.03**

(a) Animals were exposed for 4 weeks. Organ weights, given in grams and as a percentage of are means of 10 animals/group unless otherwise indicated in parentheses.

* P<0.05; ** P<0.01; *** P<0.001

reference: Krajnc *et al.*, 1984

The 4-week study described above was supplemented with 6-week studies that more closely examined TBTO's toxicity and its effects on the endocrine system. Tests concerning the endocrine system are described in section VG.4. Since no essential differences were found between males and females in the 4-week study, the 6-week experiments were performed with only male SPF-derived Wistar rats (Riv: TOX[M]). In one 6-week study, the animals were fed test and control diets (prepared as described above) containing 0 (vehicle control), 5, 20, or 80 mg TBTO/kg feed for a period of 6 weeks. Blood samples were obtained from each rat (10/dose group) from the orbital plexus after the 6-week feeding period. In addition to the hemocytometric parameters examined in the 4-week study, the concentrations of reticulocytes and thrombocytes were determined, and the isocitrate dehydrogenase (ICDH) activity and serum iron concentration were measured.

Another 6-week study was performed to determine whether the hepatic lesions observed in the 4-week study in the 320 mg/kg dose group had an infectious etiology, or whether they were due to changes in the common bile duct. In this experiment, rats (6/group) were fed dietary TBTO at 320 mg/kg feed for 6 weeks. In addition to a control group that was given, *ad libitum*, feed containing vehicle only (olive oil), another control group was fed a restricted diet based on the calculated feed consumption of the 320 mg/kg group in the 4-week study. After the test period, the pancreas, liver, and bile duct were collected from the control and test animals and evaluated histologically. To examine the possibility of infectious etiology, tissue suspensions were prepared from liver, kidney, spleen, and lung, and were tested in standard virus isolation procedures in secondary mouse embryo cells, baby hamster kidney, and mouse L-cell lines. Also, liver sections with macroscopic changes were examined microscopically for bacteria and for mouse hepatic virus.

After 6 weeks of TBTO exposure, the concentration of thrombocytes in the blood of test animals was unchanged. In the 20 and 80 mg/kg dose groups, there was only a slight (statistically insignificant) increase in the concentration of reticulocytes. In the 80 mg/kg dose group, the ICDH activity was significantly increased (P<0.05), while the serum iron concentration, and hemoglobin and hematocrit concentrations were significantly decreased (P<0.05 and 0.001, respectively). Hematocrit was also lower in the 20 mg/kg group compared to controls (P<0.05). Gross necropsy of animals exposed to 320 mg/TBTO/kg diet for 6 weeks revealed hepatic lesions in 2/12 animals. The lesions were confirmed microscopically and were associated with chronic ulcerative inflammation of the common bile duct. Adjacent exocrine pancreatic tissue showed only minimal involvement,

such as edema, slight atrophy of acini, and infiltration with mononuclear cells. Staining procedures for bacteria, and immunofluorescence for mouse hepatitis virus were negative in these lesions. Also, viruses were not isolated from suspensions of liver, kidney, spleen, and lung.

The disappearance of iron in the spleen seen in the 4-week study and the decrease in serum iron seen in this study point towards defects in iron uptake or an iron loss (e.g., by mild intestinal hemorrhage). The tendency toward an increased fraction of reticulocytes and the enhanced ICDH activity observed indicate the presence of immature erythrocytes. According to these results, the authors state that a direct hemolytic action of TBTO cannot be excluded. The authors also concluded that the liver lesions seen in this study and in the 4-week study were primarily caused by the toxicity of the bile duct system and not by intercurrent infections as a result of immunosuppression, although the role of virus infections cannot be completely excluded [Krajnc *et al.*, 1984].

oral, rat

Forty male albino Charles River rats were used to examine the toxic effects of dietary TBTO. Groups of 10 animals were fed, *ad libitum*, a diet containing 32, 100, or 320 ppm TBTO for a period of 30 days. Control animals were fed the basic commercial diet without the test chemical. Each week, individual body weights, mortality and food consumption were recorded, and the animals were observed for any changes in appearance and behavior. Animals that died during the study were necropsied at the time of death, and survivors were necropsied at the end of the 30-day feeding period following sacrifice by exsanguination.

Although mild respiratory infection was seen throughout the control and test groups, the incidence of infection appeared to be greater in the 320 ppm dose group (numbers not reported). All TBTO-treated animals exhibited growth suppression during the course of the study, with suppression increasing in severity as the levels of dietary TBTO increased; animals in the 320 ppm dose group actually exhibited weight loss. The food consumption of animals treated with 320 ppm TBTO was reduced to approximately 1/2 that of control animals for the full 30-day period. However, food consumption for the 32 and 100 ppm dose groups was comparable to that of the control group. All animals in the control group and in the 32 and 100 ppm dose groups survived the feeding period, while only 4/10 animals in the highest dose group (320 ppm) survived until day 30. Prior to death, the animals that succumbed exhibited weight loss, bloody discharge around the eyes and nose, rapid and labored respiration, bloating, and general signs of debilitation. Autopsies of these animals did not reveal any gross pathology except an almost complete lack of fat stores. Gross autopsies of survivors also did not reveal any gross pathology which could be attributed to the dietary ingestion of TBTO. The authors of this study concluded that TBTO is a moderately hazardous compound [Elsea and Paynter, 1958].

oral, rat

In a 4-week dose range-finding study, TBTO was administered to 5 male and 5 female juvenile rats (unspecified strain) in feed at concentrations of 4, 20, 100, or 500 ppm. After 4 weeks of exposure to 100 ppm TBTO, the rats exhibited a reduction in food consumption and weight gain (values not reported). Males in this dose group also had a reduction in absolute thymus weight. At the highest dose level (500 ppm), the authors reported high mortality, apathy, emaciation, reductions in thymus and lymph node weight, depletion of lymphocytes in lymphatic organs, and atrophy of thymus and lymph nodes. No substance-related effects were observed at the two lowest doses.

In a subsequent experiment, 10 rats/sex were administered TBTO via the feed for 13-14 weeks at 0 (negative control), 4, 20, and 100 ppm. In the 20 ppm dose group, male rats had slightly prolonged food coagulation times, and female rats showed a slight decrease in food consumption (without growth retardation). Animals in the 100 ppm dose group exhibited a decrease in food consumption and weight gain. Other effects seen in this dose group included the following: reduced thymus, lymph node, and thyroid gland weights; decreased adrenal weight (males only); increased serum alkaline phosphatase (males and females) and albumin concentration (females only); and decreased serum gamma-globulin levels (females only). No effects were observed in the control group, and no histological changes were seen in any group. The "no-effect" level in this study was 4 ppm [Schweinfurth, 1985].

oral, mouse

TBTO was tested in a study examining the effect of dietary triorganotin on the lymphatic tissues and blood composition of young and mature, male albino Swiss-Webster mice. In one part of the study, 15 young mice were

fed a diet containing TBTO for seven days, *ad libitum*. The test compound was dissolved in dichloromethane and added to the dry powder feed (1 ml dichloromethane/g of powder). The solvent was then evaporated off, leaving a final dietary level of TBTO of 260 μ equiv/kg diet. The test period was followed by another 7-day feeding period, in which the mice were given untreated food. Ten mice were sacrificed at day 7, following the test period, and the remaining five were sacrificed at day 14. Body weights were recorded daily and fresh tissue weights (brain, heart, liver, and spleen) were recorded at the time of sacrifice. Also, at days 2 and 4, assays of digestive enzymes were performed on all animals. For the second part of this study, groups of 4-10 mature mice were fed *ad libitum* on a comparable diet containing either 780 or 2340 mequiv TBTO/kg diet for up to 8 days. Body weights were recorded daily and blood compositions were determined on days 4 and 7. After sacrifice, brain fresh weight and dry weight, and spleen fresh weight were recorded for each animal. Also, adrenal epinephrine content, liver free-SH content, and digestive enzyme activity were determined. For both procedures, groups of 5-10 control mice were maintained on the original diet without the addition of TBTO.

Dietary TBTO, at 260 mequiv/kg, retarded both body and organ growth in young mice after 7 days of treatment. The mean (n=10) body weight of test animals was $72 \pm 2\%$ of the final mean weight recorded for control animals (20.1 ± 0.6 g). This represents an approximate mean weight gain of only 0.6 grams compared to an approximate mean gain of 4.4 grams in control animals. The reduction in heart and liver weights of young mice treated with TBTO was generally proportional to the reduction in body weight. After seven days, the mean heart weight was $82 \pm 3\%$ of the mean control weight (0.11 ± 0.01 g), and the mean liver weight was $71 \pm 3\%$ of the mean control weight (1.83 ± 0.09 g). The most pronounced effect of TBTO on organ weights was seen in the reduction of the spleen weight in treated mice. After 7 days, the mean weight was only $36 \pm 2\%$ of the mean weight of the spleens in control mice (0.15 ± 0.01 g). The brain was not significantly affected by TBTO treatment, with the mean weight after 7 days being $98 \pm 1\%$ of the control weight (0.49 ± 0.01 g). The body weights and organ weights of the young mice returned to normal after the animals were transferred to an untreated diet for 7 days.

Mature mice treated for 4 days with TBTO, at concentrations of 780 and 2340 mequiv/kg, exhibited reduction in body weight and spleen weight. The mean weight loss in mice (n = 4) treated with the lower dose of test chemical (780 mequiv/kg) was 2.7 g, compared to a 2.0 ± 0.4 gram gain in control animals. Test animals treated with the higher dose of TBTO (2340 mequiv/kg), had a mean weight loss of 6.2 grams. The mean spleen weights for mice treated with 780 and 2340 mequiv TBTO/kg diet were 86 and 63% of the mean spleen control weight, respectively. The test compound had no effect on the brain weight of mature mice at either dietary level. After four days of treatment, mature mice also exhibited a dose-dependent reduction in leukocyte count, which was associated with the reductions in spleen and body weights. The mean (n =4) leukocyte count in the lower dose group was 106% of the control value, while the count in the higher dose group was only 69% of the control. There was little change in the levels of hemoglobin and hematocrit, or in the erythrocyte count. After 3 days of treatment, dietary TBTO, at 780 mequiv/kg, had no significant effect on the adrenal epinephrine level in mature mice. No data were reported on the effects of TBTO on digestive enzyme activity or the liver nonprotein thiol content. The authors of this study concluded that these deleterious effects may have resulted directly from the absorption of toxic levels of TBTO from the gastrointestinal tract, or indirectly from malnutrition due to the unpalatability of the diet. In either case, the chemical did not have marked effects on the nervous system (no change in brain weight and no change in adrenal epinephrine content), but may have impaired the immune function as evidenced by decreased spleen weight and decreased leucocyte count [Ishaaya *et al.*, 1976].

inhalation, rat

In a subchronic inhalation study, 10 male and 10 female juvenile rats (unspecified strain) were exposed to vapors of TBTO at 0.03 and 0.16 mg/m³, or an aerosol of TBTO at 2.8 mg/m³ four hours/day for 4-5 weeks (21-24 exposures). Untreated animals served as controls. At the highest dose level (2.8 mg/m³), TBTO caused death in 5 males and 6 females. Animals in this group exhibited apathy, respiratory distress, decreased food consumption, and decreased weight gain (no data for these parameters were reported). Other effects noted in this dose group included decreases in alpha1-, total alpha-, and alpha-globulin. Histological evaluation revealed inflammatory reactions within the total respiratory tract and changes in the lymphatic organs (depletion of lymphocytes, atrophy of thymus and lymph nodes). No local or systemic toxic effects could be seen in the other dose groups, and 0.16 mg/m³ was considered the "no effect" level for rats [Schweinfurth, 1985].

dermal, guinea pig

The skin sensitization of TBTO was studied in guinea pigs of unspecified species and number using a modified Landsteiner technique. A series of ten injections, followed by a challenge injection, was carried out using a 0.1% volume/volume solution of TBTO in sesame oil. No data on controls were provided. The authors reported that although there appeared to be an increase in the severity of dermal response with continued injections, it was not possible to determine conclusively if sensitization had occurred due to the primary irritating effects of TBTO. They noted that in order to conclusively determine if TBTO is a skin sensitizer, it would be necessary to test the compound at concentrations less than 0.1% [Elsea and Paynter, 1958].

dermal, guinea pig

The skin-sensitization potential of TBTO was examined in 20 female Dunkin-Hartley guinea pigs using the Magnusson-Kligman method. Immunization consisted of an intradermal injection of 1% TBTO in olive oil, followed 24 hours later by a topical application of 5% TBTO. Challenge patch tests, conducted with 0.25% and 0.1% TBTO in olive oil, did not reveal any sensitization in the animals. No other results were reported [Poitou *et al.*, 1978].

dermal, rabbit

In a dermal toxicity test, emulsions of TBTO were applied to the skin of rabbits (unspecified strain, sex, and number) under occlusive conditions over a period of 28 days for 6 hours per day. Concentrations of 0.01-0.03% were rated as slightly to moderately irritating, 0.1% was rated as moderately irritating, and concentrations of 0.3-1.0% were found to be severely irritating [Schweinfurth, 1985].

D. Chronic/Carcinogenicity

1. Human Data

No data were found.

2. Animal Data

oral, rat

In a 106-week toxicity and carcinogenicity study, groups of 60 male and 60 female SPF-derived weanling Riv:TOX Wistar rats were fed 0 (negative control), 0.5, 5.0, or 50.0 mg TBTO/kg diet *ad libitum*. The test chemical was mixed with olive oil and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which the final experimental diets were prepared by a 1:10 mixture. An equivalent amount of olive oil was added to the control diet. Body weight and food and water consumption were monitored weekly (three measurements/week were used to calculate mean daily consumption) during the first 3 months of the study, and at 4-week intervals thereafter. For the first year, the rats were inspected daily for clinical abnormalities; during the second year, they were inspected twice daily Monday through Friday. Dead or moribund rats, as well as rats showing large tumors, were removed and autopsied. Rats that died during the first two weeks of the study were substituted with animals from reserve litters.

During weeks 13, 53, and 105, blood samples were taken from the retro-orbital plexus of 10 rats/sex/group, and a complete hematological evaluation was done on each sample. During weeks 14, 54, and 106, 10 rats/sex/group were placed in cages for 48 hours without access to food and water. Urine samples were taken from each animal during the last 16 hours, and the samples were analyzed for volume, protein and creatinine concentrations, and osmolality. A venous blood sample was also taken from each of these animals for the determination of serum creatinine. Immunological and biochemical parameters were also examined; the procedures and results for these experiments are described in section VG.1 and VG.3, respectively. After 1 or 2 years of TBTO exposure, 10 rats/sex/group were sacrificed by exsanguination and autopsied. The following organs were weighed: brain, heart, lungs, liver, spleen, kidneys, pituitary gland, thyroid, thymus (after 1 year only), pancreas, adrenals, ovaries, testes, and mesenteric and popliteal lymph nodes. Also, complete gross pathological and histological examinations were done on the entire rat. Neoplastic lesions were examined in the control and high-dose groups, and if differences were observed, the intermediate dose groups were also examined. The livers and kidneys from 5 rats/sex/group were sampled for the determination of tin content. These results are reported in section VA.2. Unless otherwise indicated, statistical significance was defined as $P < 0.05$.

During the first year, slightly increased body weights were seen in males from the lowest dose group (0.5 mg/kg); and between weeks 31 and 67, these increases were significantly different from controls. During the second year (from week 67), the body weight of males in the highest dose group (50 mg/kg) was significantly lower than controls. After week 81, females in this dose group also showed significantly lower body weights than controls. Feed consumption was increased in males from all treatment groups; in the 5 mg/kg dose group the increases were persistent and significant throughout the study, while in the other dose groups the increases were significant only between weeks 24 and 80. Feed consumption in females was affected in the 50 mg/kg dose group only; during the first 7 weeks, feed consumption significantly increased, but between weeks 20 and 60, consumption significantly decreased. Water consumption was significantly increased in males from the 5 mg/kg dose group during the entire study and in males from the 50 mg/kg dose group after week 24. TBTO treatment did not affect water consumption in females. Towards the end of the experiment, mortality increased significantly in the 50 mg/kg dose group compared to controls. For females, the increase was seen from week 96 onwards. At 106 weeks, the mortality rate in the males fed 50 mg/kg had reached 60%. By week 106, the mortality rate for the high-dosed females was approximately 46%.

Hematological changes were noted mainly at the high-dose level. Hemoglobin concentrations and hematocrit values were decreased in males after 12 months ($0.01 \leq 0.05$ and $0.001 \leq 0.01$, respectively), and in females after 24 months (not significant). Mean corpuscular volume and mean corpuscular hemoglobin decreased significantly (0.01 , $P < 0.05$) in males after 12 months. In females, the thrombocyte count was increased ($P < 0.001$) after 12 and 24 months. Lymphocytes decreased significantly in the high dose males ($0.001 \leq 0.01$ after 24 months, and in the females ($0.01 \leq 0.05$) after 12 (5 and 50 mg/kg) and 24 months (50 mg/kg). For males and females, increasing urine production with age was observed in the high dose group after 3 (males only), 12, and 24 months. The authors report that the decreases in creatine concentration observed in the 50 mg/kg dose group (both sexes) after 12 and 24 months, and in osmolality in the 50 mg/kg females after 24 months, point towards a decreased renal concentration capacity. No effects were found on protein concentration and creatine clearance.

Treatment related effects on organ weight after one year were also restricted to the high dose group. Both sexes in the 50 mg/kg dose group showed increases in absolute adrenal weight, and females exhibited non-significantly increased thyroid weight and significantly increased ovarian weight (P values and data not reported). After 2 years of TBTO-treatment, the liver and kidney weights of male and female rats were significantly increased in the 50 mg/kg dose group. Males in this dose group also showed a significant increase in heart and adrenal weight, while females also showed a significant decrease in thyroid weight. The mean pituitary weight of the high dose group was higher than controls, but not statistically significant. Differences between test and control organ weights were not observed for the following organs: brain, spleen, testes, prostate, ovaries, uterus, lungs, pancreas, and mesenteric lymph nodes. Data on organ weights are presented below in [Table 11](#).

Histopathological examination revealed treatment-related non-neoplastic changes in the liver, spleen, kidney, and thyroid. After one year of treatment, the following changes were seen: a decrease in the cell height of the follicular epithelium of the thyroid in all dose groups, with a reduced number of psammoma bodies in the 50 mg/kg dose group; a decrease in splenic iron content at 5 (females only) and 50 mg/kg; and vacuolated hepatocytes with slight bile-duct activation (characterized by hyperplasia, cellular hypertrophy, and minimal infiltration of mononuclear cells) at 50 mg/kg. After 2 years of exposure, only the thyroid changes were still present. In addition, at 2 years, the kidneys of males and females from the 50 mg/kg dose group showed a higher incidence and severity of vacuolation and pigmentation of the proximal tubular epithelium. The kidneys from high-dosed rats (sex not specified) also had a high incidence of nephrosis, which was characterized by degeneration of glomeruli and tubuli and accompanied by an advanced interstitial inflammatory reaction. Data and P values were not reported for the above non-neoplastic effects.

After 2 years of TBTO exposure, a significant number of neoplastic lesions were seen in the pituitary, adrenals, and parathyroid. The data for these tissues are presented below in [Table 12](#). In males and females from the 0.5 and 50 mg/kg dose groups, the incidence of benign tumors of the pituitary (mainly prolactinomas) was significantly elevated compared to controls ($P < 0.05$ in low dose group; $P < 0.001$ and 0.01 in males in females of high dose group, respectively). Several of these tumors caused severe central nervous system dysfunction and were considered fatal. In addition, the authors report that these tumors contributed significantly to the increased mortality in the 0.5 and 50 mg/kg dose groups. Treatment with 50 mg/kg of TBTO also resulted in a significant increase in the number of pheochromocytomas in the adrenal medullary (both sexes, $P < 0.001$), and in the incidence of parathyroid adenomas (males only, $P < 0.01$). Minimal signs of malignancy were observed in less than

20% of the pheochromocytomas; metastases were not seen. The incidence of adrenal cortical tumors, however, was significantly decreased in males from the 0.5 and 50 mg/kg dose groups ($P < 0.05$ and 0.01 , respectively). Although statistically insignificant, there was a low, non-dose-related incidence of pancreatic carcinoma. Two females from the 50 mg/kg group, and one female from the 0.5 mg/kg group had a large solid or multinodular tumor on the pancreas with widespread metastases. In all three cases, the tumors were fatal. The incidence of other tumors observed in test groups was comparable to control data.

The authors of this study concluded that a lifetime feeding of 50 mg/kg of TBTO induces toxicity in various organ systems. Many of the findings regarding mortality, body weight changes, and clinical signs were attributed by Wester *et al.*, to the increased incidence of pituitary tumors. An increase in some common tumors was found at this high dose, and is probably due to metabolic endocrine or immunological changes. Effects on the incidence of some of these tumors were also observed in the low-dose groups, but not at the intermediate dose. Because of the high spontaneous incidence of tumors in this strain of rats, the variable incidence in the treated groups, and the absence of a dose-effect relationship, the authors report that the significance of the increased incidence of tumors is questionable [Wester *et al.*, 1990a].

Table 11: Organ Weights (g) of Rats Treated with TBTO in Feed for 2 Years^a

Organ	Concentration in mg TBTO/kg							
	Contrc		0.5		5		50	
	Female	Male	Female	Male	Female	Male	Female	Male
heart	0.99	1.17	0.96	1.17	0.98	1.23	1.07	1.32**
liver	9.0	10.9	9.5	11.2	9.5	12.1	11.6**	14.8**
kidneys	2.23	2.90	2.41	2.88	2.47	3.15	2.96**	3.73**
adrenal	0.06	0.04	0.05	0.05	0.05	0.05	0.092	0.283*
thyroid	0.01	0.02	0.01	0.02	0.01	0.02	0.014*	0.024
pituitary	0.01	0.01	0.02	0.03	0.01	0.02	0.032	0.041

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; ***]

^aData have not been included for the following organs in which differences in org between control and test animals were not observed: brain, spleen, testes, prostrat uterus, lungs, pancreas, and mesenteric lymph nodes.

