TOXICOLOGICAL REVIEW

TRIBUTYLTIN OXIDE

(CAS No. 56-35-9)

In Support of Summary Information on the

Integrated Risk Information System

(IRIS)

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Washington D.C.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA (U.S. EPA, 1994c). Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development, Office of Air and Radiation, Office of Prevention, Pesticides, and Toxic Substances, Office of Solid Waste and Emergency Response, Office of Water, Office of Policy, Planning and Evaluation, and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix B.

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response information in IRIS pertaining to chronic exposure to tributyltin oxide. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of tributyltin oxide.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response (U.S. EPA, 1995a). Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

1.0 Introduction

This document presents the derivation of the noncancer dose-response assessments for oral exposure [the oral reference dose or RfD] and for inhalation exposure [the inhalation reference concentration or RfC], and the cancer hazard and dose-response assessments.

The RfD and RfC are meant to provide information on long-term toxic effects other than carcinogenicity. The Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime. The inhalation reference concentration (RfC) is a continuous inhalation exposure estimate analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is expressed in units of mg/m^3.

The carcinogenicity assessment is meant to provide information on three aspects of the carcinogenic risk assessment for the agent in question: the U.S. EPA classification, and quantitative estimates of risk from oral exposure and from inhalation exposure. The classification reflects a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000.

Development of these hazard identifications and dose-response assessment for tributyltin oxide has followed the general guidelines for risk assessments as set forth by the National Research Council (1983). Other EPA guidelines that were used in the development of this assessment include the following: The Risk Assessment Guidelines (U.S. EPA, 1987), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), Guidelines for Reproduction Toxicity Risk Assessment (U.S. EPA, 1996b), (proposed) Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1995b), the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), (proposed) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988) , the Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995c), and Guidance on Risk Characterization (U.S. EPA, 1995a).

Literature search strategy employed for this compound were based on the CASRN and at

least one common name. As a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE AND MEDLINE backfiles. EPA also considered in the development of this document any pertinent scientific information submitted by the public to the IRIS Submission Desk.

2.0 Chemical and Physical Information Relevant to Assessments

IUPAC Name	Bis-[Tri-n-butyltin]-oxide
Primary Synonym	Tri-n-butyltin oxide (TBTO)
CAS Number	56-35-9
Molecular Formula	$C_{24}H_{54}OSn_2$
Structural Formula	$(CH_3CH_2CH_2CH_2)_3Sn\text{-}O\text{-}Sn(CH_2CH_2CH_2CH_3)_3$
Molecular Weight	596.07 g
Boiling Point	220-230 °C
Melting Point	<45 °C
Density	1.17 g/cc (20 °C)
Vapor Pressure	1×10^{-3} Pa (20 °C)
Henry's Constant	2×10^{-5} kPa x m^3mol (20 °C)
Conversion Factor	1 ppb = 26.6 µg/m^3

Tributyltin compounds are used primarily as biocides. Tributyltin derivatives, which are toxic to gram positive bacteria, are combined with gram negative bactericides for use as disinfectants on surfaces such as hospital floors and sports arenas. Formulations which release tributyltin oxide or tributyltin fluoride in a controlled fashion have been proposed for use as molluscicides against the snails which serve as vectors for the transmission of schistosomiasis to humans.

Tributyltin oxide is an effective biocidal preservative for wood, cotton textiles, paper, and paints and stains for residential homes. Tributyltin oxide is added as an antifouling agent in numerous formulations of marine paints. Paints containing up to 20% tributyltin prevent the attachment and growth of barnacles, plankton, algae, and other organisms to ship hulls.

Tributylin is present in most of these antifouling formulations as an organometallic polymer such as tributyltin (methacrylic-CO-methylmethacrylate) ester, also referred to as OMP-2. Tributyltin is slowly released from the painted surface as the polymer is hydrolyzed in sea water, providing protection against encrustations for as long as 4-5 years. See the review by Boyer (1989) for additional information.

3.0 Toxicokinetics Relevant to Assessments

A large body of information demonstrates that the critical effect (the toxic effect that occurs at the lowest dose) for TBTO is depression of thymus-dependent immunological responses. No relevant information on toxicokinetics is available.