Table 12: Tumor Incidence in Rats Treated with TBTO in Feed for 2 Years^a

Organ ^b	Concentration in mg TBTO/kg							
	contrc		0.5		5		50	
	Female	Male	Female	Male	Female	Male	Female	Male
pituitary ^c	22 (3)	34 (5)	32 (5)	39* (11)	22 (4)	34 (5)	35** (1)	43*** (17)
adrenal cortex	8	13	7	5*	8	11	8	2**
adrenal medul	3	16	3	13	3	14	34***	33***
parathyroid ^d	0/54	0/39	0/44	2/50	1/40	1/51	1/44	6/43**

^a Data have not been included for the following tissues for which significant increases or decreases in tu formation were not observed: mammary gland, brain, oral cavity, pancreas, small intestine, liver, muscul system, skin, hemopoietic system, spleen, kidney, uterus, urinary bladder, ovary, and mesothelium.

^b Values for the pituitary, adrenal cortex, and adrenal medulla are for groups of 50 rats.

^c Values in brackets represent the number of tumors that were fatal.

^d The values for the parathyroid are expressed as number of rats with adenoma/number of parathyro

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

E. Reproductive Effects and Teratogenicity

1. Human Data

No data were found.

2. Animal Data

oral, rat

The teratogenic effects of TBTO were investigated using female Long-Evans rats. In two preliminary experiments, the maximum tolerated dose of TBTO in nonpregnant females was estimated to be approximately 16 mg/kg/day (see section V.B.3). Based on these results, groups of 18 pregnant rats were intubated for 14 consecutive days

(gestational days 6-20) with TBTO in corn oil at concentrations of 12 or 16 mg/kg/day. A control group received an equivalent volume of corn oil. Due to evidence of maternal toxicity (decreased maternal weight gain, and vaginal bleeding) seen in both dose groups, 12 and 16 mg/kg were determined to be above the MTD for pregnant rats. Consequently, a second prenatal exposure study was initiated. In this study, groups of 16 pregnant females were intubated with 0 (corn oil), 2.5, 5.0 or 10.0 mg TBTO/kg/day on days 6-20 of gestation. The MTD for pregnant dams was the lowest dosage which produced a greater than 10% decrease in maternal weight gain during the exposure period. In the second prenatal exposure study, maternal weight gain in the 10 mg/kg dose group was decreased by 20% from a mean vehicle control value of 95.1 ± 4.1 g to a mean test value of 76.3 ± 9.1 g. Besides one death in the high dose group, no other signs of maternal toxicity were observed in any dose group. Based on these results, the MTD of TBTO for pregnant rats was estimated to be 5-10 mg/kg/day.

Following prenatal exposure, dams from both experiments were allowed to give birth. On postnatal days (PND) 1 and 3, litters were counted and weighed, and pups were examined for external malformations. Females that had not given birth by the 24th day of gestation were killed and examined for uterine implantation. Postnatal evaluations were conducted on rats from the second prenatal exposure study only. For this study, pups were randomized and each dam was assigned 4 male and 4 female pups on PND 4. Because a limited number of pups were born to the dams in the 10 mg/kg group, pups in this group were randomized and assigned to 3 dams. Mortality and signs of toxicity were recorded daily, and the pups were weighed on PND 5, 10, 15, 20, 30, 47, 62, 84, and 110. To examine sexual maturation, male pups were examined on PND 21-25 for testes descent, and females were examined on PND 30-36 for vaginal opening. Motor activity of individual rats was monitored in figure-8 mazes daily on PND 13-21, 43, and 61. Also, the acoustic startle response was tested on PND 30, 64, and 78. On PND 110, the rats were decapitated and their brains were removed. Wet weights of the whole brain, cerebellum, and hippocampus were recorded.

In the first prenatal study, exposure to 12 or 16 mg TBTO/kg/day resulted in a reduction in litter size on PND 1 by approximately 73 and 96%, respectively ($P < 0.05$); pup viability continued to decline from PND 1 to PND 3. On PND 1, average pup weight in both dose groups was also decreased by approximately 45% compared to controls ($P < 0.05$). In this first study, pups from both dose groups were born with fetal malformations. On PND 1, 2/71 pups in the 12 mg/kg dose group were born with cleft palates; both animals were also born dead. No cleft palates were observed in the control group. In this same test group, 6/7 pups were born dead with placentas attached. In the higher dose group (16 mg/kg), only 1/8 dams littered, and only 5 pups were born alive with no malformations (P values not reported for teratogenic effects). In the second prenatal exposure study, signs of fetal toxicity were seen only the highest dose group (10 mg/kg). On PND 1 and 3, decreases were seen in litter size (50 and 63%, respectively), and in mean pup weight (68 and 66%, respectively) ($P < 0.05$). No malformations were observed in any dose group.

Postnatal evaluations of pups from this second study showed that prenatal treatment with 10 mg/kg of TBTO resulted in a 14% mortality rate by PND 21. Prenatal exposures also produced a decrease in body weight that was both dose- and age-dependent. On PND 5, body weight was significantly reduced ($P < 0.05$) in the 5 and 10 mg/kg dose groups compared to controls. By PND 10, however, only the 10 mg/kg dose group had decreased body weight. The weight of these animals remained reduced throughout PND 110. Male offspring, prenatally exposed to TBTO, did not show any significant differences in testes descent compared to controls. In females, prenatal exposure to TBTO caused a delay in the age of vaginal opening; on average, females in the 10 mg/kg dose group reached sexual maturation approximately 2 days later than controls ($P < 0.05$). Prewaning motor activity was decreased in all dose groups on PND 14, while postweaning motor activity was decreased on days 47 and 62 in the 10 mg/kg dose group only. No effect was seen on the animals' acoustic startle response following prenatal exposure. After sacrifice (PND 110), whole brain, cerebellar, and hippocampal weights were significantly reduced in the high dose group ($P < 0.05$).

These results indicate that the teratogenic and toxic effects of TBTO were persistent and significant at only high dosage levels. The authors of this study concluded that prenatal exposure to TBTO produced significant embryo/fetal toxicity in rats, at dosage levels which were maternally toxic. However, due to overt maternal toxicity, a nonspecific effect of TBTO can not be ruled out. In addition, the results of the MTD studies indicate that pregnant rats are more sensitive to the toxic and lethal effects of TBTO. The authors noted that this difference in toxicity may be due to the fact that the administered doses of TBTO were adjusted daily for maternal weight. As maternal weight increases due to increasing weight of the fetus, larger amounts of administered compound may effectively increase the maternal dose if placental transfer is limited [Crofton *et al.*, 1989].

oral. rat

In an unpublished two-generational reproduction study conducted by Biodynamics, the parental (F0) generation, which was comprised of 30 males and 30 females per dose group (unspecified strain), was administered dietary concentrations of TBTO of 0 (control), 0.5, 5.0, and 50 mg/kg. Dosing in this group was begun 10 weeks before the animals were mated and continued throughout breeding, gestation, and lactation. Thirty male and thirty female offspring per dose group (F1 generation) were mated to produce the F2 generation. For this mating, F1 generation males and females were exposed to TBTO (same concentrations as F0 generation) for 15 weeks prior to mating and again dosing was continued throughout mating, gestation, and lactation.

Preliminary data indicate that there was no compound-related mortality or body weight change in the F0 generation. After treatment with 50 mg/kg TBTO, pup weights were decreased in the F1 generation on days 7 and 14; and pup weights were decreased in the F2 generation on days 7, 14, and 21. Also, F1 male parents had reduced body weights throughout the pre-mating period, while F1 female parents showed body weight decreases only during the first 3 weeks. There were no effects on mating, pregnancy, fertility, litter size, or pup survival in either generation. The only other effect described in this report was a decrease in relative and absolute thymus weight in both sexes at 50 mg/kg TBTO [Biodynamics, 1989 as reported in WHO, 1990].

oral. mouse

The embryotoxicity of TBTO was studied using NMRI mice. On days 6-15 of gestation, groups of pregnant mice were orally administered TBTO dissolved in olive oil at concentrations of 1.2, 3.5, 5.8, 11.7, 23.4, or 35 mg/kg body weight; 35 mg/kg was previously determined in a 10-day acute toxicity test to be the highest dose to give minimal maternal mortality (LD₁₀). Under these experimental conditions, 11.7 was the lowest dose resulting in slight maternal toxicity (reduction in maternal weight). At doses up to 23.4 mg/kg, no changes were observed in the number of resorptions or in the percentage of living fetuses per litter. In the 35 mg/kg dose group, however, the resorption rate was 59% (compared to a control rate of 9.1%), one of the five litters was completely resorbed, and one of six pregnant animals died. Treatment with 1.2-11.7 mg TBTO/kg did not result in significant retardation of fetal growth. The average weight of fetuses in the 23.4 and 35 mg/kg dose groups was reduced by approximately 8% and 20%, respectively, compared to the mean control weight.

Treatment with TBTO also caused a dose-dependent increase in the frequency of cleft palate. The incidence of this structural abnormality in the 11.7, 23.4, and 35 mg/kg dose groups was 7, 24, and 48%, respectively, compared to a 0.7% incidence in controls. However, 11/14 affected mice were clustered in 1/18 litters, and 15 litters contained no affected fetuses. Up to a dose of 5.8 mg/kg, TBTO did not statistically increase the frequency of cleft palate. The authors report that since 1.7 mg/kg was the lowest dose resulting in maternal toxicity, cleft palate occurring at higher doses may be a nonspecific toxic (maternal) effect and not a teratogenic effect of TBTO. The authors also noted that cleft palate occurs spontaneously in this strain of mice. The two highest doses of TBTO also increased the frequency of irregular ossification centers of sternbrae and the frequency of minor abnormalities, such as fusion of the bases of the os occipitalis. The authors pointed out that this strain of mice has a tendency to produce these particular abnormalities as a result of nonspecific effects on the mother. They concluded that the deformities seen at high doses were most likely due to nonspecific maternal toxicity and not to the specific teratogenic action of the compound. They report that the results indicate that doses of TBTO lower than 12 mg/kg administered on days 6-15 of gestation are not teratogenic, but that at higher dose levels, "indicators" of teratogenicity were found [Davis *et al.*, 1987].

oral. mouse

In addition to the study described above, pregnant female NMRI mice (unspecified number) were administered a single oral dose via stomach tube of either 30 or 110 mg TBTO/kg body weight (in olive oil) on day 10 of gestation. The embryos, livers, and kidneys of the animals were removed 24 and 48 hours after dosing, and the tissues were processed for light and electron microscopy. Morphological techniques showed no specific lesions in the embryos 24 and 48 hours after treatment. Also, no abnormalities were seen in the kidneys of the pregnant dams. The livers, however, were damaged. After 24 and 48 hours, there was a pronounced increase in small to large lipid droplets in hepatocytes, and the sinus endothelia contained numerous fat inclusions. In addition, the rough endoplasmic reticulum was disorganized; its cavities occurred singly and irregularly, and parallel packing had disappeared. Segregation of the nucleolus was also present, with chromatin mass concentrating at the periphery. No other cell organelles exhibited changes. According to the authors, a single high dose of TBTO

affects maternal, but not fetal, tissue [Davis *et al.*, 1987].

oral, mouse

To examine the embryotoxicity of TBTO, groups of 8 pregnant Swiss albino mice were given daily doses of the test chemical dissolved in semisynthetic vegetable oil at concentrations of 0 (vehicle control), 5, 20, or 40 mg/kg by gavage on days 6-15 of gestation. On gestational day 17, the dams were sacrificed and the following parameters were examined: total implantations, number of live and dead fetuses, number of resorptions, average fetus body weight, number of stunted forms (fetuses below 2/3 average body weight), and average placental weight. Live fetuses were removed and examined for gross abnormalities and body weight changes. To assess maternal toxicity, the brain, kidneys, liver, and spleen of the dams were examined *in situ* for gross abnormalities, and weighed for organ-body weight comparisons.

No TBTO-related deaths occurred among dams in any dose group. Dams in the 40 mg/kg dose group had a significant ($P < 0.001$) decrease in maternal body weight gain, from a mean control value of 50.7 ± 3.0 g to a mean test value of 39.9 ± 3.6 g. Other clinical signs of toxicity seen in these females included piloerection, lethargy, and hunched posture. No significant changes in body weight or behavior were seen in dams from the other two dose groups. Although no changes were seen in the brain, kidney, or liver weights of treated animals, there was a significant and dose-related decrease in spleen weight compared to controls ($P < 0.05$ at 5 mg/kg, and $P < 0.01$ at 20 and 40 mg/kg). No macroscopic abnormalities or lesions were observed in any dose group.

Treatment-related reproductive effects were observed in the 40 mg/kg dose group and included an increase in the number of resorptions (81 compared to a control value of 2) and a significant decrease ($P < 0.05$) in mean fetal weight (0.787 ± 0.10 g compared to a control value of 0.993 ± 0.14 g). Totally resorbed litters were observed in 5 dams from this group, 3 of which exhibited vaginal bleeding on gestational days 8-9. TBTO at 5 and 20 mg/kg did not have any effect on reproductive performance or fetal malformations. Exposure to TBTO at 5, 20, and 40 mg/kg caused a significant increase in placental weight compared to controls (+8.1, +18.1, and +24.0%, respectively), but did not cause any external abnormalities. Consequently, the fetal/placental weight ratio showed a significant and dose-related decrease in all treatment groups ($P < 0.05$ at 5 mg/kg, $P < 0.001$ at 20 mg/kg, and $P < 0.01$ at 40 mg/kg). The authors report that this dose-dependent increase in placental weight may suggest interference by TBTO with placental exchange. No other changes were seen in dams or fetuses from any treatment group. The authors of this study concluded that these results do not show whether TBTO has a direct action on fetuses or whether the embryotoxicity seen in this study reflects maternal toxicity [Baroncelli *et al.*, 1990].

oral, rabbit

The maternal toxicity, fetal toxicity, and teratogenic effects of TBTO were examined in New Zealand white rabbits. Groups of 20 pregnant female rabbits were administered a 0.5 ml oral dose of TBTO in corn oil by gavage, at concentrations of 0.2, 1.0, and 2.5 mg/kg body weight/day, on gestational days 6-18. An additional group, serving as the vehicle control, was given equivalent doses of corn oil.

All females, with the exception of 1 from the 1.0 mg/kg dose group, survived to the end of the experiment (gestational day 29). Females that aborted during the study (3, 1, 1, and 7 animals from the control, 0.2, 1.0, and 2.5 mg/kg/day dose groups, respectively) were sacrificed and examined the day of aborting. No TBTO-related clinical abnormalities were observed in the 0.2 and 1.0 mg/kg/day dose groups. However, females in the 2.5 mg/kg/day dose group exhibited significant mean body weight loss between gestational days 6-18 when compared to controls (P values not given in this report). Postmortem examinations of the mothers did not reveal any treatment-related abnormalities. The authors attributed the increased incidence of abortions seen in the high dose group to a secondary effect of maternal toxicity. There was also a slight, but statistically non-significant, decrease in mean fetal weight in the 2.5 mg/kg/day dose group. The other two doses of TBTO (0.2 and 1.0 mg/kg/day) had no effect on fetal growth or survival. In addition, there were no differences in the type or frequency of fetal malformations between the treatment and the control groups. The authors of this study concluded that TBTO was not teratogenic and the NOEL for maternal and fetal toxicity was 1 mg/kg/day [Nemac, 1987, as reported in WHO, 1990].

in vitro, mouse

TBTO was studied for its toxic potential on prenatal development by determining the effects of this substance on limb differentiation (mouse embryos) in organ culture. To mate the animals, five female NMRI mice were caged with one male for 2 hours. If vaginal plugs were detected, the 24-hour period following mating was called day 0 of the pregnancy. At day 11 or 12, the forelimb buds of the developing embryos were dissected and cultured for 6 days in a chemically defined culture medium containing 0.0, 0.03, 0.1, 0.3, or 1.0 mg TBTO/ml (50, 170, 500, 1700 nM, respectively). Each culture contained 15-20 explants per dose. In an experimental series, each concentration was evaluated in duplicate or triplicate, and each experimental series was repeated at least twice. After 6 days, the explants were fixed and processed for histological examination.

The results of these studies show that TBTO in concentrations as low as 0.1 mg/ml caused "drastic" interference with the differentiation of mouse forelimb buds from 12-day-old embryos. Explants treated with 0.1 mg/ml of the test material showed inhibited development of the phalanges and the scapula, when compared to the controls. At 0.3 mg/ml, TBTO caused "drastic" impairment of the differentiation of the paw skeleton, scapula, and in some cases, ulna and radius. When 12-day-old limb buds were cultured in the highest dose of test chemical (1.0 mg/ml), almost no morphogenetic differentiation occurred. In these cases, only the cartilage of the humerus and parts of ulna and radius were recognizable. When 11-day-old limb buds were cultured in TBTO, the effects were more pronounced. Impairment in differentiation (abnormal paw skeleton) was seen at the lowest concentration of test chemical (0.03 mg/ml). At 0.1 mg/ml, TBTO induced abnormal "drastic" abnormalities of the paw skeleton, fusion of the metacarpal anlagen, and abnormal development of the phalanges. Eleven-day limb buds cultured with 0.3 mg/ml of test chemical, exhibited almost complete inhibition of paw skeleton development, compression and bending of the humerus, and absence of scapula formation. At the highest dose level (1.0 mg/ml), 11-day limb buds were almost completely undeveloped, and only part of the humerus formed as cartilaginous anlage.

According to the authors, the types of abnormalities observed do not allow a clear-cut distinction between a teratogenic or a cytotoxic effect, and the interference with differentiation as a result of a general toxic effect. However, the authors concluded that these data indicate a high embryotoxic potential of TBTO, which is not observed *in vivo*, presumably due to the limited exposure of the embryos because of the placental barrier and/or maternal metabolism [Krowke *et al.*, 1986].

in vitro, mouse

TBTO was studied for its toxic potential on prenatal development by determining the effects of this substance on limb differentiation in organ culture. TBTO at concentrations of 0.001-0.1 mg/ml present during the first 3 days of culture caused malformations of the skeletal elements of the limb. The lowest concentration (0.001 mg/ml) caused fusion of the carpals and metacarpals. At 0.01 mg/ml, TBTO caused fusion of the anlage of the metacarpals and reduction in the size of the scapula. Treatment with 0.1 mg/ml resulted in an overall reduction in limb bud size. The scapula anlagen and hand skeleton were reduced to rudiments in this dose group. Immunohistological staining of treated limbs demonstrated that, with increasing concentrations of TBTO, collagen type II and chondroitin sulfate PG content diminished in the cartilage of the remaining bone anlage. Also, staining for myosin showed that the development of the muscle tissue was strongly inhibited by TBTO treatment [Barrach and Neubert, 1986].

F. Genetic Toxicology

1. Prokaryotic Data

Salmonella typhimurium

The genotoxicity of TBTO was tested in a preincubation modification of the *Salmonella*/microsome test in the absence of metabolic activation and in the presence of liver S-9 from Arochlor-induced male Sprague-Dawley rats and Syrian hamsters. The assay was conducted in *Salmonella* stains TA97, TA98, TA100, and TA1535 at doses of TBTO in 95% ethanol ranging from 0.01-20.0 mg/plate. TBTO was found to be nonmutagenic in all strains of *Salmonella* tested [Zeiger *et al.*, 1988].

Salmonella typhimurium

The genotoxicity of TBTO in *Salmonella typhimurium* was tested in a fluctuation assay, three standard plate incorporation assays, and a *Salmonella*/rat hepatocyte assay. The fluctuation assay was conducted in *Salmonella* strains TA98 and TA100 using 0.03-10 mg TBTO/ml. The first plate incorporation assay was conducted in *Salmonella* strains TA98 and TA100 with concentrations of TBTO ranging from 0.00012-1170 mg/ml. The second plate incorporation assay was conducted in strains TA97, TA98, TA100, TA1530, and TA1535 at 0.5-500 mg TBTO/plate. The third plate incorporation assay was conducted with a 60-minute preincubation in the same strains as the second assay using concentrations of TBTO of 0.2-20 mg/ml. The fluctuation assay and the first two plate incorporation assays were conducted without metabolic activation and in the presence of liver S9 from Arochlor-induced rats. The third plate incorporation assay was conducted only with metabolic activation. Finally, a hepatocyte-mediated assay was conducted in *Salmonella* strains TA97, TA98, TA100, TA1530, TA1535, and TA1538 in the presence of rat hepatocytes at concentrations of 1-30 mg/ml.

The results from the plate incorporation assays showed that TBTO was nonmutagenic in all strains tested in the presence and absence of rat liver S9. No mutagenic effects were seen in any *Salmonella* strain treated with TBTO in the presence of rat hepatocytes. In the fluctuation test, however, TBTO at concentrations of 0.1-3 mg/ml was mutagenic in strain TA100 in the presence of rat liver S9. According to the authors, the fact that there was no clear dose-effect relationship may be due to the toxicity of TBTO [Davis *et al.*, 1987].

Bacillus subtilis

The genotoxicity of TBTO was tested at 58 mg/plate in a *rec*-assay in *B. subtilis* strains H17 (*rec*+) and M45 (*rec*-) without metabolic activation. At 58 mg/plate, TBTO did not induce any DNA damage and was nonmutagenic [Davis *et al.*, 1987].

Klebsiella pneumoniae

The genotoxicity of TBTO was tested in a fluctuation test without metabolic activation in *K. pneumoniae* (*ur*- *pro*-). TBTO was dissolved in dimethyl sulfoxide and tested at concentrations between 11.7-1170 mg/ml (19.7-1970 mM). Although high concentrations of TBTO caused some growth inhibition, the chemical did not induce reverse mutations at any concentration tested [Davis *et al.*, 1987].

2. Eukaryotic Data

Schizosaccharomyces pombe, Saccharomyces cerevisiae

The potential of TBTO to produce forward mutations in *S. pombe* P1 and mitotic gene conversions in *S. cerevisiae* D4 was tested in the presence or absence of a mouse liver S9 activation system at concentrations of TBTO ranging from 0.003-0.50 mg/ml. In the test for forward mutations, some increase in mutation frequency was seen at the highest concentration in the absence of metabolic activation. However, there was no indication of a dose-response relationship, and the assay was considered negative. TBTO did not induce mitotic gene conversions in *S. cerevisiae* under any test condition [Davis *et al.*, 1987].

Chinese hamster cells

The ability of TBTO to produce gene mutations in V79 Chinese hamster cells was investigated in microsome- and cell-mediated assays with and without metabolic activation. The microsome-mediated assays were conducted in the presence or absence of a rat liver postmitochondrial fraction (S15 or S9); the cell-mediated assays were done in the presence or absence of hamster embryo cells, mice epidermal cells, or human epidermal cells. TBTO, at concentrations of 0.05-0.2 mM, did not induce gene mutations to 8-azaguanine or ouabain resistance. When tested at concentrations ranging from 1.97-4.15 mM (with metabolic activation) or 0.1-0.22 mM (without metabolic activation), TBTO did not induce 6-thioguanine resistant mutations in Chinese hamster cells.

In another assay, 0.02-0.2 mM of TBTO (in an unreported vehicle) did not inhibit the metabolic cooperation between V79 Chinese hamster cells (6-thioguanine-sensitive) and 6-thioguanine-resistant mutant cells in the absence of metabolic activation [Davis *et al.*, 1987].

Chinese hamster ovary (CHO) cells

The ability of TBTO to induce chromosomal aberrations and sister chromatid exchange was tested in CHO cells with or without rat liver S9 metabolic activation. TBTO was tested in dimethyl sulfoxide at concentrations ranging from 0.0008-8.4 mM. At the highest test concentration (8.4 mM) and in the presence of metabolic activation, TBTO induced a significant increase ($P < 0.05$) in the number of chromosomal aberrations (mostly chromosomal deletions) in CHO cells after 15 hours of treatment. Also, the number of endoreduplications and polyploid cells was significantly increased ($P < 0.05$) after a 24-hour sampling time. TBTO did not increase the frequency of sister chromatid exchange in CHO cells under any test condition [Davis *et al.*, 1987].

mouse lymphoma cells

The ability of TBTO to produce gene mutations was tested in L5178Y mouse lymphoma cells (TK+/-) without metabolic activation. TBTO was dissolved in ethanol and used at concentrations ranging from 0.02-0.15 mM. Under these test conditions, TBTO did not induce either BUdR- or 6-thioguanine-resistant mutants in mouse lymphoma cells during 2 hours of treatment [Davis *et al.*, 1987].

human lymphocytes

The clastogenic potential of TBTO was tested using human lymphocytes with and without rat liver S9 metabolic activation. In the presence of S9, TBTO was tested at concentrations up to 1.0 mg/ml; in the absence of S9, TBTO was used at concentrations up to 0.1 mg/ml. These maximum concentrations were associated with a marked reduction in mitotic index (58% and 57% for 1.0 and 0.1 mg/ml, respectively). No increases in chromosomal aberrations were seen at any dose level [Reimann and Lang, 1987, as reported in WHO, 1990].

Drosophila melanogaster

The genotoxicity of TBTO was tested in an X-linked recessive lethal assay using adult Berlin K male *D. melanogaster*. The tester males were either fed 0.37 mM of the test substance dissolved in a mixture of 8% Tween 80, 8% ethanol, and 5% sucrose; or injected with 0.37 and 0.74 mM TBTO prepared as an emulsion containing 7.5% cottonseed oil, 0.6% lecithin, 0.15% pluronic F-68, and 0.7% sodium chloride. Immediately after oral treatment, the males were mated to virgin females; after treatment by injection, they were allowed to recover for 24 hours. Neither dose of TBTO increased the number of sex-linked recessive lethal mutations [Davis *et al.*, 1987].

oral, mouse

The ability of TBTO to produce chromosomal damage *in vivo* was studied in a micronucleus test using NMRI mice. Groups (numbers not given in this report) of mice were given 4 oral doses by gavage of TBTO in arachis oil at concentrations of 31.25, 62.5, 125, or 250 mg/kg body weight. Animals were sacrificed 24, 48, and 72 hours after treatment; bone marrow cells were harvested from each animal and analyzed for micronuclei in polychromatic erythrocytes (PCE).

Since the highest dose level (250 mg/kg) caused marked mortality (16/36 animals), the results from this group were not analyzed. In the 125 mg/kg dose group, 4 mice died, but 5000 PCEs were analyzed from 5 male and 5 female mice at each harvest interval. Similar analyses were conducted for the two lower dose levels. No significant increases in micronuclei were seen at any dose level at any time [Reimann and Lang, 1987, as reported in WHO, 1990].

oral, mouse

The ability of TBTO to produce chromosomal damage *in vivo* was studied in a micronucleus test using BALB/c mice. Groups of 10 male and 10 female mice were given a single oral dose by gavage of 30 or 60 mg TBTO/kg body weight, as a solution in olive oil. A control group was administered an equivalent dosage of olive oil. Half of the animals from each group (5 males and 5 females) were sacrificed 30 and 48 hours after treatment; bone marrow cells were harvested from each animal and then analyzed for micronuclei.

A significant increase ($P < 0.001$) in the number of micronucleated polychromatic erythrocytes (PCEs) was seen after 48 hours in the males of the higher dose group (60 mg/kg). Females in this dose group also had an increase in micronucleated PCEs, but the number was not statistically different from the controls. When the results for males and females were combined, the number was still significantly higher than controls ($P < 0.001$). No differences were seen between control animals and animals treated with 30 mg TBTO/kg for 48 hours. After a 30-hour treatment, no effect was seen on the frequency of micronucleated cells in the bone marrow of either dose group [Davis *et al.*, 1987].

Because the results found by Davis *et al.* conflict with those of Reimann and Lang (see above), and because of the unusually high incidence of spontaneous micronuclei seen in the Davis *et al.* study, a re-evaluation of the slides from the Davis *et al.* study was done by Schering, Incorporated. The re-evaluation did not confirm the increase in micronuclei seen in the males from the 60 mg/kg dose group after 48 hours, but did reveal a significant increase (P value not given in this report) in micronuclei in female mice. This increase was believed to be biologically non-significant due to the high variability of the control data. Also, the re-analysis stated that interpretation of a study that uses a small number of PCEs (1000) is problematic, and that no conclusions can be drawn from the Davis *et al.* study [Schering, Inc., 1986, as reported in WHO, 1990].

oral, mouse

The ability of TBTO to produce mutagenic effects *in vivo* was studied in a micronucleus test using mice of unspecified strain and sex. The mice (unspecified number) were given a single oral dose of TBTO at concentrations ranging from 31-125 mg/kg. Twenty-four, forty-eight, or seventy-two hours after dosing, the mice were sacrificed, and smears of their bone marrow were screened for the presence of micronuclei in polychromatic erythrocytes. No indication of chromosomal breakage or impairment of the spindle apparatus was observed [Schweinfurth, 1985].

G. Other Toxicological Effects

1. Immunotoxicity

oral, rat

Male Sprague-Dawley rats were used to examine the effect of short- and long-term exposure to TBTO on rat lymphoid and endocrine organs. The procedures and results for the endocrine studies are reported in section VG.4. For the short-term test, a total of 90 animals received a single dose (100 mg/kg body weight) of TBTO dissolved in olive oil. Control animals received an equivalent volume (1 ml/kg) of olive oil. "At least 4 animals each" were examined at 3, 6, and 12 hours, and at 1, 2, 3, 4, 6, 8, 10, 14, and 21 days. At each time point, the thymus and spleen were weighed and examined histologically. For the long-term study, groups of 10 rats received daily doses of TBTO dissolved in olive oil at concentrations of 0 (vehicle control), 3, 6, or 12 mg/kg five days a week, for 13 or 26 weeks. After the test period, 5 animals from each group were weighed and sacrificed, and the spleen, lymph nodes, and thymus were removed, weighed, and examined histologically.

After receiving a single dose of TBTO, test animals showed a reduction in relative thymus and spleen weight (P values not reported). The greatest reduction in thymus weight occurred four days after dosing; the mean relative weight of the thymus was 66 ± 9 mg/100 g body weight in the test group compared to a mean control value of 273 ± 61 mg/100 g. The greatest reduction in spleen weight was seen after day 2, when the mean value in the test group was 210 ± 36 mg/100 g compared to a mean control value of 323 ± 29 mg/100 g. The spleen recovered from the weight loss by day 10, while the thymus recovery was still incomplete on day 21. Histological evaluation revealed pyknosis and karyorrhexis in the thymic cortex within a few hours after treatment. At 48 hours, macrophages and phagocytized cellular debris were observed in the thymus. The macrophages changed to foam cells, and reached a peak 4 days after dosing at the expense of native thymic cells. Regenerated thymocytes were present in the cortex 6 days after dosing. The first sign of injury in the spleen and lymph nodes was karyorrhexis in the germinal center after 24 hours. In the next 48 hours, lymphocytes were decreased in number around the postcapillary venules and in lymphoid follicles.

Following long-term exposure, animals in the highest dose group (12 mg/kg) showed a significant reduction ($P < 0.003$) in body weight. The average weights after 13 and 26 weeks respectively, were 310.8 ± 21.2 and 355.8

± 36.0 g, compared to respective control values of 423.8 ± 58.8 and 438.6 ± 33.0. After 26 weeks, the mean body weight of animals in the 6 mg/kg dose group (382.0 ± 42.3 g) was also significantly lower (0.003<P<0.01) than the mean control value. A significant (P<0.003) and dose-related reduction in relative thymus weight was seen following dosing for 13 and 26 weeks. In the rats given 0, 3, 6, and 12 mg TBTO/kg/day for 13 weeks, the relative thymus weights were 68.2 ± 6.4, 62.9 ± 9.6 (not significant), 34.0 ± 10.9, and 31.3 ± 6.8 mg/100 g body weight, respectively. After 26 weeks of dosing, the respective weights were 44.9 ± 3.3, 31.3 ± 6.1, 27.8 ± 4.2, and 24.8 ± 4.4 mg/100 g. Despite the considerable reductions in thymus weight, the only histological observation was a slight reduction in the width of the thymic cortex. A significant reduction (0.01<P<0.05) in relative spleen weight was also seen after 26 weeks of TBTO exposure in the 12 mg/kg dose group, and after 13 weeks of exposure in the 6 mg/kg dose group. The authors of this study concluded that long-term, oral administration of TBTO causes profound changes in the thymus, spleen, and lymph nodes in the male rat, while a single exposure produces only transient effects [Funahashi *et al.*, 1980].

oral, rat

In a 4-week toxicity study (see section VC.2), SPF-derived Wistar rats (Riv: TOX[M]) were used to examine the effects of dietary TBTO administration on rat immunoglobulin titers. Technical grade TBTO was mixed with olive oil, and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 dilution. Groups of 10 rats/sex were fed final dietary concentrations of 5, 20, 80, or 320 mg TBTO/kg feed. Control animals received feed containing equivalent amounts of olive oil. After 4 weeks of exposure, the rats were sacrificed, and blood samples were taken from the abdominal aorta for the estimation of serum IgG and IgM titers. IgG, as a percentage of the control value, was significantly reduced in males of the 80 mg/kg dose group (P<0.05) and males and females of the 320 mg/kg group (P<0.001). IgM was significantly increased at 80 and 320 mg/kg for males (P<0.01) and females (P<0.001). As described in section VC.2, thymus atrophy, reduced thyroid weight, lymphocyte depletion of spleen and lymph nodes, and lymphopenia were observed. These results, according to the authors, may suggest an impairment in the function of T-helper cells as a result of the immunotoxicity of TBTO [Krajnc *et al.*, 1984].

oral, rat

In a study conducted by Vos *et al.*, 1984, SPF-derived Riv:TOX Wistar weanling rats were used to examine the immunotoxic effects of short-term oral exposure to TBTO. To study the parameters of specific and nonspecific resistance, several *in vitro* and *in vivo* function tests (described below) were performed using 0 (negative control), 20, or 80 mg TBTO/kg diet. A dose of 320 mg/kg was also included in the test for serum IgM and IgG. The animals were fed the test and control diets *ad libitum* throughout the test period (minimum of 6 weeks) and during the function tests. The following parameters were evaluated (immune parameters 1-8 examine thymus-dependent immunity, and parameters 9-12 examine nonspecific resistance):

1) *Total serum IgM and IgG*

Groups of 8 rats were administered 0, 80, or 320 mg TBTO/kg diet for 6 weeks. Blood was taken on days 0, 3, 7, 14, 28, and 42, and the IgG and IgM concentrations were determined by ELISA. The results showed no effect on serum IgM and IgG levels during the first week of TBTO exposure. However, changes in these levels appeared on day 14 and were more pronounced after longer exposure. At day 14, serum IgM was moderately increased and serum IgG was decreased in the 320 mg/kg dose group. After 28 days of exposure, the mean IgM concentration in the 320 mg/kg dose group was significantly higher (P<0.05) than in the control group, and the mean IgG levels in the 80 and 320 mg/kg dose groups were significantly lower (P<0.05 and P<0.001, respectively). The mean IgM concentration was significantly increased (P<0.01) in the 80 mg/kg dose group only after 42 days of TBTO exposure. The effect of the 320 mg/kg dose was not evaluated at this time point due to mortality. Vos *et al.* report that these changes in serum titers indicate that TBTO alters the humoral responses upon natural stimulation, reflecting the summation of multiple antigenic stimuli on the B-cell system.

2) *Antibody responses to Sheep Red Blood Cells (SRBC), ovalbumin, tetanus toxoid, and Trichenella spiralis*

To examine the suppression of T-helper-cell function, the IgG and IgM responses to various antigens (SRBC, ovalbumin, tetanus toxoid, and *T. spiralis*) were examined following 6 weeks of TBTO exposure at 0, 20, or 80 mg/kg. Groups of 10 rats were immunized intraperitoneally with 0.5 ml of a 20% SRBC suspension, followed by a booster injection 15 days later. Using a hemagglutination assay, the primary and secondary antibody responses

were determined on days 10 and 20 after primary immunization, respectively. Separate groups of sera were treated with 24 mM 2-mercaptoethanol (ME) for 1 hour to determine the primary and secondary ME-resistant hemagglutination titers. In another study, groups of 9-10 animals were immunized either intravenously with 5 Lf tetanus toxoid plain, or subcutaneously with ovalbumin and 0.05 ml of complete H37Ra adjuvant containing killed *Mycobacterium tuberculosis*. Blood samples were collected from the animals on day 21 after primary immunization with tetanus toxoid, and on days 15, 21, and 28 after primary immunization with ovalbumin. Finally, groups of 10 and 6 animals were infected with 1000 *T. spiralis* larvae (see parameter 5), and sera were collected on days 21 and 42 after infection. The IgM and IgG titers against ovalbumin, tetanus toxoid, and *T. spiralis* were determined by ELISA.

The antibody titers against SRBC showed that the primary antibody response was the same in control and TBTO-exposed rats. The secondary response, however, was suppressed in the highest dose group (80 mg/kg), with the ME-resistant (presumably IgG) hemagglutination titer significantly lower ($P < 0.05$) in the test group than in the controls (sixfold reduction). The mean IgM and IgG titers to ovalbumin, and the IgM titer against *T. spiralis* were the same in the treated and control groups; and the titers to tetanus toxoid were marginally different from controls, but not statistically significant. The IgG titer against *T. spiralis* (parameter 4) was significantly different ($P < 0.05$) from controls in only the 20 mg/kg dose group, 21 days after immunization. Since an absence of an antibody response was previously seen in the athymic nude rat, Vos *et al.* state that the results indicate that TBTO exposure suppresses thymus-dependent antibody synthesis.

3) *Delayed-type hypersensitivity (DTH) reactions to ovalbumin and tuberculin*

After 6 weeks of exposure to 0, 20, or 80 mg/kg TBTO, groups of 9-10 rats were immunized with the ovalbumin solution described in parameter 2. The animals were intradermally challenged with 5 mg ovalbumin in 0.02 ml PBS injected into the central pinna of the right ear 3 weeks after immunization, and with 10 mg tuberculin in 0.1 ml PBS injected into the shaved right flank 4 weeks after immunization. As controls, the left pinna or the left flank was injected with 0.02 and 0.1 ml of PBS, respectively. The thickness of the earskin and flankskin was measured 24, 48, and 72 hours after challenge; the results were expressed as the thickness of challenged skin minus that of solvent-treated control skin. From this test, it was observed that TBTO treatment caused a dose-related suppression of the 24-, 48- and 72-hour DTH reactions to ovalbumin and tuberculin. Significant reductions occurred in the 20 and 80 mg/kg dose group, but suppression was more severe in the high-dose group. According to the authors, the suppression of DTH reactions indicates an impairment in cell-mediated immunity.

4) *Resistance to Trichinella spiralis infection*

In two experiments, groups of 10 (experiment 1) or 6 (experiment 2) animals were infected with 1000 *T. spiralis* larvae after a 6 (experiment 1) or 7 (experiment 2) week exposure to TBTO (0, 20, or 80 mg/kg). In the first study, the yield of muscle larvae in the carcass was determined by the digestion method 6 weeks after infection. Sera from these animals were collected on days 21 and 42 after infection, and IgE titers were determined using a passive cutaneous anaphylaxis (PCA) assay. In the second experiment, the expulsion of adult worms from the small intestine was measured on days 8, 10, 12, and 14 after infection with a sedimentation method. Also, to examine the inflammatory reaction around the muscle larvae, the tongues of the animals were processed for histological examination 14 days after infection.

TBTO exposure reduced, in a dose-related fashion, the resistance to *T. spiralis* infection, as shown by increased yield of muscle larvae with reduced inflammatory reaction around larva-containing muscle cells, impaired expulsion of adult worms from the small intestine, and suppression of the IgE response. Six weeks after infection with *T. spiralis*, the counts of muscle larvae in the 20 and 80 mg/kg dose groups showed a dose-related and significant increase over controls ($P < 0.01$ and $P < 0.001$, respectively). Eight days after infection, no difference was seen between worm expulsion in control and treatment groups; however, significantly higher intestinal worm counts were found after 10 days in the high dose group ($P < 0.05$), and after 12 and 14 days in both treatment groups ($P < 0.05-0.001$). Also, worm expulsion in the control group was nearly complete at day 12 (less than 1% of the value at day 8), while expulsion in the high-dose group was markedly impaired (62 and 33% at days 12 and 14, respectively, in comparison to day 8). IgE titers against *T. spiralis* were reduced in a dose-related fashion, with significant suppression occurring in the 80 mg/kg dose group 21 and 42 days after infection ($P < 0.001$ and $P < 0.05$, respectively) and in the 20 mg/kg dose group 21 days after infection ($P < 0.05$). Histological examination of the tongues of animals killed 14 days after infection revealed a moderate to strong reaction around the nurse

cells (larva-containing muscle cell) in the control group. The reaction, consisting of mononuclear cells and eosinophilic granulocytes, was slightly reduced in animals from the 20 mg/kg dose group. In the 80 mg/kg dose group, the inflammatory reaction was much less, consisting of decreased numbers of both cell types. The number of connective tissue mast cells did not differ between control and treatment groups.

Since increased counts of muscle larvae, reduced inflammatory reaction in parasitized musculature, retarded expulsion of adult worms, and suppressed serum IgE titers were also seen in the athymic nude rat in previous studies, the authors concluded that TBTO exposure suppressed the thymus-dependent immunity.

5) *Mitogenic responses of thymus and spleen cells*

After 6 weeks of exposure to TBTO (0, 20 or 80 mg/kg), the thymus and spleen were removed from groups of 5-6 and 6 animals, respectively. The parathymic lymph nodes were removed, cell suspensions were prepared from each organ, and the total number of viable nucleated cells per organ was determined. A total of 1×10^6 thymus cells or 5×10^5 spleen cells were cultured in triplicate. The mitogenic responses of thymus and spleen cells to concanavalin A (Con A), phytohemagglutinin (PHA), pokeweed mitogen (PWM), or lipopolysaccharide (LPS-spleen cells only) were examined by measuring the incorporation of tritiated thymidine into the DNA. The mitogenic responses per whole thymus or spleen were also calculated.

Exposure to TBTO at 80 mg/kg for 6 weeks significantly suppressed the incorporation of tritiated thymidine into DNA of thymocytes upon stimulation with the T-cell mitogens PHA ($P < 0.05$) and Con A ($P < 0.01$), and the T- and B-cell mitogen PWM ($P < 0.05$). At this concentration, TBTO also significantly decreased ($P < 0.05$) tritiated thymidine incorporation in unstimulated cultures. At 20 mg/kg, TBTO significantly reduced ($P < 0.05$) the thymocyte response to PHA and PWM. Since exposure to TBTO at 80 mg/kg significantly reduced ($P < 0.05$) the number of viable thymocytes, all derived values per whole thymus in this dose group were also suppressed (35-50% of control values).

Changes in the mitogenic responses of spleen cells were only observed in the highest dose group (80 mg/kg). Exposure to TBTO at this concentration significantly reduced tritiated thymidine incorporation upon stimulation with PHA ($P < 0.05$), and significantly increased tritiated thymidine incorporation in unstimulated cultures ($P < 0.01$) and in cultures stimulated with PWM and LPS ($P < 0.5$ and $P < 0.001$, respectively). As in thymus cells, exposure to TBTO at 80 mg/kg also significantly depressed ($P < 0.05$) the number of viable nucleated spleen cells in treatment groups. In calculating the response per whole spleen, DNA synthesis was significantly decreased ($P < 0.01$) after stimulation with PHA and Con A in the 80 mg/kg dose group compared to the control group. There were no differences seen in the responses (per whole spleen) to PWM and LPS.