Some recent studies suggest that the mechanism of the immunotoxic effects is related to induction of apoptosis, programmed cell death, within the thymus. Raffray and Cohen (1991) demonstrated that thymocytes in culture showed cellular changes consistent with apoptosis at concentrations of TBTO that did not affect cell viability. Raffray et al. (1993) showed that these effects occur independently of a requirement for protein synthesis and do not require fully conserved energetics (that is, the effects occur despite depression of ATP levels to less than 20% of control values). Raffray and Cohen (1993) demonstrated a correlation between reduction of thymus weight in animals given a single oral dose of TBTO and evidence of apoptosis (increased DNA fragmentation) in thymic cell isolates (principally thymocytes) isolated from the animals during the period of thymic involution. These workers also showed that dibutyltin, the major metabolite of tributyltin, is less effective in inducing apoptosis in vitro, suggesting that the in vivo toxicity is directly attributable to tributytin. Pieters et al. (1994) reviewed the accumulated evidence and ideas regarding the mechanisms involved in the induction of thymic atrophy.

Organotin compounds, including tributyltin, have recently been shown to induce apoptosis in immortalized neuronal cell lines (Thompson et al., 1996). There is, however, no correlation between these data and on TBTO induced neurotoxicity in vivo.

Data from another group of researchers suggest that the toxicity of TBTO could be mediated by alteration in the structure of mitochondria and depression of ATP synthesis (Hara et al., 1994; Yoshizuka et al., 1992a; Yoshizuka et al., 1992b).

4.0 Hazard Identification

4.1 Studies in humans

No information was located regarding toxicity of tributyltin oxide in humans following oral exposure. Human data summarized by Boyer (1989) suggest that tributyltin oxide is a potent non-allergenic dermal irritant. There are several case reports claiming irritation of the respiratory tract following acute inhalation exposure of people to tributyltin oxide (Anon., 1991; Hay and Singer, 1991; and Shelton et al., 1992). None of these reports, however, contains sufficient

information to characterize the exposure-response relationship for the reported effects.

4.2 Prechronic and Chronic Studies and Cancer Bioassays in Animals

Oral Studies

Monkeys

Effects of tributyltin oxide (purity 96%) on hematology and serum chemistry were assessed in groups of 3 and 4 adult male cynomolgus monkeys that ingested doses of 0 and 0.160 mg/kg, respectively, 6 days/week for 22 weeks (0 and 0.14 mg/kg-day) (Karrer et al., 1992). The tributyltin oxide was dissolved in vegetable oil and added to Tween 80-augmented pear juice that the monkeys drank. Study endpoints consisted of clinical observations, body weight, and standard hematology and clinical chemistry indices, including serum immunoglobulin (IgM and IgG) levels.

A progressive decrease in total leukocyte counts occurred during the first 10 weeks of exposure [significantly ($p<0.05$) lower than controls at weeks 8 and 10; 67% of control value at week 10]. Leukocytes subsequently increased and were similar to controls between weeks 10 and 16, but decreased again between weeks 16 and 20 (61.5% of control value at week 20, $p<0.05$). No significant alterations in differential leukocyte count, serum immunoglobulins or other study parameters were observed. Based on decreased total leukocyte levels, 0.14 mg/kg-day (the only dose tested) is a LOAEL in monkeys.

Dogs

Groups of 4 male and 4 female Beagle dogs were treated with tributyltin oxide [purity 95.9% (Batch 1) or 97.4% (Batch 2)] in arachis oil by gavage in dosages of 0, 0.2, 1 or 5 mg/kg-day for 12 months (Schuh, 1992). Study endpoints included clinical signs of toxicity, body weight, food consumption, ophthalmoscopy, hematology, serum chemistry (including immunoglobulins), urinalysis, electrocardiology, neurological responses, organ weights, gross pathology and histology. Gross findings were microscopically examined only "if necessary for clarifying a diagnosis." Histological examinations of liver, kidney, heart, brain, spinal cord, spleen, lymph nodes (mesenteric and iliac), adrenals, pituitary and intestine were performed on all animals; other tissues were examined only in the control and high dose groups.

Five dogs (2 male, 3 female) in the high dose group were sacrificed in moribund condition during weeks 32-47. Effects in these animals included clinical signs (apathy, atactic gait, emaciation and dehydration), severely reduced food intake and body weight loss, changes in clinical chemistry and urine indices (e.g., increased serum GPT, GGT and inorganic phosphate, and decreased serum albumin, urine pH and urine specific gravity), and histopathology (e.g., hepatocellular ballooning and single-cell degeneration, and atrophy of bone marrow, spleen, testis and epididymis). Other changes in treated dogs included decreased numbers of circulating reticulocytes and lymphocytes and serum levels of immunoglobulins in the low and high dose groups, and increased serum alkaline phosphatase and total alpha globulins and atrophy of lymph