6) *Cell surface marker analysis of splenic lymphocytes*

After 9 weeks of TBTO exposure (0, 20, or 80 mg/kg), the spleens were removed from groups of 5-6 animals, cell suspensions were made, and the number of viable nucleated cells was determined. Monoclonal antibodies were used to detect the T-, B-, T-helper, and T-suppressor cells of splenic lymphocytes. Results were expressed as percentages of lymphocytes positive for the different cell surface determinants, the T:B ratio, and the ratio between the subsets with the phenotype mediating T-helper and T-suppressor functions. Based on the total number of spleen cells and the relative counts, the numbers of different lymphocyte subpopulations were also calculated per whole spleen.

Cell surface analysis of splenic lymphocytes showed that a 9-week exposure to bis(tri-n-butyltin) oxide at 80 mg/kg significantly ($P < 0.01$) increased the percentage of B-cells and significantly ($P < 0.01$) suppressed the relative count of T-cells (nearly half the control value) resulting in a significantly reduced ($P < 0.01$) T:B ratio. No effects were observed in the 20 mg/kg dose group. Also, no changes were seen in the relative percentages of T-helper and T-suppressor cells, or on the T-helper:T-suppressor ratio in either dose group. Calculations per whole spleen showed that the total number of viable spleen cells was significantly ($P < 0.01$) reduced after a 9-week exposure to 80 mg/kg of the test chemical. The total number of B-cells in this dose group appeared unchanged, while the total numbers of T-, T-helper, and T-suppressor cells were significantly suppressed ($P < 0.01$) to 52-60% of the controls.

7) *Cell count and cell viability of thymus, spleen, and bone marrow*

Groups of 4 weanling rats were fed diets containing TBTO at 0, 80, and 320 mg/kg. After 3, 8, or 20 days of exposure, the animals were sacrificed and cell suspensions were made of thymus, spleen, and femoral bone marrow. For each suspension, nucleated cells were counted and cell viability was determined with a dye exclusion test. Also, the thymus morphology was examined histologically in male rats exposed to 320 mg/kg of the test chemical for 3 days.

Dietary exposure to 320 mg/kg for only 3 days strongly reduced the cell count in the thymus to 6% of the control value. The number of thymocytes was further decreased to approximately 2% and 1% of the control value after 8 and 20 days of TBTO exposure, respectively. In the 80 mg/kg dose group, cell counts were significantly reduced ($P < 0.01$) after 8 and 20 days of exposure. Thymocyte viability was also reduced in a dose-related fashion, with significant differences seen at day 8 for the 80 mg/kg dose group ($P < 0.05$), and at days 3, 8, and 20 for the 320 mg/kg dose group ($P < 0.01$ after 3 days, and $P < 0.001$ after 8 and 20 days). Histological observations of the thymus support these results. In treated animals, atrophy of the thymic cortex, with evidence of thymocyte destruction, was observed. Also, as a result of the loss of cortical thymocytes, the cortico-medullary junctions became less distinct.

Although less pronounced, exposure to TBTO at 320 mg/kg also significantly reduced the cell count in the spleen. After 3, 8, and 20 days of exposure, the numbers of spleen cells in treated animals were approximately 31, 32, and 20% of the control values, respectively. Treatment with TBTO at this concentration also significantly lowered spleen cell viability after 3, 8, and 20 days compared to controls ($P < 0.05$, $P < 0.001$, $P < 0.01$, respectively). At the 80 mg/kg dose level, however, the spleen cell count was significantly reduced ($P > 0.05$) at day 20 only, and no changes were observed in cell viability.

No significant changes were seen in the cell count and cell viability of the femoral bone marrow after 3 days of exposure to either dose level. However, the cell count was significantly reduced in the 320 mg/kg dose group after 8 and 20 days of TBTO exposure ($P < 0.05$, and $P < 0.01$, respectively), and after 20 days in the 80 mg/kg dose group ($P < 0.05$).

8) *In vitro cytotoxicity for thymus and bone marrow cells*

Thymocytes and bone marrow cells from two rats were collected and cultured in duplicate. The cultures were exposed to concentrations of TBTO in ethanol of 0.08, 0.4, 2.0 and 10.0 mg/ml, and cell viability was determined after 1.5, 6, and 24 hours of incubation using the trypan blue exclusion test. The results showed that TBTO was cytotoxic to both thymus and bone marrow cells at even the lowest concentration. Also, cell viability was reduced as early as 1.5 hours after exposure. P values were not reported.

9) *Clearance of Listeria monocytogenes*

After 7 (experiment 1) or 6 (experiment 2) weeks of exposure to TBTO at concentrations of 0, 20, or 80 mg/kg, groups of 5 animals were injected with 7×10^5 *L. monocytogenes* bacteria. For the determination of nonspecific phagocytosis and killing, the animals were sacrificed 1 and 2 days after injection, spleens were removed and homogenized, and the number of viable bacteria per spleen was determined. In experiment 1, the bacterial counts were significantly decreased ($P < 0.05$) in the high dose group the first day after infection in comparison to control counts. After the second day, however, the bacterial counts in the spleen were significantly increased in both dose groups ($P < 0.001$ for the 20 mg/kg dose group, and $P < 0.01$ for the 80 mg/kg dose group). In the second experiment, no differences were seen in bacterial counts the first day after infection in either dose group, and marked increases were seen the second day after infection in both dose groups. The increases were statistically significant, however, in only the 80 mg/kg dose group ($P < 0.001$).

10) *In vitro phagocytosis and killing of L. monocytogenes by adherent peritoneal and spleen cells*

Spleen and peritoneal cells were collected from groups of 5-6 rats after 6 and 10 weeks of exposure to 20 or 80 mg/kg TBTO, respectively. Cell suspensions were prepared, and the suspensions were cultured in duplicate for both the phagocytosis and the killing tests. The total number of viable cells harvested and the total number of adherent cells per dish were determined. The number of bacteria phagocytized per adherent cell and the number of bacteria killed per adherent cell were also calculated.

After 10 weeks, 80 mg/kg TBTO significantly reduced ($P<0.05$) the number of viable peritoneal cells. However, the number of adherent cells, and the amount of bacterial phagocytosis and killing (expressed on a cell for cell basis) were not altered by TBTO treatment. Six weeks of TBTO exposure reduced the total number of viable spleen cells in the 80 mg/kg dose group ($P<0.10$). The total number of adherent cells (1% of the cells in suspension) was also lower in the 80 mg/kg dose group compared to controls ($P<0.10$). In contrast to the study with peritoneal cells, TBTO feeding seemed to affect splenic bacterial phagocytosis and killing on a cell for cell basis; the number of bacteria phagocytized in the 80 mg/kg dose group was 70% of the control value ($P<0.10$), and the number of bacteria killed was 82% of the control value (P value not reported). According to the authors, the data indicated that the adherent cells from the spleen are less efficient in bacterial phagocytosis and killing than the population of adherent peritoneal cells. From this *in vitro* study, the authors concluded that the impaired *in vivo* splenic bacterial clearance is due to the reduction in both the numbers of adherent cells and bacterial digestion on a cell for cell basis.

11) *Natural cell-mediated cytotoxicity of spleen and peritoneal cells*

After 6 weeks of exposure to 20, or 80 mg/kg TBTO, spleen (obtained from groups of 6 or groups of 8 rats) and peritoneal cells (obtained from groups of 7 rats) were harvested and cultured for use as effector cells in a ^{51}Cr -release assay with a xenogeneic murine YAC lymphoma target cell line. The cytotoxicity of the peritoneal cells was determined in the total, the nonadherent (natural killer or NK cells), and the adherent (cytotoxic macrophages) cell populations. As the adherent fraction of the spleen shows very low cytotoxic activity, only the total spleen population was assessed. For spleen cells, two separate experiments were carried out: experiment one used spleen cells obtained from groups of six rats, and experiment 2 used cells obtained from groups of 8 rats. Specific release was calculated per culture of spleen and peritoneal cells, and per total number of viable nucleated spleen cells.

The spontaneous killing activity (NK activity) of spleen cells was high in the total cell population and depended on the effector:target (E:T) ratio. In both experiments using spleen cells, the activity was lower in the 80 mg/kg dose group, the difference being statistically significant in experiment 1 at an E:T ratio of 100. Since the number of viable spleen cells was significantly reduced ($P<0.05$) in the high dose group, the NK activity calculated for the whole spleen was significantly depressed in both experiments at both E:T ratios. The values ranged from 59-72% of the control activity. TBTO exposure did not affect the number of viable cells harvested from the peritoneal cavity, nor did it affect the cytotoxic activity of the total population and the nonadherent fraction at either E:T ratio. However, the cytotoxic activity of the adherent peritoneal cells against YAC lymphoma target cells was significantly suppressed ($P<0.05$) in the 20 and 80 mg/kg dose groups, at both E:T ratios.

12) *Susceptibility to endotoxin*

After 6 weeks of TBTO exposure, rats were intravenously injected with *E. coli* lipopolysaccharide (LPS) dissolved in 0.5 ml of PBS. Groups of four rats received 10 or 30 mg LPS and the mortality due to endotoxin shock was scored after 24 and 48 hours. All four animals from the 0, 20, and 80 mg/kg dose groups died from endotoxin shock within 24 hours after injection of 30 mg LPS. In the control and 20 mg/kg dose groups, 1/4 rats died after administration of 10 mg LPS. The authors concluded that TBTO did not render the animals more susceptible to endotoxin.

The authors of this study concluded that short term exposure of weanling rats to TBTO suppressed various parameters of the thymus-dependent immunity, apparently as a result of a direct cytotoxic effect on thymocytes. Also, parameters of the nonspecific resistance were affected as shown by suppressed activity of NK cells and macrophages [Vos *et al.*, 1984].

oral, rat

An additional investigation was carried out by Vos *et al.* to determine if the immune function suppression observed following short-term exposure to TBTO (described above) also occurred after long-term treatment. To examine the immunotoxic effects of long-term exposure to TBTO, function studies for specific and nonspecific resistance were performed on weaned and aged male SPF-derived Riv:TOX Wistar rats. The study with weanling rats is described first below. In this investigation, 3-4-week-old weanling rats were exposed to diets containing 0 (negative control), 0.5, 5.0, or 50 mg TBTO/kg for 4-6 months and 15-17 months prior to the function tests described below⁹ (TBTO exposure continued during the function tests). For each different test, each time point,

and each concentration, separate groups of animals were used, with the exception of the repeat study with *Trichinella spiralis* (see parameter 3 below). The immune parameters examined in the weanling rats and the results of these tests are as follows (immune parameters 1-5 examine thymus-dependent immunity, or specific resistance, and parameters 6-7 examine nonspecific resistance):

Weanling rats:

1) *IgM and IgG responses to sheep red blood cells*

After 16 months of TBTO exposure, groups of 9-10 rats were injected with 0.5 ml of a 20% sheep red blood cell (SRBC) suspension, and then given a booster injection 15 days later. Blood was collected on days 6 and 20 after the primary immunization, and the primary and secondary IgM and IgG responses were measured using ELISA. Only results for the primary IgM and the secondary IgG antibody titers to SRBC were reported; neither were significantly altered in weanling rats exposed to TBTO.

2) *IgM and IgG responses to ovalbumin/DTH reactions to ovalbumin and tuberculin*

After 6 and 15 months of exposure, groups of 9-12 rats were subcutaneously immunized with ovalbumin in PBS mixed with 0.05 ml of complete H37Ra adjuvant containing killed *Mycobacterium tuberculosis*. Blood was collected 22 days after immunization, and the IgM and IgG responses to ovalbumin were determined by ELISA. The 24-, 48-, and 72-hour delayed-type hypersensitivity (DTH) reactions to ovalbumin and tuberculin were determined 3 and 4 weeks after immunization as described above (Vos *et al.*, 1984). Neither the IgM and IgG responses to ovalbumin, nor the 24-, 48-, and 72-hour DTH reactions to ovalbumin and tuberculin were affected by exposure to TBTO for 6 or 15 months.

3) *Resistance to T. spiralis infection*

The resistance of weanling rats (9-12/group) to an infection of 1000 *T. spiralis* larvae was measured after 5.5 (two experiments) and 16.5 (one experiment) months of exposure to TBTO. The animals in the second 5.5 month group, prior to *T. spiralis* infection, were also immunized with ovalbumin in H37Ra adjuvant. For all three experiments, muscle larvae yield was measured by the digestion method, and serum IgG and IgM titers were determined by ELISA 42 days after infection. Twenty-two days after infection, serum IgE titers were measured by passive cutaneous anaphylaxis (PCA). In the 16.5-month experiment, the muscle larvae were also counted using a histological examination of the animal's tongue, and the inflammatory reaction around the larvae was evaluated.

The IgM and IgG responses to *T. spiralis* antigens were unaltered. However, IgE responses were significantly suppressed in the 5 and 50 mg/kg dose group compared to the controls ($P < 0.01$ and $P < 0.001$, respectively) following 5.5 and 16.5 months of exposure. Also, six weeks after infection with *T. spiralis*, there was a significant ($P < 0.001$) and dose-related increase in the number of muscle larvae counted in rats treated with 5 or 50 mg/kg of the test chemical. Histological examination of the tongue revealed a reaction consisting of mononuclear cells and eosinophilic granulocytes around individual muscle larvae, which were moderately reduced in animals from the 50 mg/kg dose group.

4) *Mitogenic responses of thymus and spleen cells*

Body, thymus, and spleen weights in weanling rats were recorded after 4.5 months of TBTO exposure. After 4.5 and 16 months of exposure, the thymus and spleen were removed, suspensions were made, and the number of viable nucleated cells was determined. The response of thymus and spleen cells to the mitogens, phytohemagglutinin (PHA), concanavalin A (con A), pokeweed mitogen (PWM), or *Escherichia coli* lipopolysaccharide was measured after 4.5 months (thymus and spleen cells) and 16 months (spleen cells only¹⁰) of test chemical exposure as described above (Vos *et al.*, 1984). TBTO exposure for 4.5 months was found to significantly reduce ($P < 0.05$) thymus weight in the 50 mg/kg dose group only. Body and spleen weights were not significantly reduced at any dose level. Also, TBTO treatment for 4.5 months did not alter the response of thymus cells to T-cell mitogens, and treatment for 4.5 and 16 months did not affect the response of spleen cells to T- and B-cell mitogens.

5) *Cell surface marker analysis of mesenteric lymph node cells*

After 6 and 18 months of exposure, cell suspensions were made of the mesenteric lymph nodes removed from 5-8 weanling rats, and the number of viable nucleated cells was determined (the 0.5 mg/kg dose group was not examined). T- and B-lymphocytes were quantified using monoclonal antibodies to rat cell surface determinants, and the results were expressed as percentages of lymphocytes positive for the different cell surface determinants, the total number of positive cells per lymph node, and the T:B ratio.

Using monoclonal antibodies, a dose-related shift was detected in T- and B-cell numbers in the mesenteric lymph nodes. Treatment for 6 and 18 months with 50 mg/kg of the test compound significantly reduced ($P < 0.05$) the relative count of T-lymphocytes, while treatment with 5 and 50 mg/kg significantly increased ($P < 0.01$) the percentage of B-lymphocytes. As a result, the T:B ratio was also significantly reduced ($P < 0.05$) in the 5 and 50 mg/kg dose groups. Based on the relative counts and the total number of nucleated cells per lymph node, the T- and B-lymphocyte populations were also calculated per whole node. As the number of nucleated cells was reduced in the high dose group, the total number of B-lymphocytes appeared unaltered, while T-cell numbers were approximately 50% of the control values after 6 and 18 months of exposure.

6) Clearance of *Listeria monocytogenes*

Following 5 and 17 months of exposure, the clearance of *L. monocytogenes* bacteria was examined in groups of 5 animals as described above (Vos *et al.*, 1984). At 50 mg/kg, TBTO exposure for 5 and 17 months reduced macrophage function, as indicated by a significant reduction ($P < 0.001$) in the splenic bacterial clearance. No changes were seen in the other dose groups.

7) Natural cell-mediated cytotoxicity of spleen and peritoneal cells

As described above (Vos *et al.*, 1984), the natural killer (NK) activity in spleen obtained from groups of 5-6 animals was determined following 4.5 and 16 months of TBTO exposure; the cytotoxicity of peritoneal cells was determined following 4.5 months of exposure. No changes were seen in NK activity after 4.5 months. However, 16 months of TBTO treatment suppressed NK activity in spleen cells in all dose groups at effector to target cell ratios of 50 and 100. TBTO had no effect on the number of viable nucleated spleen cells in either experiment, or on the spontaneous cytotoxicity of adherent and nonadherent peritoneal cells.

Aged rats:

In another study, 1-year-old male rats were fed the experimental diet, at the same concentrations of TBTO, for 5 months prior to function tests. Body, thymus, and spleen weights were determined following the exposure period. The immune parameters examined to investigate thymus dependent immunity and nonspecific resistance in aged rats exposed for 5 months included the following: the resistance to a *T. spiralis* infection; the clearance of *L. monocytogenes*; and the natural cell-mediated cytotoxicity of spleen cells. The procedures were similar to those described above for weanling rats, with one exception: two experiments were done to test the resistance to *T. spiralis*. In one, the expulsion of adult worms was examined by quantifying the recovery of worms from the small intestine 12 days after infection using a sedimentation method. In the second, the yield of muscle larvae was determined 42 days after infection using both the digestion method and the histological method.

After 5 months of treatment, TBTO, at 50 mg/kg, significantly reduced ($P < 0.01$) the thymus weight in 1-year-old rats from a mean value of 67 ± 28 mg (12 control rats) to a mean value of 41 ± 9 mg (12 experimental rats). Body and spleen weight were not affected by TBTO treatment. A 50 mg/kg dose of TBTO also significantly impaired ($P < 0.01$, mean value of 5-9 animals) worm expulsion. Using the digestion method, no differences were observed in the muscle larvae counts between control and test animals. However, histological examination showed a significant increase ($P < 0.001$) in larvae at the 50 mg/kg dose level, compared to controls. Treatment with TBTO at the 50 mg/kg level for 5 months significantly increased bacterial counts in the spleen 2 days after infection (data and P values not reported). The natural cell-mediated cytotoxicity was slightly decreased in one experiment at a TBTO concentration of 50 mg/kg. However, this parameter was not affected in a second experiment.

According to the authors, these results show that long-term exposure of young and aged rats to TBTO suppressed the host resistance to a bacterial (*L. monocytogenes*) and, especially, to a parasitic (*T. spiralis*) infection. The results were less pronounced in aged rats. Based on the results of this study, Vos *et al.* report that, with the aging of rats, the immune system becomes less sensitive to the effects of TBTO. Also, the authors

conclude that the data demonstrate the sensitivity of these challenge models since alterations were detected at dose levels at which no other effects were found [Vos *et al.*, 1990].

oral, rat

In a long-term toxicity and carcinogenicity study (see section VD.2), the effect of dietary TBTO on serum immunoglobulin concentrations was examined in SPF-derived Riv:TOX Wistar rats. The test chemical was mixed in olive oil and homogenized with feed to yield final dietary concentrations of 0 (negative control), 0.5, 5, and 50 mg TBTO/kg feed; animals were fed *ad libitum*. After 14, 55, and 106 weeks of exposure, a heparinized venous blood sample was drawn from 10 rats/sex/group, and serum IgG, IgM, and IgA levels were determined in each sample.

Serum IgM and IgG (mean values for 9-10 rats/group reported) increased in both control and treated rats over time. In the 50 mg/kg dose group, the IgM levels were significantly higher than those seen in the control group at all time points ($0.01 \leq 0.05$ for males and females tested after 55 weeks and females tested after 14 weeks; $0.001 \leq 0.01$ for males and females tested after 106 weeks and males tested after 14 weeks). A slight, but significant ($P < 0.05$) increase over controls was also seen at 5 mg/kg in males and females. In females from the 50 mg/kg dose group, IgG values were significantly lower than controls ($0.001 \leq 0.01$) after 14 and 55 weeks. In the high dose group, IgA values (mean values for 7-10 rats/group reported) were significantly higher than controls after 55 ($0.01 \leq 0.05$ for females and $0.001 \leq 0.01$ for males) and 106 weeks ($0.001 \leq 0.01$ for females and $P < 0.001$ for males). According to the authors, the increases in IgM and the decreases in IgG seen in the highest dose group may be attributed to the immunotoxicity of TBTO. Other findings from this study (reported in section VD.1), including lymphocytopenia, were consistent with these immunotoxic effects [Wester *et al.*, 1990a].

oral, rat

The immunotoxic effects of subacute oral dosing with TBTO were examined in adult and pre-weanling male Fisher rats. Five groups of adult rats (unspecified number) were dosed by oral gavage with peanut oil (negative control), or TBTO in peanut oil, at 1.25, 2.5, 5 or 10 mg/kg/day for 10 consecutive days. At each concentration, separate groups of rats were immunized with a single intravenous injection of 0.5 ml of 10% sheep red blood cells (SRBC) in sterile saline on the eighth day of this 10-day dosing schedule. Two additional groups of adults (unspecified number) were treated intermittently 3 times/week, for a total of 10 doses of TBTO in peanut oil, at 5, 10, or 20 mg/kg per dose. All doses were administered in volumes of 0.25 ml/100 g. As a positive control, the same number of adult rats were similarly dosed by oral gavage with 6 mg/kg per dose cyclophosphamide (CY).

In two additional experiments, an unspecified number of pre-weanling rats were dosed intermittently by oral gavage with peanut oil or TBTO in peanut oil in a volume of 5 ml/g, beginning at 3 days of age and then 3 times/week until 24 days of age, for a total of ten doses. In the first experiment, the pre-weanling rats were administered 0.0 (peanut oil negative control), 2.5, or 5 mg/kg of the test chemical, and in the second experiment, the pups received 0.0 or 10 mg/kg doses of TBTO in peanut oil.

For adults and pups, six rats in each group were sacrificed 48 hours after the last dosing, and the spleen, thymus, and inguinal, axillary, cervical, and mesenteric lymph nodes were removed. The body, spleen, and thymus weights were recorded. For all animals, the following immune function tests were performed:

1) The lymphoproliferative (LP) responses of the splenic lymphocytes were determined for the T-cell mitogens, phytohemagglutinin (PHA) and concanavalin A (con A); the B-cell mitogen, *Salmonella typhimurium* mitogen (STM); and the T- and B-cell mitogen, pokeweed mitogen (PWM).

2) Natural killer (NK) cell activity of splenocytes was determined in a 4-hour ^{51}Cr release assay using W/Fu-G1 rat lymphoma and YAC-1 mouse lymphoma target cells.

3) The mixed lymphocyte reaction (MLR) was evaluated using responder (dosed rat) and stimulator (Wistar/Furth rat) lymph node lymphocytes which were treated with mitomycin C. The results for the MLR assay are expressed as net counts per minute (CPM), subtracting the CPM of responder only cultures from the CPM of responder plus stimulator cultures.

4) Cytotoxic T lymphocyte (CTL) responses were examined *in vitro* with lymph node cells obtained from TBTO-dosed (responder) and Wistar/Furth (stimulator) rats. The CTL activity was measured as the percent specific ⁵¹Cr release in a 4-hour ⁵¹Cr release assay using W/Fu-G1 rat lymphoma target cells. CTL viable counts, for final E:T ratios, were determined using a modified pronase method.

5) The primary immune response was determined only in rats immunized with 10% sheep red blood cells (SRBC) in sterile saline using a direct plaque-forming cell (PFC) assay 4 days after the immunization.

Data for the immune function assays were analyzed using Dunnett's *t*-test, with a $P < 0.05$ was defined as significant.

Adult rats: Ten daily doses of TBTO administered to adult rats caused a dose-dependent and significant decrease in thymus weight, but no change in spleen or body weight. At 5 and 10 mg/kg, the test chemical significantly suppressed the LP responses to the T-cell mitogens, when compared to the peanut oil control. No changes were seen in the LP responses to PWM or STM, the NK cell activity or the MLR of test animals from any dose group. Adult rats dosed with CY for 10 consecutive days (positive control) displayed reduced thymus and spleen weights, decreased NK cell activity (against both target cells), and suppressed mitogen LP and MLR responses. As in the test groups, the body weights in the positive control group were unaffected. Immunization of rats with SRBCs on the eighth day of the 10-day dosing schedule resulted in an enhancement in the primary PFC response at doses of TBTO of 2.5 and 5 mg/kg. A similar immunization in the positive control group resulted in the suppression of the PFC response to SRBCs.

Intermittent exposure of adults to TBTO over a three-week period, resulted in a dose-related and significant reduction in thymus weights in comparison to controls. Unlike daily dosing of adults, intermittent dosing also caused a slight, but significant, reduction in spleen weight at dosages of 10 and 20 mg/kg. LP responses were significantly reduced at 5, 10, and 20 mg/kg for con A, at 10 and 20 mg/kg for PHA and STM, and at 20 mg/kg for MLR. NK cell activity and the *in vitro* generation of CTL from spleen cells obtained from treated rats were not affected by intermittent dosing with TBTO. Intermittent dosing with CY caused reductions in spleen and thymus weights, but did not cause suppression of NK cell activity. In fact, treatment with CY, according to this dosing schedule, enhanced NK cell activity against YAC-1 mouse lymphoma cells. The LP responses following intermittent dosing with CY were similar to those observed following daily dosing.

Weanling rats: Pre-weanling rat pups dosed intermittently over the first 3 weeks of life with 10 mg/kg TBTO in peanut oil showed a significant reduction in body weights when compared to controls, for up to 10 weeks after the last treatment. The body weights of pups dosed at 2.5 or 5 mg/kg did not differ from controls at any time after exposure to the test compound. In the 3-week-old rats, the thymus weights were significantly reduced in the 5 and 10 mg/kg dose group, while the spleen weights were significantly increased in the 10 mg/kg dose group. NK cell activity was significantly reduced at 5 and 10 mg/kg per dose against W/Fu G-1 rat lymphoma cells, and at 10 mg/kg per dose against YAC-1 mouse lymphoma cells. The LP responses of 3-week-old rats to Con A, PHA, and the T- and B-cell pokeweed mitogen (PWM), were significantly reduced in both the 5 and 10 mg/kg dose groups, while the responses to STM and MLR were significantly reduced only in the 10 mg/kg dose group.

The remaining rats in each group were examined at 1-3 week intervals, for up to 10 weeks after their last exposure, in order to determine if any of the observed alterations in the immune functions were reversible. All of the immune functions that were examined in adult rats dosed either intermittently or daily were no different from controls within 1-3 weeks after the last exposure to TBTO. However, pups dosed intermittently with 10 mg/kg per dose TBTO displayed suppressed LP responses to mitogens 7 weeks, but not 3 or 10 weeks, after treatment. These same rats had reduced body weights compared with controls ($P < 0.05$) 10 weeks after the last exposure to TBTO.

The doses of TBTO required to suppress the LP and MLR responses, to suppress the NK cell activity, and to reduce the body weight in pre-weanling pups, were lower than the doses needed to induce these effects in adults. In fact, none of the doses tested on adult rats altered the animals' body weight or NK cell activity. According to the authors, examinations of these immune parameters indicate that the developing rat is more sensitive to the immunosuppressive effects of TBTO than the mature rat. Because several of the observed immune effects resulted from exposure of pre-weanling rats to a dosage that also caused a reduction in body weight, the authors suggest that TBTO may also be acting as a developmental toxicant [Smialowicz *et al.*, 1989].

oral, rat

Adult male Fischer 344 rats were used to examine the immunotoxic effects of subacute oral exposure to TBTO. For the following immune function assays, groups of rats were dosed by oral gavage with TBTO in peanut oil for 10 consecutive days at 1.25-15 mg/kg/day in a volume of 2.5 ml/kg. Positive control groups were dosed with cyclophosphamide (CY) in water at 0.75-6 mg/kg/day in a volume of 2.5 ml/kg. Negative control groups (0 mg/kg) were dosed with an equal volume of peanut oil or water.

To determine the primary antibody response to a T-cell-dependent antigen, groups of 6 rats were immunized with an intravenous injection of 10% sheep red blood cells (SRBC) on day 9 of the 10-day dosing with the test chemical (0, 2.5, 5, 10, or 15 mg/kg), or CY (0, 0.75, 1.5, 3, or 6 mg/kg). To determine the primary antibody response to a T-cell independent antigen, groups of 6 rats were immunized with an intravenous injection of 40 mg/ml trinitrophenyl lipopolysaccharide (TNP-LPS) on day 9 of dosing with the test chemical (0, 1.25, 5, or 10 mg/kg), or CY (0, 0.75, 1.5, 3, and 6 mg/kg). The plaque-forming cell responses (PFC) to SRBC and TNP-LPS were determined by a standard direct PFC assay on day 4 following dosing. In separate groups of rats dosed with 0 or 10 mg/kg of test chemical, the PFC response was determined on days 3-6 following immunization with SRBCs.

To examine the delayed-type hypersensitivity (DTH) response to bovine serum albumin (BSA), a T-cell-dependent antigen, separate groups of 8 rats were immunized with a subcutaneous injection of 100 mg BSA in Freund's complete adjuvant on day 4 of dosing, or 1 day after dosing, with either TBTO (0 and 7.5 mg/kg) or CY (0 and 6 mg/kg). Seven days after immunization, the rats were challenged with an injection of 2% heat-aggregated BSA in the left hind footpad. As a control, the right hind footpad was injected with an equal volume of saline. Footpad swelling was measured at 24, 48, and 72 hours, and the DTH response was recorded as the difference between the thickness of the BSA-injected footpad and the saline-injected footpad. After the last measurement, the rats were bled and serum antibody titers were determined by ELISA. Finally, splenic lymphocyte populations were analyzed in animals dosed for 10 days with TBTO at 0 (peanut oil) or 10 mg/kg. The numbers of T-helper/ inducer (CD4), T-suppressor/cytotoxic (CD8), and B-lymphocytes were counted using immunofluorescence staining.

Ten-day oral dosing with TBTO at 5, 10, or 15 mg/kg resulted in a significant enhancement ($P<0.05$) of the PFC response to SRBCs, and the greatest enhancement was seen in the 10 mg/kg dose group. Enhancement of the PFC response was not associated with a shift in the kinetics of the primary antibody response to SRBCs. The peak response occurred on day 4 after immunization in both the negative control (peanut oil) and 10 mg/kg dose groups. The mean number of PFCs was always greater in groups of rats dosed with TBTO compared to rats dosed with peanut oil on days 3-6 post immunization, and significant enhancement ($P<0.05$) occurred on days 3 and 4. Conversely, rats dosed daily with CY for 10 days with 3 or 6 mg/kg had significantly ($P<0.05$) reduced PFC responses. No changes were observed in the PFC response to TNP-LPS in rats dosed with TBTO. However, rats dosed with 6 mg/kg of CY showed a significantly suppressed response to TNP-LPS ($P<0.05$). Exposure of rats to TBTO did not affect the DTH or antibody responses to BSA. Exposure to CY at 6 mg/kg, however, significantly suppressed ($P<0.05$) the DTH and antibody responses to BSA when rats were immunized either during or after dosing. Evaluation of splenic lymphocyte subsets revealed that rats dosed at 10 mg/kg/day with TBTO had a significantly reduced ($P<0.05$) CD8 population. The mean percentages of CD4 cells and B-lymphocytes were reduced, but not significantly.

From these results, the authors concluded that T-lymphocytes are a primary target for TBTO-induced immune alterations and that the enhancement of the PFC response to SRBC in TBTO-exposed rats may be mediated by alterations in the suppressor (CD8-positive) T-lymphocyte population [Smialowicz *et al.*, 1990].

oral, rat

Weanling male SPF-derived Wistar RIV:Tox rats were used to examine the effects of dietary TBTO on the natural killer (NK) activity in the rat lung. TBTO was dissolved in olive oil, and mixed in the diet at concentrations of 0 (negative control), 20, or 80 mg/kg. Groups of 6 animals were fed the test and control diets *ad libitum* for 6 weeks, and each animal was weighed weekly. After the 6-week feeding period, the animals were sacrificed and the weight of the thymus, spleen, mesenteric lymph nodes, liver, and kidneys were measured. For determination of NK activity, cytotoxic cells were isolated from rat lungs by enzymatic dispersion, and the suspensions were purified to separate the adherent and nonadherent (lymphoid) cell populations. The nonadherent cells were then

used as the effector cells in a ⁵¹Cr-release assay with a YAC lymphoma target cell line. The effector:target cell ratios were 25, 50, and 100. Cytotoxicity (NK activity) directed against the target cells was calculated per culture and per lung.

At 80 mg/kg, TBTO caused a decrease in body, spleen, and thymus weights compared to controls (93, 89, and 80% of the mean control value, respectively). Exposure to dietary TBTO decreased NK activity in the rat lung. At both 20 and 80 mg/kg, TBTO significantly depressed ($P < 0.05$ and 0.01 , respectively) NK activity as expressed per culture at an effector:target ratio of 100. The overall effect of TBTO per culture was also significantly different from controls ($P < 0.05$). The number of cells isolated per lung was significantly increased after exposure to 20 mg TBTO/kg feed. Consequently, the decrease in net NK activity per lung after TBTO exposure was unclear. Only the overall effect was significant ($P < 0.05$). The authors concluded that TBTO significantly affects the natural killer activity of lymphoid cells in the rat lung [Van Loveren *et al.*, 1990].

oral, rat

Male and female Sprague-Dawley rats were used to examine the immunotoxic effects of dietary TBTO and to determine the chemical's no observed effect level (NOEL). TBTO was mixed in appropriate amounts with 50 ml olive oil and then incorporated in 10 kg of diet. In four separate experiments, groups of 10 male and 10 female rats each were given 0.5, 2.0, 5.0, or 50.0 mg test chemical/kg diet. Control animals (10 males, 10 females) in each experiment were fed only the powdered diet mixed with olive oil. Clinical signs were recorded twice daily, and body weight and food and water consumption were noted weekly.

In the first experiment, blood samples were taken from the retro-orbital sinus of 10 males and 10 female rats per dose group after 28 days of treatment. The following blood parameters were measured: hemoglobin, mean corpuscular hemoglobin, packed cell volume, erythrocyte count, total and differential leukocyte counts, mean corpuscular volume, and platelet count. Also, the following clinical chemistry parameters were measured: blood urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. After 4 weeks of TBTO exposure, 10 males and 10 females per group were sacrificed and necropsied. Brain, liver, spleen, thymus, and iliolumbar and mesenteric lymph nodes were removed and weighed. Histological evaluations were performed for thymus and for iliolumbar and mesenteric lymph nodes. Quantitative evaluations were done for the thymus by measuring the thickness of the thymic cortex of five thymic lobules (1 section), and for the mesenteric lymph nodes by counting primary and secondary follicles (3 sections). Total cell count and cell viability of splenic and thymic cells were determined in five animals per group using the trypan blue exclusion test.

Humoral immune response was assessed using a direct plaque-forming cell (PFC) assay. After 31 days of treatment, 10 males and 10 females per dose group were immunized by an intravenous injection of sheep erythrocytes. On day 36, the animals were sacrificed, spleens were removed and homogenized, and hemolytic plaques were counted. Cell-mediated immune response was assessed using a delayed-type hypersensitivity (DTH) against bovine serum albumin (BSA). After 29 days of treatment, 10 males and 10 females per group were immunized with a subcutaneous injection into the base of the tail of BSA in Freund's complete adjuvant mixed in saline. On day 36, the footpad thickness was measured and the animals were given a booster injection of BSA into the same footpad. The footpad thickness was measured again 24 hours after the booster. Finally, 10 males and 10 females from each dose group were inoculated intravenously with a suspension of *Listeria monocytogenes* bacteria (strain EGD) after 31 days of TBTO exposure. On day 35, spleens were removed and homogenized, serial dilutions were plated and incubated for 48 hours at 37°C, and the number of bacteria per spleen was recorded.

No treatment-related clinical signs were observed during the test period. A slight and inconsistent weight loss was seen in males from the 50 mg/kg dose group, associated with a slight decrease in food and water consumption (P values not reported). No changes in hematology and biochemistry were noted in animals treated with TBTO. Changes in organ weights were noted only in males from the 50 mg/kg dose group. These animals showed a slight decrease in absolute liver weight and a slight increase in relative brain weight (P values not reported), which can be attributed to the lower body weight of these animals. Males in this dose group also showed a significant decrease ($P \leq 0.01$) in relative thymus weight (-30%) and in the total number of thymus cells compared to control animals. No macroscopic changes were noted at necropsy, and there were no significant changes in primary and secondary follicles of mesenteric lymph nodes. The thymic cortex thickness of males in the 50 mg/kg dose group, however, was slightly decreased compared to controls ($0.01 < P \leq 0.05$).

A significant increase ($P \leq 0.05$) in PFC was observed in males from the 2.0 and 50.0 mg/kg dose groups (+42.3 and +36.8%, respectively). No dose-related trends were present, however, and all values remained within the range of individual historical control data. Therefore, the authors concluded that this change was not compound-related. No significant changes were observed in the DTH reactions of treated animals compared to controls. The clearance of *L. monocytogenes* was moderately impaired only in the highest dose group (50 mg/kg), which showed an increase number of bacteria per spleen. According to the authors, these results confirm that the thymus is a target organ of TBTO immunotoxicity. The authors also concluded that under the conditions of these experiments, the dietary concentration of 5 mg/kg, equivalent to a dose of 0.5 mg/kg body weight, represents a no observed effect level (NOEL) for immunotoxicity in the Sprague-Dawley rat [Verdier *et al.*, 1991].

oral, mouse

In a prechronic study described in section VC.2, the effect of dietary TBTO on lymphatic tissues and blood composition was examined in young and mature, male albino Swiss-Webster mice. In one part of the study, 15 young mice were fed a diet containing 260 mequiv TBTO/kg feed for seven days, *ad libitum*. The test compound was dissolved in dichloromethane. The test period was followed by another 7-day feeding period, in which the mice were given untreated food. Ten mice were sacrificed at day 7 following the test period, and the remaining five were sacrificed at day 14. Fresh spleen weights were recorded at the time of sacrifice. For the second part of this study, groups of 4-10 mature mice were fed *ad libitum* on a comparable diet containing either 780 or 2340 mequiv TBTO/kg feed for up to 8 days. Blood compositions were determined on days 4 and 7, and spleen fresh weights were recorded for each animal after sacrifice. For both procedures, groups of 5-10 control mice were maintained on the original diet without the addition of TBTO.

Both mature and young mice treated with TBTO exhibited reductions in spleen weight. After 7 days of treatment, the mean spleen weight in young test mice was only $36 \pm 2\%$ of the mean weight of the spleens in control mice (0.15 ± 0.01 g). After 4 days, the mean spleen weights for mature mice treated with 780 or 2340 mequiv TBTO/kg feed were 86 and 63% of the mean spleen control weight, respectively. After four days of treatment mature mice also exhibited a dose-dependent reduction in leukocyte count. The mean ($n = 4$) leukocyte count in the lower dose group was 106% of the control value, while the count in the higher dose group was only 69% of the control. The authors of this study concluded that the chemical may have impaired the immune function as evidenced by decreased spleen weight and decreased leucocyte count [Ishaaya *et al.*, 1976].

oral, mouse

In an unpublished study by Biodynamics examining the effects of TBTO on immune function, groups of CD-1 mice (10/sex) were fed diets containing 0 (negative control), 4, 20, 80, or 200 mg TBTO/kg diet for 3 months. After the feeding period, males and females of the 200 mg/kg dose group showed significant increases ($P < 0.05$) in spleen weight; this effect was also seen in the 80 mg/kg dose group, but the increases were not statistically significant. There was a significant and dose-related increase ($P < 0.01$) in absolute liver weights among males and females from the 80 and 200 mg/kg dose groups, and in adrenal weight in males administered 200 mg/kg TBTO. Although not statistically significant, other effects included decreased thymus weight in the high dose group (males and females) and increased leukocyte counts in the 80 and 200 mg/kg dose groups (males and females). The authors also reported that histological changes were seen in the livers of males and females from the 80 and 200 mg/kg dose groups. The NOEL in this study was 20 mg TBTO/kg diet (approximately 4 mg/kg body weight/day) [Biodynamics, 1989, as reported in WHO, 1991].

oral, dog

In an unpublished study by Schering, Incorporated, the effects of TBTO on immune function were examined in groups of beagle dogs following oral administration. Five groups composed of 2 dogs/sex/group received doses of TBTO in arachis oil by gavage according to the following dosing schedule:

group 1: controls;

group 2: 0.1 mg TBTO/kg body weight/day for 5 weeks, followed by 0.2 mg/kg/day for 4-5 weeks, then 10 mg/kg/day for 8-9 weeks;

group 3: 0.5 mg/kg/day for 5 weeks, then 1 mg/kg/day for 13-14 weeks;

group 4: 2.5 mg/kg/day for 5 weeks, then 5 mg/kg/day for 13-14 weeks.

All males in groups 2 and 4 died because of mis-dosing to the lungs. At weeks 9 and 18, significant increases were seen in leukocyte and neutrophil counts in group 4. Leukocyte counts were also significantly increased ($P < 0.01$) in group 2 at week 13, and leukocyte, neutrophil, and lymphocyte counts were reduced, but not significantly, in group 2 at 18 weeks. At the end of the study, thymus weight was reduced in the two female survivors of group 2 (mean value of 0.9 ± 0.1 gram compared with a control value of 4.0 ± 0.6 grams); however, slight increases in thymus weights were seen in groups 3 and 4 (P values not given in this report). The two survivors in group two also had reduced iliac and mesenteric lymph node weights, and increased spleen weights (P values not given in this report). Relative liver weights increased in a dose-related manner (33.1, 41.7, 49.9, and 57.6 g/kg body weight in groups 1, 3, 4, and 2, respectively), and this increase was accompanied by cytoplasmic vacuolation of hepatocytes in group 2. Histological changes in lymphoid organs were also seen in group 2; the spleen (particularly the periarteriolar lymphocyte sheaths), lymph nodes, and thymus had reduced lymphocyte numbers [Schering, Inc., 1989, as reported in WHO, 1991].

oral, chicken

The immunosuppressive effects of TBTO on the lymphoid organs of Studler-Cornish tetralinear hybrid chickens were examined after subchronic oral dosing. Two groups of eight test animals were administered TBTO in a vegetable oil:water emulsion (1:3) at concentrations of 2 or 10 mg/kg body weight/day for ten days (2 and 10 ppm, respectively). A group of eight control animals were not administered the test compound. After 24 hours from the last administration and after 16 hours of starvation, all animals were sacrificed, and the thymus, bursa *Fabricii*, and left adrenal were removed and weighed. For each animal, the total protein, nucleic acids, and glycogen contents were determined in the thymus and bursa *Fabricii*, and the ascorbic acid content was determined in the adrenals. The data are presented as the mean values of 8 animals for each group.

Daily doses of 10 ppm TBTO induced thymus involution, as indicated by the significant decreases ($P < 0.05$) in thymus weight and in thymus RNA and DNA content of test animals. The mean thymus weight of chickens treated with 10 ppm of the test compound was 0.46 ± 0.12 g, compared to a mean control value of 0.84 ± 0.09 g. Animals in the 10 ppm dose group also showed a significant ($P < 0.05$) increase in total protein content of the thymus compared to controls. In the 2 ppm dose group, the only thymus modification observed was the significant ($P < 0.05$) increase in the glycogen content compared to controls.