nodes in the mid and high dose groups. A NOAEL and/or LOAEL based on immunosuppression or other effects cannot be clearly identified due to deficiencies with respect to study conduct and reporting. Study deficiencies include (1) irregular procedures and sampling procedures that are suggestive of significant protocol deviations, (2) data suggestive of exposure of control animals to the test material [i.e., tin was found in the urine of control animals after the first dose and after 52 weeks of dosing, and the level of urinary tin increased with time in both control and test groups], (3) apparently incomplete and lack of analyses of dosing solutions for the test and control groups (suggesting possible significant dosing errors), (4) considerable variation in animal body weights (and likely ages) in test and control groups (precluding reliable analyses of body weight, food consumption and other study parameters), (5) insufficient histopathology examinations (not performed on all gross lesions and inconsistently performed on lower dose animals when findings were noted at higher doses), and (6) incomplete tabulations of test and pre-test results precluding comprehensive assessment and comparison of all relevant data.

Rats

In a carcinogenicity/chronic toxicity study, groups of 60 male and 60 female rats were exposed to dietary tributyltin oxide for 2 years (Wester et al., 1990, 1988, and 1987). Based on estimates of average body weight and food consumption from reported data, ingested dosages are approximately 0.019, 0.19 or 2.1 mg/kg-day in males and 0.025, 0.25 or 2.5 mg/kg-day in females. Endpoints that were evaluated included clinical abnormalities, survival, body weight, and food and water consumption. Hematology, urinalysis, clinical chemistry (including immunoglobulins IgG, IgM and IgA) and endocrinology (thyroxin and free thyroxin, thyrotropin, luteinizing hormone, follicle stimulating hormone, insulin) were evaluated in 10 rats/sex/dose after approximately 3, 12 and 24 months (endocrinology not assessed at 3 months). Organ weights and histology were evaluated in 10 rats/sex/dose after 12 and 24 months, and histology also was evaluated in all moribund rats as well as rats surviving until 24 months.

No treatment-related adverse changes were found in males or females at the lowest dose. Food consumption was slightly increased in all dose groups in males throughout the study (P value not reported). Water consumption was increased at the mid and high dose groups in males after week 24 (approximately 20 and 40% higher than controls, respectively). Urine production was increased at the high dose at 12 and 24 months (males only at 3 months, quantitative data not reported), creatinine concentration was decreased in the high dose group at 12 and 24 months, and urine osmolarity was decreased in high dose females at 24 months. No changes were found in urinary protein concentration or serum creatinine clearance. The changes in water intake and urinary indices are suggestive of impaired renal concentrating capacity and may be associated with age-related degenerative changes in the kidney.

Hematological changes included significantly increased thrombocyte levels in mid and high dose females at 24 months [30.9% ($p<0.01$) and 45.5% ($p<0.001$) higher than controls, respectively] and in high dose females at 12 months (27.3% higher than controls, $p<0.001$)]. The increase in thrombocytes is not considered adverse. Minor changes in total and differential leukocyte counts did not show a consistent response with increasing dose or exposure time and

are not considered biologically significant. Significant ($p<0.05$ or 0.01) changes in other hematologic and related indices occurred only in high-dose rats at 12 months (not found at 24 months), including decreased hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin levels in males, and increased serum isocitrate dehydrogenase levels (indicative of young erythrocytes) in females.

Serum immunoglobulin levels were significantly increased ($p<0.05$, Student's t-test) in the high dose group. Concentrations of IgA were increased in both sexes after 12 and 24 months; at 24 months, levels of IgA were 508% of the control value in males ($p<0.001$), and 294% of the control value in females ($p<0.01$). Concentrations of IgG were significantly ($p<0.01$) reduced in females after 3 months (42% of the standard serum value compared to 69-71% in controls and other treated groups) and 12 months (80% compared to 124-127%), but not after 24 months or in males. Concentrations of IgM were increased in both sexes after 3, 12 and 24 months; at 24 months, IgM level was 258% of the standard serum value in males ($p<0.01$), and 240% of the standard value in females ($p<0.01$).

Other effects occurred predominantly in high-dose rats, including increased mortality after approximately week 90 and 96, respectively. At termination survival in females in the high dose group was 54% versus 74% in controls; survival in males in the high dose group was 40% versus 60% in controls. Body weight gain was reduced (P values not reported) in high dose males and females after week 67 and 81, respectively; terminal body weights at this dose were approximately 13% (male) and 9% (female) lower than controls.