The RNA content of the bursa *Fabricii* was significantly decreased ($P < 0.05$) in animals treated with both concentrations of TBTO. Treatment with 2 ppm of TBTO significantly increased the weight of the bursa *Fabricii*, from a mean weight of 0.53 ± 0.09 g (control) to a mean weight of 0.72 ± 0.03 g. No other modifications were seen in this organ in either dose group. Animals administered 2 ppm of TBTO also showed a significant increase ($P < 0.05$) in adrenal weight from a mean control weight of 23.37 ± 3.09 g to a mean weight of 30.75 ± 1.04 g. In the 10 ppm dose group, the content of ascorbic acid measured in the left adrenals was significantly higher ($P < 0.05$) than the content determined for control animals.

The authors of this study concluded that the higher dose of TBTO induced thymic depression in the chickens. Also, the differences noted in the effects of the test chemical on the thymus and the bursa *Fabricii* were most likely due to their differences in "reactivity," (the thymus had a higher "reactivity"). According to the authors, the adrenal modifications noticed did not reflect a stress situation, since the ascorbic acid content was not decreased and the weight of the gland was increased. These results confirm the direct action (not adrenal mediated) of TBTO on the lymphoid glands [Guta-Socaciu *et al.*, 1986].

inhalation, rat

In a study conducted by Schering, Incorporated, the effects of inhaled TBTO on immune function were studied in SPF Wistar rats. Groups of rats (10/sex) were exposed to 0 (negative control), 0.03, 0.165, or 2.8 mg TBTO/m³ for 4 hours/day, 5 days/week for 21-24 exposures. The two lowest doses (0.03 and 0.165 mg/m³) were administered as filtered vapors, while the highest dose (2.8 mg/m³) was administered as an aerosol (90% of the

particles were less than 5 mm in diameter).

After 2 and 4 weeks, TBTO exposure did not affect the lymphocyte and total leukocyte count of the animals. Five males and six females from the highest dose group that died during the study exhibited thymolysis and lymphocyte depletion of the thymus-dependent areas of the spleen and lymph nodes (P values not given in this report). These effects were not seen in the survivors. Three survivors, however, had an increased number of macrophages containing nuclear debris in the thymic cortex. Although absolute and relative organ (thymus, spleen, iliac lymph nodes) weights were not significantly changed in survivors, the weights were not recorded for animals that were sacrificed or died during the study. Histological signs of toxicity were limited to inflammatory reactions within the respiratory tract. Finally, food intake and body weight gain were significantly reduced ($P < 0.01$) in males from the 2.8 mg/m^3 dose group [Schering, Inc., 1983, as reported in WHO, 1991].

⁹ Although similar to the parameters described in the previous study (Vos *et al.*, 1984), the function tests conducted in this investigation used different time-points and vary in experimental design. For this reason, they have been described again using the same level of detail as the previous study.

¹⁰ The thymus was not investigated after 16 months because of strong physiological involution.

2. Neurotoxicity

oral, rat

To examine the effects of TBTO on the nervous system, 15 male Sprague-Dawley rats were administered 0.01 ml/kg of TBTO in olive oil via a stomach tube for 28 days. Another group of 15 rats, serving as the vehicle control, were administered an equivalent volume of olive oil for 28 days. After the dosing period, the nervous system of each animal was examined histologically. The results showed that nerve fibers from treated rats exhibited edema, demyelination, and axon destruction. These changes were observed in sensory nerve fibers, mainly on the pedunculus cerebellaris inferior, on the columna posterior and the cornu posterior of the spinal chord, and on peripheral nerve fibers. Preliminary studies done by the authors with ^{113}Sn -triphenyltin chloride suggest that high molecular tin compounds, such as TBTO, may be passed through the blood-brain barrier and may have a direct effect on the nervous system [Iwasaki *et al.*, 1976].

oral, rat

To examine the neurotoxic effects of TBTO, rats (unspecified strain, sex, and number) were given oral doses of 1 mg/kg-25 mg/kg TBTO in arachis oil over 10 days. The animals were examined daily for behavioral abnormalities, and light microscopic examinations were made of the brain and spinal cord after the dosing period. No evidence of morphological damage to the central nervous system was seen in the animals, even after treatment with large doses of TBTO (25 mg/kg) that produced toxic effects on other organs [Schweinfurth, 1985].

oral, rat

In a teratogenicity study described in section VE.2 and VB.2, the neurotoxic effects of prenatal and postnatal exposure to TBTO were examined in Long Evans rats. In one part of the study, groups of 16 pregnant females were intubated with 0 (vehicle control), 2.5, 5.0 or 10.0 mg TBTO/kg/day on days 6-20 of gestation, and then allowed to give birth. In the second part of the study, groups of 10 dams, which had not been previously exposed to TBTO, were allowed to litter; and on postnatal day (PND) 5, groups of two pups (1 male and 1 female) from each litter were administered a single oral dose of TBTO in corn oil at concentrations of 0 (vehicle control), 40, 50, or 60 mg TBTO/kg. Motor activity was monitored in figure-8 mazes daily on PND 13-21, 43, and 61 for the prenatal exposure group; and on PND 13-21, 47, and 62 for the postnatal exposure group. The acoustic startle response was tested in prenatally exposed rats on PND 30, 64, and 78; and in postnatally exposed rats on PND 22, 47, and 62. Rats from the prenatal and postnatal exposure groups were sacrificed on PND 110 and 64, respectively, and their brains were removed. Wet weights of the whole brain, cerebellum, and hippocampus were recorded for each animal.

Following prenatal exposure to TBTO, preweaning motor activity was significantly decreased in all dose groups

on PND 14, while postweaning motor activity was significantly decreased on days 47 and 62 in the 10 mg/kg dose group only (*P* values not reported). The acoustic startle response was not affected by prenatal exposure to TBTO at any dosage. Postnatal exposure to TBTO at any dosage did not affect preweaning or postweaning motor activity, but did produce transient effects on the acoustic startle response. On PND 22, there was a decrease in the amplitude of the response in all dose groups, but there were no significant differences on PND 47 and 62. Prenatal exposure to 10.0 mg/kg TBTO caused a significant reduction (*P*<0.05) in whole brain, cerebellum, and hippocampus weight. TBTO also reduced adult brain weight in pups following postnatal exposure. In the highest dose group (60 mg/kg) whole brain and cerebellum weights were significantly decreased (*P*<0.05) compared to controls. The authors of this study concluded that both prenatal and postnatal exposure to TBTO produced transient alterations in growth and postnatal behavior, but did not produce the magnitude and persistence of the neurotoxic effects reported previously (see O'Callaghan and Miller, 1988 above) [Crofton *et al.*, 1989].

inhalation, mouse

Male Swiss mice were exposed to an aerosol of TBTO in olive oil for either a single 1-hour period or seven 1-hour periods on successive days, using TBTO concentrations in air of 0, 42, 84, 170, or 240 ppm (0.05-0.4 mg/liter). Exploratory behavior was scored over a 5-minute period 2 hours after the single exposure was completed or 24 hours after the last of the seven exposure periods. The lower two exposure doses caused significant increases in exploratory behavior (17.01% and 5.04% for 42 and 84 ppm, respectively), while the higher two doses reduced exploratory behavior (-17.8% and -37.6% for 170 and 340 ppm, respectively). Corresponding changes (decreases) were seen in the levels of brain amines (dopamine, noradrenaline, 5-hydroxy-tryptamine) [Truhaut *et al.*, 1979].

intraperitoneal, rat

The effect of postnatal exposure to TBTO on the developing rat central nervous system was examined in Long-Evans rats. On postnatal day (PND) 5, groups of 8 pups were intraperitoneally injected with TBTO in corn oil at concentrations of 2.0, 3.0, 4.0, or 5.0 mg/kg. A control group of pups received an equivalent volume (10 μ l/g body weight) of corn oil. Animals were weighed and sacrificed by decapitation on PND 13, 22, or 60. Brain, thymus, and spleen were removed and weighed, and the brain was dissected. Each brain region (hippocampus, cerebellum, and forebrain) was weighed and homogenized. In each homogenate, four different neurotypic and gliotypic proteins were quantified using radioimmunoassay. The four proteins evaluated were: 1) p38, a synaptic vesicle-associated protein; 2) neurofilament (NF) 200, an intermediate filament protein of the neuronal cytoskeleton; 3) myelin basic protein (MBP), an oligodendroglia and myelin-sheath associated protein; and 4) glial fibrillary acidic protein (GFAP), an intermediate filament protein of astrocytes.

A single dose of TBTO on PND 5 caused dose-dependent decreases in body weight and in wet weights of the whole brain, cerebellum, hippocampus, and forebrain. At PND 13, the lowest dosages (2.0 and 3.0 mg/kg) significantly decreased (*P*<0.05) brain weight without affecting body weight, while a 4.0 mg/kg dose of TBTO significantly decreased (*P*<0.05) both brain and body weight compared to controls. Treatment with 5 mg TBTO/kg resulted in approximately 40% mortality by PND 3. The deficits in brain and body weight seen at PND 13 persisted until PND 60 in the 4.0 mg/kg dose group only. Also, at all time points, the most affected region of the brain was the cerebellum and the least affected region was the hippocampus. Thymus weight was significantly decreased (*P*<0.05) in the 4 mg/kg dose group at PND 13 and 22. Spleen weight was significantly decreased (*P*<0.05) in this same group on PND 22 and 60, and in the 2 mg/kg dose group on PND 13.

The decreases in brain and body weight were not associated with histological evidence of altered brain development, but they were accompanied by large dose- and region-dependent decreases in p38 and MBP. There were decreases in both total (per tissue) and concentration (per mg) levels of these proteins in the cerebellum and forebrain, but not in the hippocampus. At PND 22, values of p38 and MBP in the cerebellum were decreased as much as 33 and 32%, respectively. Although the exact numbers for each dose group were not reported, these decreases were seen at dose levels that did not affect brain, thymus, or body weight. At dosages that did not affect body weight, however, the effects of TBTO on protein levels did not persist through adulthood (PND 60). Reductions in NF-200 were smaller in magnitude and were limited to PND 13 (10% decrease) and 60

(12% decrease) in the forebrain, and to PND 60 (11% decrease) in the hippocampus. The high dosage of TBTO (4 mg/kg) accounted for all but the PND 13 effects. Total GFAP levels were also reduced by TBTO treatment at several time points in all three regions. However, with the exception of the cerebellum, these effects were restricted to the high dose group (4 mg/kg). The authors of this study concluded that TBTO, when administered orally on PND 5, is toxic to the developing nervous system [O' Callaghan and Miller, 1988].

intraperitoneal, mouse

The effect of TBTO on animal behavior was investigated using male Swiss OF mice. An unspecified number of mice were given intraperitoneal injections of TBTO dissolved in olive oil at concentrations of 0 (negative control), 0.5, 1, 2, and 4 mg/kg. These doses were determined not to be lethal in a prior LD50 study. Thirty minutes after dosing, the motility and curiosity of the mice were observed using a photo-electric cell acetimeter and an automated floor with holes. Changes in continued avoidance behavior were also measured in pre-conditioned animals. Thirty-minutes and 2 hours after dosing, rectal temperature was measured, and motor coordination was examined using the turning rod test. To examine changes in narcosis, the mice were given intraperitoneal injections of 75 mg/kg hexobarbital 30 minutes after dosing with TBTO. Four separate groups of 3 male Swiss OF mice were used to determine any delayed effect on activity following intravenous administration with 2 mg/kg TBTO. Four groups of 3 control animals were also employed for this determination.

A significant reduction ($P < 0.05$) in the motility of mice in the 4 mg/kg dose group was observed 30 minutes after dosing with TBTO. No effect on motor coordination was observed at any dose level, no change in narcosis was induced by hexobarbital, and no effect on rectal temperature was noted. Activity measurements following a single TBTO injection did not indicate a delayed effect. The authors concluded that under these conditions, TBTO does not affect the central nervous system [Poitou *et al.*, 1978].

3. Biochemical Toxicology

oral, rat

In a 4-week toxicity study (see section VC.2), SPF-derived Wistar rats (Riv: TOX[M]) rats were used to examine the effects of dietary TBTO on the rat biochemistry. Technical grade TBTO was mixed with olive oil, and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 dilution. Groups of 10 rats/sex were fed final dietary concentrations of 5, 20, 80, or 320 mg TBTO/kg feed. Control animals received feed containing only equivalent amounts of olive oil. After 4 weeks of exposure, blood samples were taken from the orbital plexus of each animal for the estimation of creatinine concentrations and serum activity levels of alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase (AP), and creatinine kinase (CK). The rats were then sacrificed, and blood samples were taken from the abdominal aorta for the estimation of lactate, pyruvate, and total triglyceride levels.

A dose-related and significant increase in serum ALAT activity was present in groups receiving 20 (males only), 80, or 320 mg/kg ($P < 0.5$, 0.01, and 0.001, respectively). The ASAT activity was also significantly increased in the 80 mg/kg (females only, $P < 0.05$) and 320 mg/kg (males and females $P < 0.05$, and 0.001, respectively) dose groups compared to controls. The CK and AP activities showed no significant differences. After sacrifice, biochemical analyses of the blood, sera, and liver homogenates showed that lactate and pyruvate concentrations in blood, and triglyceride levels in sera, were not significantly different from control values. However, liver glycogen levels in males and females were significantly decreased ($P < 0.01$) in the 320 mg/kg dose group. According to the authors, this reduction may reflect the nutritional status of the animals. No conclusions were made concerning the other results [Krajnc *et al.*, 1984].

oral, rat

In a long-term toxicity and carcinogenicity study (see section VD.2), the effects of dietary TBTO on plasma enzyme activity were examined in SPF-derived Riv:TOX Wistar rats. The test chemical was mixed in olive oil and homogenized with feed to yield final dietary concentrations of 0 (negative control), 0.5, 5, and 50 mg TBTO/kg feed; animals were fed *ad libitum*. After 15, 55, and 106 weeks of exposure, a heparinized venous blood sample was drawn from 10 rats/sex/group and the plasma activities of the following enzymes were determined: alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), and creatinine kinase (CK). Glucose and urea levels were also measured in these blood

samples. Isocitrate dehydrogenase (ICDH) activity was measured in blood samples taken from the retro-orbital plexus of 10 rats/sex/group after 13, 53, and 105 weeks of exposure.

Increased enzyme activity levels were generally seen only in the highest dose group (50 mg/kg). When compared to control levels, AP activity was significantly increased in males and females of this dose group after 3 ($0.001 \leq P < 0.01$), 12 ($P < 0.001$), and 24 months ($0.001 \leq P < 0.01$) of TBTO exposure. ASAT activity was also significantly increased at each time point in males from the 50 mg/kg dose group ($0.001 \leq P < 0.01$ at 12 months, and $P < 0.001$ at 3 and 24 months). In females, however ASAT activity was significantly increased only after 24 months ($0.01 \leq P < 0.05$). ALAT activity in plasma from the high-dose group was significantly increased in males ($0.001 \leq P < 0.01$) and females ($P < 0.001$) after 3 months, and in males ($P < 0.001$) after 24 months. There were no treatment-related changes in LDH or CK activities, or in blood glucose or urea concentrations. In blood taken from the retro-orbital plexus, ICDH activity was significantly increased in females from the 50 mg/kg dose group after 3 and 12 months ($P < 0.001$, and $0.001 \leq P < 0.01$, respectively). The authors concluded that TBTO, at 50 mg/kg, affects biochemical activities in the rat; no other conclusions were made [Wester *et al.*, 1990a].

oral, rat

Male Sprague-Dawley rats were used to determine the ability of TBTO to interact with cytochrome P-450, and to produce alterations in heme metabolism in the rat small intestine. TBTO was dissolved in corn oil and administered by gavage at a single dose of 100 mmol/kg of body weight. Control animals received an equivalent volume of corn oil. After 16 hours, all animals were sacrificed, intestinal microsomal fractions were prepared, and the following assays were performed: determination of benzo(a)pyrene hydroxylase activity (nmoles 8-hydroxybenzo(a)pyrene/mg protein per hour), determination of heme oxygenase activity (nmoles bilirubin/mg protein per hour), and measurement of cytochrome P-450 content (nmoles/mg protein). Results are reported as mean values of at least three separate experiments including a total of at least six animals.

When heme oxygenase activity was measured in intestinal microsomes, it was found that oral administration of TBTO produced a significant increase in heme oxygenase activity as compared with control animals ($P < 0.01$). After 16 hours, the enzyme levels in the test preparations showed a 4-fold increase over controls levels. The intestinal cytochrome P-450 concentration was 40% lower in the TBTO-treated samples than in the control microsomes. Also, the activity of benzo(a)pyrene hydroxylase was substantially lower (80%) in TBTO-treated animals than in controls ($P < 0.01$). The authors concluded that due to the intestine's role as a barrier to systemic exposure to ingested foreign chemicals, and to the increasing potential for oral exposure of humans to TBTO, the above effects may have important toxicological implications [Rosenberg *et al.*, 1984].

oral/subcutaneous, rat

Male Sprague-Dawley rats were used to determine the effects of TBTO on intestinal and hepatic heme metabolism with respect to route of administration, and to examine the ability of TBTO to produce time- and dose-dependent alterations in the heme metabolism in the small intestine following only oral administration. To determine the effects of TBTO with respect to route of administration, groups of rats (at least 3/group) were administered either a single subcutaneous injection of TBTO (50 mmol/kg body weight) dissolved in ethanol, or a single oral dose (via gavage) of the test compound (100 mmol/kg) suspended in corn oil. Forty-eight hours after dosing, the animals were sacrificed, and heme oxygenase activity and cytochrome P-450 content were determined in small intestine and liver microsomes. To determine the effects of TBTO on intestinal heme metabolism with respect to time, TBTO in corn oil was administered by gavage in a single dose (100 mmol/kg body weight) to groups of at least six animals. The animals were sacrificed immediately following dosing (4 hours) and on days 1-6 after dosing. Intestinal microsomal fractions were prepared, and the heme oxygenase, ALAS, and AHH activities, as well as the cytochrome P-450 content, were measured. To determine the dose-dependent effects of TBTO on heme metabolism in the small intestine, groups of rats (at least 3 rats/group) received a single oral dose of the test substance in corn oil at concentrations of 25, 100, or 250 mmol/kg body weight. After 24 hours, the animals were sacrificed, intestinal microsomes were prepared, and the cytochrome P-450 content, and heme oxygenase and AHH activities were measured. For each experiment, vehicle control animals received an equivalent volume of either corn oil or ethanol.

Comparison of TBTO effects with respect to route of administration indicates that the site of action of TBTO can be shifted from the liver to the small intestinal epithelium when the compound is administered orally. Following a

subcutaneous injection of TBTO, hepatic heme oxygenase activity was increased 2.5-fold over the controls ($P<0.01$), with a 50-60% reduction in cytochrome P-450 content ($P<0.02$). When TBTO was given orally, heme oxygenase activity and cytochrome P-450 content in the liver were not affected. In addition, although parenteral administration of the test compound did increase the heme oxygenase activity slightly in the small intestine (40%), oral treatment was more effective, increasing the intestinal heme oxygenase activity more than 2.5-fold compared to controls. Oral administration of TBTO also lowered the cytochrome P-450 content in the intestine by 25%.

When intestinal heme metabolism was examined with respect to time, it was found that a single parenteral dose of TBTO (100 mmol/kg) elevated heme oxygenase activity 2- to 3-fold by 24 hours. The heme oxygenase activity remained at this level throughout the following 3 days and gradually returned to normal by day 6. A single dose of TBTO also caused an initial decrease (25% of control value) in intestinal ALA-synthase activity at 4 hours, which returned to control levels by 24 hours. The ALA-synthase levels then increased 2-fold over control levels 2 days after dosing, and returned to normal levels within approximately 6 days. After a single dose of TBTO, the cytochrome P-450 content and AHH activity decreased rapidly within the first 4 hours, and remained below control levels for up to 3 days after treatment (<60% and 75% of control values, respectively). Both the cytochrome P-450 content and the AHH activity levels returned to normal by day 6.

After 24 hours, oral administration of TBTO produced significant dose-dependent alterations in the cytochrome P-450 content and AHH activity in the small intestinal epithelium. The lowest dose (25 mmol/kg) produced a 2-fold increase in heme oxygenase activity ($P<0.01$), while cytochrome P-450 content and AHH activity were reduced by almost 10 (not significant) and 50% ($P<0.05$), respectively, compared with controls. At 100 mmol/kg, TBTO increased heme oxygenase activity approximately 3-fold above controls ($P<0.01$), and lowered cytochrome P-450 content approximately 35% ($P<0.05$) compared to controls. AHH activity was also lowered in this dose group to levels less than 20% of controls ($P<0.01$). The maximum dose of TBTO (250 mmol/kg) reduced AHH activity to barely detectable levels (<5% of controls; $P<0.01$).

The authors of this study concluded that direct exposure of the gastrointestinal tract to TBTO results in substantial changes in heme metabolism that are both dose- and time-dependent. In addition, they stated that these results further define the actions of TBTO on the regulation of cytochrome P-450 content and functional activities in intestinal epithelium [Rosenberg and Kappas, 1989a].

oral/subcutaneous, rat

Male Sprague-Dawley rats were used to determine the ability of TBTO to interact with cytochrome P-450, and to produce alterations in heme oxygenase, the rate-limiting enzyme in the oxidative catabolism of heme to bile pigments. TBTO was dissolved in ethanol and administered subcutaneously in a single dose of up to 30 mg/kg body weight, or suspended in corn oil and administered by gavage in a single dose of up to 60 mg/kg. Vehicle control animals for each procedure received an equivalent volume of either corn oil or ethanol. At specified times after treatment (see below) animals were sacrificed and liver, kidney, and/or intestinal microsomes were prepared. The assays performed on these samples included the determination of heme oxygenase, aniline hydroxylase, ethylmorphine N-demethylase, and arylhydrocarbon hydroxylase activities, and the measurement of cytochrome P-450 levels.

To determine the dose-dependent effects of TBTO on hepatic heme oxygenase activity and cytochrome P-450 content, groups of rats (unspecified number) received subcutaneous injections of the test substance in ethanol at concentrations of 0 (negative control), 1.0, 3.8, 7.5, 15, or 30 mg/kg body weight. Hepatic microsomal fractions were prepared, and all assays were completed 72 hours after injection. To determine the effects of TBTO on hepatic heme oxygenase and cytochrome P-450 with respect to time, groups of 3-6 rats received a single injection of 30 mg/kg TBTO in ethanol. Hepatic microsomal fractions were prepared and the assays were performed at days 0-4, day 6, and day 9. Another test compared the effects of TBTO on hepatic, intestinal and renal heme metabolism with respect to route of administration. In this procedure, TBTO was given either subcutaneously (30 mg/kg body weight), or by gavage (60 mg/kg body weight), in a single dose, to 3-6 rats per group. Heme oxygenase and cytochrome P-450 values were determined 48 hours later for all microsomal fractions. Finally, tissue tin levels in the brain, kidney, and liver were measured 48 hours after subcutaneous (30 mg/kg) or oral (60 mg/kg) administration of TBTO. These results are presented in section VA.2.

The results of the dose-dependent study show that, at 72 hours, both hepatic heme oxygenase activity and cytochrome P-450 content were dose-dependently altered by TBTO. A single dose of TBTO of 3.8 mg/kg produced a 45% reduction in hepatic cytochrome P-450 content, while the two highest doses examined (15 and 30 mg/kg) lowered the cytochrome P450 content by 65% of control levels. Hepatic heme oxygenase activity was elevated in each dose group, with the greatest increase (3-fold over controls) seen at the highest dose administered (30 mg/kg). When hepatic heme oxygenase activity and cytochrome P-450 content were examined with respect to time, it was found that a single parenteral dose of TBTO (30 mg/kg) produced elevated (4-fold) heme oxygenase activity in the liver within 24 hours. This value remained elevated through day 6 following dosing. Hepatic cytochrome P-450 content and function (measured by the activity of aniline hydroxylase and ethylmorphine N-demethylase) were lowered by almost 65% between 48 and 72 hours after the single dose.

The effects of oral vs. subcutaneous administration of TBTO on the hepatic and small intestinal heme metabolism are reported in the study above (Rosenberg and Kappas, 1989a). In addition to re-reporting the results for the small intestine and liver, Rosenberg and Kappas (1989b) also reported that neither treatment regimen altered heme oxygenase activity or cytochrome P-450 content in the kidney at 48 hours, or at any other time point examined (unpublished observations).

In conclusion, the authors found that TBTO causes a substantial induction of heme oxygenase activity and concomitant alterations in the content and functional activities of hepatic and intestinal cytochrome P-450. Also, since the target tissue was dependent on the route of administration, and total tissue tin content did not correlate with the observed effects on heme metabolism, the authors point to the possibility that route of administration-dependent metabolite formation contributes to the ultimate biological effects of this compound [Rosenberg and Kappas, 1989b].

oral/subcutaneous, rat

Male Sprague-Dawley rats were used to determine the ability of TBTO to produce alterations in heme oxygenase activity and cytochrome P-450 content in the liver and small intestinal epithelium following oral or subcutaneous administration. This study also examined the effects of TBTO on organ and body weights, and on total tin concentrations. For each procedure, TBTO was either dissolved in ethanol and administered subcutaneously in a single dose, or suspended in corn oil and administered as a single dose by gavage. In either case, the test animals received 50 mmol of test chemical/kg body weight. Control animals received an equivalent volume (1.0 ml/kg) of either corn oil or ethanol. Each test and control group consisted of at least three animals (exact numbers not reported). Forty-eight hours after dosing, all animals were sacrificed, liver and intestinal microsomes were prepared, and the following analyses were performed: determination of heme oxygenase activity (nmoles bilirubin/mg per hour), measurement of cytochrome P-450 content (nmoles/mg protein), and measurement of tin concentrations in the liver (these results are also reported in section VA.2). Each animal was weighed at the time of dosing and at the time of sacrifice, and organ weights (liver, kidney, small intestine) were recorded after sacrifice.

Following administration of TBTO by either route, average daily food consumption and body weight gain were "substantially" reduced in test rats. Body weight loss of rats receiving an oral or subcutaneous dose was -6.7 ± 2.0 g and -2.0 ± 2.1 g, respectively, while body weight gain in control rats was 22.7 ± 3.8 g. Average daily food consumption was 24.8 g/rat in the control group, 13.0 g/rat in the test group receiving an oral dose, and 16.5 g/rat in the test group receiving a subcutaneous dose of the test chemical. Epithelial cell weight in the liver was significantly increased ($P < 0.05$) after oral dosing, from a mean weight of 1.69 ± 0.01 g (control) to a mean weight of 2.16 ± 0.07 g. However, this increase was not accompanied by observable changes in the underlying cell morphology of the proximal small intestine. No other significant changes were seen in organ weights.

TBTO, administered subcutaneously, induced a significant increase ($P < 0.05$) in heme oxygenase activity in the liver; enzyme activity levels in test samples were increased almost 3-fold over control levels. Subcutaneous administration also reduced the hepatic cytochrome P-450 content by 60%. No significant changes were seen, however, in the heme oxygenase activity or cytochrome P-450 content of the liver after oral administration of TBTO. Total tin content of the liver was lower in rats exposed to the test compound subcutaneously, than in rats exposed to the chemical orally (2.29 ± 0.36 and 20.18 ± 4.79 mg Sn/g dry weight, respectively). Tin was undetectable in the livers of control animals. In the small intestine, oral treatment with TBTO produced an increase (not statistically significant) in heme oxygenase activity and a significant reduction in the content of

cytochrome P-450 ($P < 0.05$) compared to controls. A subcutaneous injection of TBTO, however, did not result in any significant changes in small intestinal heme oxygenase activity or cytochrome P-450 content.

These results demonstrate that TBTO interacts with hepatic and intestinal heme metabolism in a manner that is dependent on the route of administration. The toxicity produced in the liver can be circumvented when the compound is given by gavage, suggesting that intestinal "first pass" metabolism of TBTO produces a metabolite that is less toxic to the liver. Also, the change in target tissue from the liver to the small intestine that is seen when route of administration is altered from subcutaneous to oral occurs despite a much higher content of tin accumulating in the liver after oral treatment compared to subcutaneous treatment. According to Rosenberg, 1990, these observations may indicate that route of administration-dependent metabolite formation contributes to the biological tissue-specific effects that occur in rats in response to TBTO [Rosenberg, 1990].

subcutaneous, rat

Male albino-Wistar rats were used to examine the effects of TBTO on several enzymic activities in the liver and kidney, and biogenic amine levels in the brain, after a single subcutaneous injection. Three groups of six rats were administered TBTO dissolved in ground nut oil at a concentration of 25 mmole tin/0.5 ml/100 g body weight. Six control animals received the same dose of ground nut oil without the test substance. All animals were starved overnight and sacrificed 24 hours after administration. The liver, kidney, and brain of each rat were removed immediately and processed for biochemical evaluation. Liver and kidney homogenates (5% w/v) were examined for changes in the activity of the following enzymes: lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), monoamine oxidase (MAO), adenosine triphosphatase (ATPase), and alkaline phosphatase. Brain homogenates (5% w/v) were tested for changes in the levels of the following biogenic amines: gamma-aminobutyric acid (GABA), dopamine, acetylcholine, and MAO. Total protein content was also determined in each homogenate.

A significant ($P < 0.05$) increase in the activity of alkaline phosphatase and ATPase and a decrease in MAO activity were produced by TBTO in both the liver and kidney homogenates. LDH and SDH activity in the liver and kidney were unchanged by administration of the test substance. In the brain homogenates of animals treated with TBTO, a significant ($P < 0.05$) decrease was seen in GABA and dopamine levels when compared to the levels in control homogenates. MAO activity in the brain was also significantly less ($P < 0.05$) in test animals than in controls. Acetylcholine levels were not changed by treatment with the test substance.

According to the authors, the increase in alkaline phosphatase activity is due to a general response of the liver and kidney to overcome the insult produced by the test compound, and possibly to an accelerated membrane transport function related to anion hydroxide exchange across the lipid biomembranes mediated by TBTO. Increased activity of ATPase is correlated with an increase in membrane bound ATPase after treatment with the organotin, which might also be correlated with an acceleration in membrane transport function by the TBTO ions. The decrease in MAO activity in the liver and brain may be the result of an interaction of organotin ions with the sulfhydryl component of the enzyme. Impairment of MAO activity is an indication of abnormalities of the central nervous system. The depletion of GABA and dopamine in the brain enhances the neurotoxic effects of the test compound and results in the altered behavior of the rats (hyperactivity). The authors concluded that TBTO exerts its toxic action on the oxidative phosphorylation mechanism and the central nervous system by impairing the various enzymic activities and the concentration of biogenic amines [Dwivedi *et al.*, 1985].

subcutaneous, rat

To examine the potential of TBTO to induce corneal edema, groups (unspecified number) of male Wistar rats were given an intramuscular injection of 1 ml/kg of TBTO (unspecified solvent). Untreated male rats served as controls. The animals were sacrificed 1, 2, 3, 6, 8, or 20 hours after injection, and all corneas were isolated, fixed, and processed for electron microscopy. Four hours after injection with TBTO, marked swelling of mitochondria could be seen in the corneal endothelial cells. At 6 hours, the corneal edema appeared in the endothelial layer and stroma. By X-ray microanalysis, tin peaks were obtained from swollen mitochondria in the endothelial cells. The edematous swelling of the corneal tissue became more advanced at 20 hours.

To investigate the action of TBTO on mitochondria, respiration control rates of liver mitochondria were measured

polarographically. There were no differences in the rates of state 3 (ADP-stimulated) respiration between TBTO injected rats and controls. However, the rates of state 4 respiration were markedly increased in treated rats (P value not reported). According to the authors, these results indicate that TBTO uncoupled the oxidative phosphorylation in mitochondria and induced the corneal edema by disturbance of the active pump function of endothelial cells [Yoshizuka *et al.*, 1990].

intraperitoneal, rat

Female Osborne-Mendel rats were used to examine the effects of a lethal dose of TBTO on tissue amine levels. Groups of 18 rats were fasted for 18 hours and then given an intraperitoneal injection of the test chemical in corn oil. The final concentration of TBTO was 10 mg/kg, which had been determined to be lethal (LD100) in a LD50 study. Control animals were injected with an equivalent volume of corn oil. Animals were sacrificed 2, 24, or 48 hours after dosing, and the adrenals, hearts and brains were removed. Noradrenaline and adrenaline levels were determined in all organ samples, and 5-hydroxytryptamine levels were determined in brain samples.

A lethal dose of TBTO significantly reduced noradrenaline levels in the brain and heart 2-48 hours after dosing ($P < 0.05$ for the brain, and $P < 0.01$ for the heart). Noradrenaline levels in the adrenals were significantly reduced ($P < 0.05$) after 24 and 48 hours. Adrenaline levels in the heart were significantly increased ($P < 0.01$) 24 and 48 hours after treatment with TBTO, while adrenaline levels in the adrenals were significantly decreased ($P < 0.05$) at 2, 24, and 48 hours. 5-Hydroxytryptamine levels in the brain were significantly reduced ($P < 0.05$) 48 hours after dosing [Robinson, 1969].

dermal, guinea pig

Hartley guinea pigs (unspecified sex) were used in a skin painting study to determine the potential of TBTO to induce secondary Faconi syndrome, a renal tubular disturbance characterized by glucosuria, amino aciduria, phosphaturia, and hypophosphatemia. Two groups of test animals (10 animals per dose group) were painted with 0.1 ml of 10 mg/kg or 40 mg/kg of TBTO dissolved in ethanol. The test compound was applied to the shaved backs of the animals once a day for 50 days. Control animals ($n=10$) were treated with 0.1 ml of absolute ethanol using the same procedure. For each animal, urine was collected before treatment and then weekly or biweekly for the duration of the 50-day test period. Sera and plasma were collected from all animals on day 50. Analyses were conducted on each urine sample to measure the protein concentration, the sugar content, and the lysozyme content. The electrolyte concentrations (sodium, potassium, inorganic phosphate, chloride, and calcium) in the sera and urine samples were measured, and complete amino acid analyses were conducted. In addition, microsomes were prepared from fresh kidneys obtained from both control and test animals, and cytochrome P-450 and 1-25-dihydroxy vitamin D levels were measured from the kidney and sera, respectively. Animals were weighed at the beginning and end of the study, and histological examinations were made of kidney specimens.

By the end of the study, control animals weighed more than animals in either test group (590 ± 60 g {control} versus 463 ± 48.5 g and 365.0 ± 10.9 g for the 10 mg/kg and 40 mg/kg dose groups, respectively). Histological changes seen in kidneys obtained from animals in the 10 mg/kg and 40 mg/kg dose groups include swelling, degeneration, and destruction of the tubular epithelium. No remarkable lesions were found in the glomerulus and no abnormalities were seen in control animals. The amount of sugar excreted into the urine was significantly higher ($P < 0.05$) in the 10 and 40 mg/kg dose groups than in the control group. However, the increases in urinary protein and lysozyme content were insignificant in comparison to the controls.

Temporal examination of the urinary excretion of electrolytes indicate that in the 10 mg/kg dose group, excretion of sodium, chloride, and phosphate was maximum on day 50 and significantly higher than the concentrations seen in the control group ($P < 0.01$ and $P < 0.001$). For these animals, urinary excretion of potassium and calcium did not differ significantly from the control levels. For animals treated with 40 mg/kg TBTO, urinary excretion of sodium, chloride, and calcium was significantly higher than controls ($P < 0.001$) at day 50. At this time, calcium excretion was insignificantly higher, and phosphate excretion was insignificantly lower in these animals than in control animals. Measurements of serum electrolytes at day 50 show that serum sodium levels in the 10 mg/kg and the 40 mg/kg dose group were significantly higher ($P < 0.01$ and $P < 0.05$, respectively) than the serum sodium levels in the control group. Animals treated with 10 mg/kg TBTO also had significantly lower ($P < 0.001$) sera phosphate levels than control animals. No other differences in serum electrolyte levels were observed.

After 50 days, urinary excretion of several amino acids was significantly increased ($P < 0.05$, 0.01, or 0.001) in

animals treated with 10 mg/kg and 40 mg/kg TBTO compared to control animals. These amino acids included threonine, glycine, alanine, citrulline, cystathionine, cystine, isoleucine, b-amino-n-butylic acid, lysine, and arginine. In the serum samples, some amino acid levels were significantly ($P < 0.05$, 0.01 , or 0.001) lower in test animals than in controls. Animals treated with 10 mg/kg TBTO had lower levels of cystathionine, isoleucine, histidine, and arginine, while animals treated with 40 mg/kg had lower levels of histidine and cystathionine. These amino acids were excreted in greater amounts in the urine of TBTO-treated animals than in the urine of control animals. Finally, the levels of cytochrome P-450 measured in the kidney and of 1-25-dihydroxy vitamin D measured in the serum were significantly ($P < 0.5$) lower in animals treated with 10 mg/kg TBTO compared to controls.

These results indicate that secondary Facioni syndrome was demonstrated in animals treated with a low dose (10mg/kg/day) of TBTO. However, animals treated with a high dose (40 mg/kg/day) of the compound showed different changes in the excretion of some electrolytes, such as calcium and inorganic phosphate. This seems to suggest that high doses of TBTO induced different metabolic and renal injuries than low doses [Mori *et al.*, 1984].

in vitro, rat

An unreported number of male Sprague-Dawley rats were used to examine the capacity of organotins, including TBTO, to inhibit the uptake of radiolabelled 5-hydroxytryptamine (5-HT-14C) and to stimulate the release of preloaded 5-HT-14C and endogenous 5-HT by rat platelets. TBTO was dissolved in acetone or ethanol (unspecified) to yield a final concentration of 1×10^{-5} M. Platelet rich plasma (PRP) was obtained by centrifuging blood samples taken by cardiac puncture from rats under light ether anesthesia, and the PRP supernatant was diluted with Tyrode's solution. For uptake inhibition studies, aliquots of PRP (200 μ l) were preincubated with TBTO for 14 minutes. Radiolabeled 5-HT was added to each tube and the incubation was continued for 10 minutes. After the uptake of 5-HT-14C was stopped by the addition of ice-cold Tyrode-EDTA, the PRP sample was centrifuged for 10 minutes, and aliquots of the supernatant were radioassayed by liquid scintillation counting. To determine the percentage of inhibition, the uptake by TBTO-treated platelets was compared to that of controls. For release studies, platelets were preloaded with 5-HT-14C, washed, and resuspended in Tyrode's solution. The samples were incubated with TBTO for 10 minutes and centrifuged. Release was determined by radioassay of the supernatant, and percentage release was calculated by comparing TBTO-treated samples to control samples. Some platelet samples treated with TBTO were subjected to HPLC analysis for endogenous 5-HT content or were processed for scanning electron microscopy.

The results of this study show that, of the organotins tested, TBTO was the most active inhibitor of 5-HT uptake and the most potent inducer of 5-HT release. HPLC analysis of platelet extracts show that, although TBTO decreased the levels of endogenous 5-HT, the levels were not significantly different from controls. Because the percentage of 5-HT-14C released (94.3%) differed from the percentage of endogenous 5-HT released (79.5%), the authors concluded that the release of 5-HT-14C from preloaded platelets may not be quantitatively indicative of endogenous 5-HT release. Scanning electron microscopy revealed an increased platelet aggregation and shape change in TBTO-treated samples compared to vehicle-treated controls. This, according to the authors, could have caused the decreased endogenous 5-HT levels. The authors also suggested that the action of TBTO on rat platelets was due, in part, to their known ability to interfere with ATPase-mediated systems [Johnson and Knowles, 1983].

in vitro, rat

An unreported number of male Sprague-Dawley rats were used to examine the effects of organotins, including TBTO, on rat platelet aggregation mechanisms and the interaction of the chemicals with physiological inducers of aggregation. Compounds used to induce aggregation were ADP, which was dissolved in 0.9% saline to yield a concentration of 2.0 mM, and collagen, which was dissolved in 0.01 N acetic acid at 0.1 mg/ml. TBTO was dissolved in ethanol and diluted with saline to final concentrations ranging from 0.5-10 mM. Platelet rich plasma (PRP) was obtained by centrifuging blood samples taken by cardiac puncture from rats under light ether anesthesia. The PRP supernatant was diluted with Tyrode's solution to 300,000 platelets/ml.

To examine the effect of TBTO on ADP-induced aggregation, aliquots (0.5 ml) of PRP were incubated with 10 ml TBTO at final concentrations of 1, 5, or 10 mM for 1, 3, or 5 minutes before the addition of 10 ml of the ADP preparation. To examine the effect of TBTO on collagen-induced aggregation, equivalent aliquots of PRP were

incubated with 10 ml of TBTO at final concentrations of 0.5, 0.625, 0.75, 0.875, 1.0, 2.5, and 5.0 mM for 1 minute before the addition of the collagen preparation. The appropriate solvent controls were tested with ADP and collagen. The change in light transmission caused by aggregation of the platelets induced by ADP and collagen was recorded on a strip chart recorder. From these tracings, shape change and primary and secondary aggregation patterns could be detected. The effect of TBTO on aggregation was determined by comparing aggregation patterns of TBTO-treated PRP samples to solvent-treated PRP control samples. In the case of collagen-induced aggregation, the time of onset of aggregation was also compared to controls. To determine whether TBTO induced aggregation, PRP was incubated with 10 mM of the compound for 6 minutes.

TBTO inhibited both ADP- and collagen-induced aggregation. The inhibition was dependent on both the incubation time and on the concentration of test chemical used. For example, the inhibition of ADP-induced aggregation increased from 5-30% after a one-minute incubation with 5 mM of TBTO to 80-100% after a 5-minute incubation. In addition, an increase in TBTO concentration from 1-5 mM resulted in a 30% increase in the inhibition of ADP-induced aggregation. ADP-induced aggregation curves following incubation of platelets with TBTO indicated that shape change and primary aggregation were present, but secondary aggregation did not occur. TBTO, at a concentration of 5 mM, caused complete inhibition of collagen-induced aggregation. Marked inhibition (60-80%) was also observed at a concentration of 2.5 mM. The collagen-induced aggregation curves suggested that only shape change occurred. At lower concentrations (2.5 to 1 mM), the inhibitory potency of TBTO was dramatically decreased; concentrations of TBTO less than 1 mM caused relatively little inhibition of collagen-induced aggregation. These lower concentrations of TBTO did, however, increase the latent period for induction of aggregation by collagen. TBTO, at 10 mM, also directly induced platelet aggregation. According to the authors, this induction may be related to the ability of TBTO to release platelet vesicular stores of ADP and 5-HT.

The authors concluded that TBTO has the potential to disrupt normal hemostatic mechanisms in mammalian platelets. However, at blood TBTO concentrations necessary for induction of hemostatic dysfunction, other physiologically adverse effects of the organotins may be more significant [Knowles and Johnson, 1986].

4. Effects on the Endocrine System

oral, rat

In a 4-week toxicity study (see section VC.2), SPF-derived Wistar rats (Riv: TOX[M]) were used to examine the effects of dietary TBTO on rat insulin and glucose levels. Technical grade TBTO was mixed with olive oil, and portions (50 ml oil/kg feed) were homogenized with feed to yield graded premixes from which final experimental diets were prepared by a 1:10 dilution. Groups of 10 rats/sex were fed final dietary concentrations of 0 (negative control), 5, 20, or 80, mg TBTO/kg feed. After 4 weeks of exposure, the rats were sacrificed, and blood samples were taken from the abdominal aorta, and serum glucose and insulin levels were measured.