Clinical chemistry changes in high dose males included significantly (predominantly $p<0.01$ or 0.001) increased serum alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase at 3, 12 and 24 months. Alkaline phosphatase levels also were increased in high dose females, but there were no consistent changes in alanine aminotransferase or aspartate aminotransferase. The increases in serum enzymes were less than two-fold higher than control values and are not considered adverse in this study.

Absolute liver, kidney, adrenal gland (male only) and heart (male only) weights were increased and thyroid weight (female only) was decreased in high dose rats at study termination; relative organ weights were not reported. The liver weight was increased 36% and 29% in males and females, respectively; the kidney weight was increased 29% and 33% in males and females, respectively; the adrenal weight in males and females was increased 630% and 44%, respectively; the heart weight in males was increased 13%; and the thyroid weight in females was decreased 26%.

Treatment-related nonneoplastic histological changes occurred in the liver, spleen and thyroid of high dose males and females. Histologic effects after 12 months included slight bile duct changes (characterized by hyperplasia, cellular hypertrophy and minimal infiltration of mononuclear cells or by cholangiofibrosis), decreased hemosiderin content in spleen (qualitative analysis only), and decreased thyroid follicular epithelial cell height. Examination after 24 months

showed that only the thyroid histologic changes persisted. There were no accompanying significant changes in concentrations of serum thyroid hormones. The incidence and severity of age-related degenerative changes in the kidney [nephrosis and vacuolation and pigmentation of the proximal tubular epithelium (suggestive of iron and/or lipofuscin)] were increased in high dose males and females after 24 months.

Based on the constellation of changes observed at the highest dose, the LOAEL for chronic toxicity is 2.1 mg/kg-day and the NOAEL is 0.19 mg/kg-day.

Mice

Tributyltin oxide (purity 97.1%) was fed to groups of 50 male and 50 female CD-1 mice in dietary concentrations of 0, 5, 25 or 50 ppm for 18 months in a study primarily designed to assess carcinogenicity (Daly, 1992). Based on food consumption and body weight data, mean compound intake was reported to be 0, 0.7, 3.7 or 7.7 mg/kg-day in males and 0, 0.9, 4.8 or 9.2 mg/kg-day in females. Other endpoints that were evaluated included clinical observations, limited hematology (total and differential WBC counts and RBC morphology in 10 mice/sex/group at 12 and 18 months), organ weights, gross pathology and histology. Clinical chemistry and immunologic assays were not performed.

Statistically significant decreases in survival occurred in treated mice of both sexes. In males, survival after 18 months was 67, 52, 42 and 42% in the control, low, mid, and high dose group, respectively ($p<0.05$, all doses). The overall survival of the low dose males (52%) was within the range of the controls (45-78%). Because the difference in survival between the low dose and control males became apparent late in the study (beginning at 15 months) and was marked at termination (54% versus 71% in controls), the decreased survival in the low dose males is considered treatment-related. Survival in females at 18 months was 59, 48, 40 and 27% in the control, low, mid, and high dose group, respectively ($p<0.05$ except for low dose group). No information on cause(s) of death was available. Other treatment-related effects included significantly decreased food consumption and increased absolute and relative liver weights in females at the high dose. Incidences of gross liver enlargement and discoloration were slightly increased in both sexes in all dose groups. The gross liver changes are not considered biologically significant because of the slight changes and absence of hepatic histopathologic alterations. Increased incidences of common spontaneous non-neoplastic lesions, particularly glomerular/interstitial amyloidosis of the kidney, were found. Incidences of renal amyloidosis were increased in females in all dose groups (50, 67.7 and 78.4%, respectively, compared to 34.8% in controls) but not in males. The progression of this lesion appeared to be more rapid in both sexes at the two highest doses, indicating a compound-related effect. This study identifies a FEL of 0.7 mg/kg-day (the lowest dose tested) based on decreased survival.

Inhalation Studies

Schweinfurth and Gunzel (1987) summarized the results of several short term inhalation studies in laboratory animals. After a single four hour exposure of rats to aerosols of TBTO,

signs of irritation (nasal discharge, lung edema, and congestion of the pulmonary circulation) and enteritis were observed. The LC_{50} was 77 mg/m^3 (total particles) or 65 mg/m^3 (particles with a diameter <10 µm). In guinea pigs exposed to aerosols of TBTO in olive oil at 200 mg/m^3 and above, death occurred within one hour of exposure. Ten male and ten female rats were exposed to almost saturated vapors of TBTO without a single death occurring during exposure for seven hours or the following 14-day observation period. Only minor clinical signs (slight nasal discharge directly after exposure) were noted. For this study the authors reported no information on particle size or the endpoints evaluated.