Serum glucose levels in males were significantly decreased ($P < 0.01$) in the 320 mg/kg dose group. This dose group also had reduced sera insulin levels; 6/17 test samples had insulin concentrations that were significantly lower than controls ($P < 0.01$), while 11/17 samples had concentrations that were below the limit of detection (2 mIU/liter). No changes in sera insulin or glucose were seen in any other treatment group. Despite an inhibition of insulin release, the authors concluded that TBTO was not a diabetogenic agent since the data on serum glucose concentrations, serum triglyceride levels (see section VG.3), and urinary ketone bodies (see section VC.2) do not support diabetogenic action. Krajnc *et al.* stated that the absence of measurable concentrations of insulin in most sera from animals in the high dose group may be attributable to the marked decrease in feed intake (see section VC.2). It may also reflect a decrease in the basal metabolic rate rather than a direct effect on the pancreas [Krajnc *et al.*, 1984].

The 4-week study described above and in section VC.2 was supplemented with 6-week studies that more closely examined the effect of TBTO on the endocrine system. Since no essential differences were found between males and females in the 4-week study, these experiments were performed with only male SPF-derived Wistar rats (Riv: TOX[M]). For all experiments, the animals were fed test and control diets (prepared as described above)

containing 0 (vehicle control), 20, or 80 mg TBTO/kg feed for 6 weeks. In one experiment, the adrenal, pituitary, and thyroid organs from 10 rats/group were removed weighed, and processed for immunohistochemical and histological evaluations after the 6-week feeding period. In a second experiment, blood samples were taken from groups of 8 animals by decapitation after the 6-week feeding period; the concentrations of insulin, thyroxin (T4), thyrotropin (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH) and corticosterone were measured by radioimmunoassay. In a third study, a silicon rubber cannula was inserted into the jugular vein of each animal (8/group) after 6 weeks exposure to TBTO. Two to three days after surgery, the animals were fasted overnight and intravenously injected with glucose (4mmol/kg body weight). Serial blood samples were taken before glucose injection and 3, 5, 10, 15, and 20 minutes after injection; serum insulin concentrations were measured for each blood sample. The response to hypothalamic releasing hormones was tested in the same animals 48 hours after the glucose tolerance test. The animals were administered thyrotrophin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LHRH), 48 to 72 hours apart. The concentrations of TSH (response to TRH), and LH and FSH (responses to LHRH) were measured in venous blood samples taken before administration and 8, 20, and 60 minutes after administration. Serum concentrations were plotted against time, and the areas above the zero time values were calculated and statistically evaluated. All animals received control and experimental diets during the entire test period.

Insulin concentrations in serum obtained from decapitated animals showed a significant decrease in both the 20 and 80 mg/kg dose groups ($P<0.05$ and 0.01 , respectively). However, the time course of insulin release, following glucose administration, did not show any significant differences from control groups. In serum taken after decapitation, concentrations of T4 and TSH were significantly decreased at 80 mg/kg ($P<0.01$ and 0.05 , respectively), while the serum LH concentration was significantly increased ($P<0.01$); no differences in FSH and corticosterone concentrations were observed. After TRH administration, the TSH release showed a tendency toward reduced secretion at 80 mg/kg; the area under the zero time values was significantly decreased ($P<0.01$). Twenty minutes after TRH administration, serum TSH concentrations were significantly decreased ($P<0.05$) in the 20 and 80 mg/kg dose group when compared to controls. Conversely, the release of LH and FSH following administration of LHRH was enhanced in the 80 mg/kg dose group; the area under the zero time values was significantly increased ($P<0.01$ and $P<0.05$, respectively).

No treatment-related effects were observed in the weight of the adrenals or the pituitary gland, but the absolute and relative weights of the thyroid were significantly decreased ($P<0.05$) in the 80 mg/kg dose group (relative weight of 0.0049 ± 0.0007 g compared to a control value of 0.0068 ± 0.0017 g). Also, histopathology shows decreased thyroid activity in this dose group, as indicated by flattening of the epithelial lining of the thyroid follicles. Immunocytochemical staining of the pituitary showed a reduction in the number, and staining intensity, of TSH-producing cells in the 80 mg/kg dose group, and to a lesser degree in the 20 mg/kg dose group, compared to controls. There was also a dose-related increase in the number of cells staining strongly for LH. These findings correlate with the serum TSH and LH values. No effect was observed on FSH-, GH- (growth hormone), and ACTH (adrenocorticotropin)-producing cells, nor on the pancreatic insulin and glucagon-producing cells.

According to the authors, the increased release of LH and FSH, and the decreased release of TSH indicate that TBTO does affect the endocrine system. However, the authors concluded that 6-week feeding at 80 mg/kg does not result in stimulation of the pituitary-adrenal axis; no effect was seen on ACTH-producing cells in the anterior pituitary, or on pars intermedia and adrenal histology. Also, serum corticosterone concentration and adrenal weight were unaffected. Despite the inhibition of insulin release, the authors of this study concluded that TBTO was not a diabetogenic agent. In support of this conclusion, they point to the findings that the insulin response to glucose challenge did not show treatment-related effects, and no influence was seen on the production of immunoreactive insulin and glucagon in the pancreatic islets [Krajnc *et al.*, 1984].

oral, rat

In a long-term toxicity and carcinogenicity study (see section VD.2), the effects of dietary TBTO on serum hormone levels were examined in SPF-derived Riv:TOX Wistar rats. The test chemical was mixed in olive oil and homogenized with feed to yield final dietary concentrations of 0 (negative control), 0.5, 5.0, and 50.0 mg TBTO/kg feed; animals were fed *ad libitum*. After 12 and 24 months of TBTO exposure, the concentrations of thyroxin (T4), free thyroxin (FT4), thyrotropin (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH), and insulin were measured by radio-immunoassays in serum obtained from 10 rats/sex/group. No effects were observed on serum levels of TSH, LH, FSH, T4, FT4, or insulin. The FT4:T4 ratio, however, was significantly decreased in the

50 mg/kg dose group for both sexes at 12 months ($P < 0.001$) and for males at 24 months ($0.001 \leq P < 0.01$). After 12 months, a significant decrease in this ratio was also seen in males ($0.01 \leq P < 0.05$) and females ($0.001 \leq P < 0.01$) in the 5 mg/kg dose group. These results were inconsistent with those found in the previous study (Krajnc *et al.*, 1984), and the authors were unable to account for the inconsistency [Wester *et al.*, 1990a].

oral, rat

Male Sprague-Dawley rats were used to examine the effect of short- and long-term exposure to TBTO on rat lymphoid and endocrine organs. The procedures and results for the lymphoid studies are reported in section VG.1. For the short-term test, a total of 90 animals received a single dose (100 mg/kg body weight) of TBTO dissolved in olive oil. Control animals received an equivalent volume (1 ml/kg) of olive oil. "At least 4 animals each" were examined at 3, 6, and 12 hours, and at 1, 2, 3, 4, 6, 8, 10, 14, and 21 days. At each time point, the pituitary and adrenals were weighed, and the pituitary, adrenals, and thyroid were examined histologically. The pituitary was also stained immunohistochemically for ACTH (adrenocorticotrophin) and TSH (thyroid stimulating hormone). Blood samples were taken from the ascending aorta at each time point, and serum TSH and T4 (thyroxine) levels were determined by radioimmunoassay. To test the adrenocortical function, 25 mg/100 g of synthetic ACTH was intramuscularly injected into the animals 24 or 96 hours after dosing; the serum concentrations of cortisol were determined at 8, 16, and 24 hours after injection. For the long-term study, groups of 10 rats received daily doses of TBTO dissolved in olive oil at concentrations of 0 (vehicle control), 3, 6, or 12 mg/kg five days a week, for 13 or 26 weeks. After the test period, 5 animals from each group were sacrificed, and the adrenals and pituitary were removed, weighed, and examined histologically. The thyroid was also examined for microscopic changes.

Twelve hours after a single dose of TBTO, adrenal weight was increased from 14.3 ± 0.8 mg/100 g to 15.8 ± 3.8 mg/100 g body weight. Adrenal weight reached a maximum on the second day (20.9 ± 4.3 mg/100 g body weight). The amount of stainable lipids in the adrenal cortex decreased during the first 24 hours, while hydropic vacuoles increased. After 48 hours, the amount of lipids increased, and the cortex became thickened. Histological changes in the adrenals returned to normal by day 14. The thyroid follicles showed signs similar to those produced by hypophysectomy (distension with colloid and flat epithelial cells). These changes were severe after 72 hours, but had returned to normal within 14 days. Absolute pituitary weight was increased slightly, but not significantly, 1 and 2 days after dosing. One day after dosing, a significant increase ($P < 0.01$) in relative pituitary weight was observed, with the weight increasing from 3.78 ± 0.23 to 4.19 ± 0.35 mg/100 g body weight. After 3 hours of dosing, the staining intensity for ACTH and pars intermedia cells was markedly decreased. At 6 hours, the reactions were only slightly positive and the cells were atrophic; and after 72 hours, the staining of ACTH cells began to increase in intensity. Seventy-two hours after dosing, the serum concentrations of TSH and T4 were reduced to 1/2 and 1/6 of the control values, respectively. Serum cortisol levels increased to twice the control value 96 hours after treatment with TBTO. Intramuscular injection of ACTH 8 hours after dosing led to increased cortisol levels in the blood of both treated and control rats. Sixteen-hours after ACTH stimulation, cortisol levels in treated rats decreased, while levels in controls increased.

Long-term exposure to TBTO caused significant increases in adrenal weight in the 6 ($0.01 < P < 0.05$ at 13 weeks and $0.003 < P < 0.01$ at 26 weeks) and 12 mg/kg ($P < 0.003$) dose groups. After 13 and 26 weeks, the mean relative weights in the 12 mg/kg dose group were 17.8 ± 1.5 and 15.9 ± 1.9 mg/100 g, respectively; the mean weights in the 6 mg/kg group were 15.3 ± 2.6 and 13.5 ± 2.6 mg/100 g, respectively. These compare to respective control values of 11.2 ± 1.7 and 10.1 ± 1.3 mg/100 g. The weight increase in the adrenals was associated with marked increases in stainable lipids. Pituitary weight significantly increased in all dose groups after 26 weeks of TBTO exposure ($0.003 < P < 0.01$ at 3 and 6 mg/kg, and $P < 0.003$ at 12 mg/kg), and in the high dose group after 13 weeks of exposure ($P < 0.003$). After 26 weeks, the mean relative weight of the pituitary in the 0, 3, 6, and 12 mg/kg groups was 2.24 ± 0.21 , 2.53 ± 0.16 , 2.76 ± 0.32 and 2.80 ± 0.39 mg/100 g, respectively. In the pars distalis of the pituitary, PAS-positive cells were numerous and a majority of the cells were highly vacuolated. In the pars intermedia, vacuolar changes and focal necrosis were observed after 13 weeks of treatment. After 26 weeks, various sized cysts that contained a colloid-like substance were present. Microscopic examination of the thyroid revealed large colloid-filled follicles lined by flat epithelial cells throughout the tissue [Funahashi *et al.*, 1980].

5. Cytotoxicity

In vitro, *Saccharomyces cerevisiae* /human KB cells

The cytotoxicity of TBTO was evaluated by determining the concentration necessary to induce a 50% inhibition of cell growth (ID₅₀) using *S. cerevisiae* and human KB cells. Experimental cell populations were incubated for 72 hours with serial dilutions (concentrations not reported) of TBTO in acetone, while controls were incubated for 72 hours with only acetone. The inhibition of cell growth was determined by comparing the total number of viable cells in the treated cultures with the total number of viable cells in the control cultures. Dose-response curves obtained from the data show that cell growth was inhibited by TBTO and the percent of inhibition was dose-dependent. The ID₅₀ values determined after the 72 hour incubation period were 0.18 mg/ml and 0.12 mg/ml for *Saccharomyces cerevisiae* and KB cells, respectively [Mochida *et al.*, 1988].

In vitro, hamster

The cell detachment and cloning efficiency of baby hamster kidney cells (BHK-21 C13) were used as parameters to evaluate the cytotoxicity of TBTO and other organotins. Cell detachment indicates irreversible damage to the cytoskeleton, while cloning efficiency examines growth of single cells, reattachment of cells, and subsequent clone formations. TBTO was dissolved in acetone and pH-adjusted in a 10% mixture with MEM. Experimental cell populations used in the cell detachment assay or the cloning efficiency assay were incubated with unspecified concentrations of TBTO for 4 hours or 6 days, respectively. The control cultures were incubated for an equivalent time with 1% acetone. The IC₅₀ (concentration at which there was a 50% reduction in cloning efficiency) for TBTO was 5×10^{-7} mol/liter (0.3 mg/ml medium). The CD₅₀ (50% effect on cell detachment) was 3×10^5 mol/liter (18 mg/ml) [Reinhardt *et al.*, 1982].

H. Aquatic Toxicity

TBTO has been shown experimentally to be highly toxic to both marine and fresh water organisms, affecting growth, development, and mortality. The following LC₅₀ values were obtained from tables summarizing the acute toxicity of TBTO on aquatic organisms [WHO, 1990]:

	Organism	Duration	LC₅₀ (mg/L)
Marine	Bivalves (adult)	96-hour	38-290
		48-hour	300-1800
	Bivalves (larval)	48-hour	1.6-23
	Crustaceans	96-hour	1.0-41
	Fish	96-hour	1.5-36
Fresh Water	Snails	24-hour	30-100
	Clam	24-hour	2100
	Water Fleas (juvenile)	48-hour	4.7-70
	Fish	96-hour	13-240

Other toxic effects of TBTO in aquatic species include the following:

Inhibited growth oyster spat (*Crassostrea virginica* and *Crassostrea gigas*) [Waldock and Thain, 1983; Lawler and Aldrich, 1987]; and juvenile mussels (*Mytilus edulis*) [Stromgren and Bongard, 1987].

Induced shell thickening in the oyster [Waldock and Thain, 1983] and the freshwater clam (*Anodonta cygnea*) [Machado *et al.*, 1989].

Sublethal doses inhibited metamorphosis and growth of mud crab larva (*Rhithropanopeus harrisi*) [Laughlin *et al.*, 1983].

Reduced egg production in female copepods (*Acartia tonsa*) [Johansen and Mohlenberg, 1987].

Inhibited limb regeneration and caused deformities in regenerated limbs of the fiddler crab (*Uca pugilator*) [Weis and Kim, 1988] and brittle star (*Ophioderma brevispina*) [Walsh *et al.*, 1986].

Induced gill pathology at toxic concentrations in the mummichog (*Fundulus heterclitus*) [Pinkney *et al.*, 1989].

Caused abnormalities in developing hindlimbs of axolotl larvae (*Ambystoma mexicanum*) [Scadding, 1990].

Induced imposex in female mud snails (*Nassarius obsoletus/Ilyanassa obsoleta*) [Smith, 1981].

Caused chronic toxic effects (inflammation of oral and skin epithelium, vacuolation of hepatocytes and retinal pigment epithelium, tubulonephrosis and glomerulopathy, and keratitis) in fish (*Oryzias latipes* and *Poecilia reticulata*) [Wester *et al.*, 1990b].

VI. STRUCTURE ACTIVITY RELATIONSHIPS

The various organotin compounds may be represented by the general formula $R_nSnX_{(4-n)}$ [Schweinfurth, 1985], where R is an alkyl or aryl group and X is a halogen or hydroxyl [Clayton and Clayton, 1981]. The type of organic substituent (R), and the degree of substitution (n) have a considerable effect on the toxicity of the organotins, while the "anionic" group (X) has less importance [Schweinfurth, 1985].

Five studies were conducted to examine the carcinogenicity of three structurally related compounds; tributyltin fluoride, triphenyltin acetate, and triphenyltin hydroxide.

A 6-month study of the carcinogenicity potential of tributyltin fluoride (TBTF) was conducted in male Swiss white mice. Groups of 50 mice were treated with 15 mg of 5 and 10% TBTF in propylene glycol 3 times/week for a period of six months. The authors of this study concluded that TBTF did not have any carcinogenic effects [Sheldon, 1975].

Strain A/St or Strain A/J mice were administered intraperitoneal injections of triphenyltin hydroxide 3 times/week for 8 weeks in two separate laboratories. The doses of the test compound ranged from 0.5-2.5 mg/kg. In laboratory A (Strain A/St), females dosed with 2.5 mg/kg triphenyltin hydroxide had a significantly higher ($P \leq 0.05$) incidence of tumors when compared to vehicle (tricaprylin) controls; 40% of the females in the test group had tumors compared to 11% of the females in the control group. No other significant differences were seen in either laboratory at any of the doses tested [Maronpot *et al.*, 1986].

In a 2-year bioassay examining the carcinogenicity of triphenyltin hydroxide, Fisher 344 rats and B6C3F1 mice were administered 37.5 or 75 ppm of the test compound in feed for 78 weeks. There were no significant increases in tumor incidence in dosed rats or mice compared to controls [NCI, 1978].

In a bioassay of pesticides for tumorigenicity, hybrid mice (C57BL/6 x CsH/Anf and C57BL/6 x AKR) were used to examine the carcinogenic potential of triphenyltin acetate. On days 7-28 of age, the mice were given daily doses of 0.464 mg/kg of the test compound in 0.5% gelatin, via a stomach tube. After this time, the mice were given the test compound with the diet, at a concentration of 1206 ppm, for approximately 18 months. Necropsy of the animals revealed that oral treatment with triphenyltin acetate did not increase the incidence of tumors in mice when compared to the untreated controls [Innes *et al.*, 1969].

The other study examining tributyltin fluoride was also negative [NCI, 1988b].

No other data were found on the carcinogenicity of structurally related compounds including tributyltin, tributyltin benzoate, tributyltin chloride, tributyltin fluoride, tributyltin linoleate, tributyltin methacrylate, and tributyltin naphthenate [WHO, 1990; RTETCS, 1991; Cancerlit, 1991; IRIS, 1991].

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APPENDIX I. ON-LINE DATABASES SEARCHED

	DATE OF SEARCH TIME PERIOD	
BRS:		
HZDB	March, 1991	
DIALOG:		
Agricola	March, 1991	1970-1991
Biosis Previews	March, 1991	1969-1991
CAB Abstracts	March, 1991	1972-1991
Cancerlit	March, 1991	1963-1991
Chem Bus Newsbase	March, 1991	1984-1991
Chemical Exposure	March, 1991	1974-1991
Chemical Regs. & Guidelines	March, 1991	
Chemical Safety	March, 1991	1981-1991
CIN	March, 1991	1974-1991
Compendex Plus	March, 1991	1970-1991
Embase	March, 1991	1974-1991
Enviroline	March, 1991	1970-1991
Environmental Bibliography	March, 1991	1974-1991
Federal Register	March, 1991	1977-1991
Fine Chemicals	March, 1991	
FSTA	March, 1991	1969-1991
Int'l Pharm. Abstracts	March, 1991	1970-1991
IHS Int'l Standards	March, 1991	
Life Sciences Collection	March, 1991	1978-1991
Medline	March, 1991	1966-1991
NIOSH	March, 1991	1973-1991
NTIS	March, 1991	1964-1991
Piers Imports and Exports	March, 1991	1989-1991
PTS Newsletter	March, 1991	1987-1991
PTS Prompt	March, 1991	1972-1991
Pollution Abstracts	March, 1991	1970-1991
Scisearch	March, 1991	1974-1991
Trade and Industry ASAB	March, 1991	1983-1991
Toxline	March, 1991	1965-1991
TSCA	March, 1991	
MEAD:		
Nexis/Lexis-BNA ENV	March, 1991	
NLM:		
Chemid	March, 1991	

Chemline	March, 1991	
RTECS	March, 1991	
IRIS	March, 1991	
Toxline 65	March, 1991	1965-1980
Toxline	March, 1991	1981-1991
Toxlit	March, 1991	1981-1991
Toxlit 65	March, 1991	1965-1980
Dart	March, 1991	1989-present
Eticback	March, 1991	1950-1989
Emicback	March, 1991	1950-present

STN:

CA Previews	March, 1991	Updated weekly
Chemlist	March, 1991	1979-present
Registry	March, 1991	
CSCHEM	March, 1991	

CIS:

TSCAPP	April, 1991	
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APPENDIX II. SAFETY INFORMATION

HANDLING AND STORAGE

TBTO is corrosive [Aldrich, 1990], but stable under normal laboratory conditions. TBTO is incompatible with oxidizers [Lenga, 1988; Pfaltz & Bauer, 1985] and acids [Pfaltz & Bauer, 1985]. Irritating fumes or vapors may develop if compound is exposed to elevated temperatures or open flame [Pfaltz & Bauer, 1985]. Decomposition products include toxic fumes of carbon monoxide, carbon dioxide [Lenga, 1988], and tin/tin oxides [Lenga, 1988; Pfaltz & Bauer, 1985].

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 wash 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: DO NOT INDUCE VOMITING. Corrosive chemicals will destroy the membranes of the mouth, throat, and esophagus and, in addition, have a high risk of being aspirated into the victim's lungs during vomiting, which increases the medical problems.

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. IMMEDIATELY transport the victim to a hospital.

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 goggles

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 cartridge.

EXTINGUISHANT

Dry chemical, carbon dioxide or halon extinguisher.

MONITORING PROCEDURES

An analytical method for monitoring organotin compounds (as Sn) is reported in the NIOSH Manual of Analytical Methods, Method No. 5504. This method involves sampling using a filter and sorbent tubes. The sample is desorbed using an acetic acid/acetonitrile solution. The measurement technique employs an atomic absorption spectrophotometer with a graphite furnace accessory [Eller, 1987].

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 TBTO is spilled the following steps shall be taken:

1. If a liquid solution is spilled, use vermiculite, sodium bicarbonate, sand, or paper towels to contain and absorb the spill.
2. Clean the spill area with dilute alcohol (approximately 60-70%) followed by a strong soap and warm water washing.
3. 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.