An inhalation study was conducted in rats for 29-32 days (Schweinfurth and Gunzel, 1987). Rats (10 males and 10 females per dose) were exposed in "nose only" chambers for 4 hours to doses of 0, 0.03 (vapor), 0.16 (vapor) or 2.8 (aerosol) mg/m^3, 5 days per week for a total of 21-24 treatments. At the highest dose, severe toxic effects were produced. Mortality was 5/10 in males and 6/10 in females. In addition inflammatory reactions in the total respiratory tract (not specified further) and histological changes (not further specified) in the lymphatic organs were observed. No local or systemic changes were observed at the lower doses. The authors, however, did not report what endpoints were evaluated.

Oral Studies for Carcinogenicity

Rats

In a carcinogenicity/chronic toxicity study, groups of 60 male and 60 female rats were exposed to dietary tributyltin oxide for 2 years (Wester et al., 1990, 1988, and 1987). Based on estimates of average body weight and food consumption from reported data, ingested dosages are approximately 0.019, 0.19 or 2.1 mg/kg-day in males and 0.025, 0.25 or 2.5 mg/kg-day in females. Food consumption in males was slightly increased in all dose groups throughout the study (P values not reported). Increased mortality occurred in the high dose group after approximately week 90 in males and week 96 in females. At termination survival in females in the high dose group was 54% versus 74% in controls; survival in males in the high dose group was 40% versus 60% in controls. Body weight gain was reduced (P values not reported) in the high dose males and females after week 67 and 81, respectively; terminal body weights in the high dose group were approximately 13% (male) and 9% (female) lower than controls.

Neoplastic lesions were examined in the control and high-dose groups, and if differences were observed, the intermediate-dose groups were also examined for those tumor types. Increased incidences of benign pituitary tumors, pheochromocytomas in the adrenal medulla, and parathyroid adenomas were noted. These data are shown below.

Concentration of TBTO (mg/kg diet)	Total Pituitary Tumors for Groups of 50 Rats	
	Female	Male
0	22	34
0.5	32*	39*

5	22	29
50	35**	43***

Statistical analysis was carried out according to Peto, one-tailed and values marked with asterisks differ significantly from control values (* P<0.05; **P<0.01; ***P<0.001).

Concentration of TBTO (mg/kg diet)	Total Pheochromocytomas for Groups of 50 Rats	
	Female	Male
0	3	16
0.5	3	13
5	3	14
50	34***	33***

Statistical analysis was carried out according to Peto, one tailed. Values marked with asterisks differ significantly from the corresponding control values (*P<0.05; **P<0.01; ***P<0.001).

Concentration of TBTO (mg/kg diet)	Number of Adenomas/Number of Parathyroids Examined	
	Female	Male
0	0/64	0/39
0.5	0/44	2/50
5	1/40	1/51
50	1/44	6/43**

The value marked with asterisks differs significantly (chi-square test) from the corresponding control value (**P<0.01).

There are increases in the incidence of some benign spontaneous tumors at the high dose in some endocrine tissues. According to the authors, these tumors normally occur in this stain of rats with high and variable background incidence (Kroes et al., 1981; Wester et al., 1985). The reported background occurrence of pituitary tumors in females was 32% and 55% and in males was 34% and 66%; the reported background occurrence of pheochromocytomas in females was 10% and 12% and in males was 26% and 44%. The authors reported no data on the background occurrence of parathyroid tumors.

There was no significant endocrine imbalance documented in the study. No significant change was observed in the serum levels of TSH, LH, FSH, insulin, total T4, or free T4. There was, however, a decrease in the free T4:total T4 ratio for both sexes at 12 and 24 months in the high dose group, and after 12 months at the mid dose group. Although the pituitary tumors stained for the presence of prolactin, there was no correlation between the serum level of prolactin or the occurrence of hyperplastic or neoplastic mammary tissue and the presence of

pituitary tumor.

Although the data on tumor occurrence in this study are questionable, the tumors in these endocrine organs are of unknown biological significance for a human health risk assessment. The results are also inconclusive because of the increased mortality at the high dose and because the dose spacing reduces the statistical power of the study.

Mice

Tributyltin oxide (purity 97.1%) was fed to groups of 50 male and 50 female CD-1 mice in dietary concentrations of 0, 5, 25 or 50 ppm for 18 months (Daly, 1992). Based on food consumption and body weight data, mean compound intake was reported to be 0, 0.7, 3.7 or 7.7 mg/kg-day in males and 0, 0.9, 4.8 or 9.2 mg/kg-day in females. Statistically significant decreases in survival occurred in treated mice of both sexes. In males, survival after 18 months was 67, 52, 42 and 42% in the control, low, mid, and high dose group, respectively ($p<0.05$, all doses). Survival in females at 18 months was 59, 48, 40 and 27% in the control, low, mid, and high dose group, respectively ($p<0.05$ except for low dose group). No information on cause(s) of death was available. There were no statistically significant increases in the incidence of any tumors or groups of tumors in males or females. TBTO is not carcinogenic in this study in mice.

4.3 Reproductive/Developmental Studies

Oral Studies

Reproductive Studies

A two-generation reproduction study was performed in which groups of 30 male and 30 female Crl:CD(SD)BR rats (F0 generation) were fed tributyltin oxide (purity 97.1%) in dietary concentrations of 0, 0.5, 5 or 50 ppm for 10 weeks prior to mating and during cohabitation (7 days), with exposure of females continuing during gestation and lactation (Schroeder, 1990). Groups of 30 male and 30 female F1 rats were fed the parental diets for 15 weeks and mated to produce the F2 generation. Based on food consumption and body weight data, mean compound intake during the premating period was 0, 0.02, 0.29 and 2.95 mg/kg-day for F0 males; 0.03, 0.34 and 3.43 mg/kg-day for F0 females; 0, 0.03, 0.36 and 3.98 mg/kg-day for F1 males; and 0.04, 0.44 and 4.42 mg/kg-day for F1 females. Other endpoints evaluated in F0 and F1 adults included clinical observations, dates of mating and parturition, gestation duration, maternal behavioral abnormalities, organ weights, gross pathology, histopathology and numbers of implantations. Evaluation of F1 and F2 offspring included numbers of live and dead pups, body weight and clinical observations at birth and throughout the preweaning period, sex distribution, and gross pathology on dead and selected weaned pups (histology was not evaluated).

Body weight gain was significantly ($p<0.05$) reduced in high dose F1 males and females (approximately 19% and 15% lower than controls, respectively) at the beginning of the premating growth period, and remained reduced in males throughout the entire (15-week) premating period

(>8%, $p<0.01$). No significant changes in body weight gain occurred in F1 males during the postmating period, although body weight was significantly lower than controls at week 38 (>8%, $p<0.01$) at the high dose. No treatment-related effects on food consumption or gross or histopathology were found in either sex or generation. Absolute and relative thymus weights were slightly but not significantly ($p>0.05$) lower than control values in F0 males at the high dose (8% and 8%, respectively) and F0 females at the high dose (13% and 17%), and significantly ($p<0.01$) lower than controls in F1 males at the high dose (38% and 31%) and F1 females at the high dose (28% and 26%). No histological changes in the thymus were found. The lack of thymic histopathology does not necessarily indicate that the decreases in thymus weight are not adverse, because decreased thymus weight could be due to immunologically significant reduced numbers of lymphocytes with no accompanying tissue pathology. Based on decreased thymus weight, the LOAEL for parental toxicity is 2.95 mg/kg-day in males and 3.43 mg/kg-day in females. The NOAEL for parental toxicity is 0.29 mg/kg-day in males and 0.34 mg/kg-day in females.

Compound-related reproductive effects and developmental effects were limited to decreased pup body weight during lactation in both generations at the high dose. Body weights were significantly lower than controls on lactation days 7, 14 and 21 in F1 offspring (10, 14 and 17%, respectively) and F2 offspring (14, 17 and 20%, respectively). Other indices were comparable to control values in both generations. Based on the lack of effects on reproductive parameters, the NOAEL for reproductive toxicity is 4.42 mg/kg-day (the highest dose tested). Based on decreased pup weight during lactation, the LOAEL for developmental toxicity is 3.43 mg/kg-day and the NOAEL is 0.34 mg/kg-day.

Developmental Studies

Rats

Groups of 24 mated female CD Sprague-Dawley rats were treated with tributyltin oxide (purity 96.9%) in corn oil by gavage at doses of 0, 5, 9 or 18 mg/kg-day on days 6-19 of gestation (Schroeder, 1981). The doses are based on analyses of dosing solutions (data not reported); original assigned doses were 6, 12 and 24 mg/kg-day. The dams were sacrificed on gestation day 20. Maternal endpoints assessed included clinical signs, body weight, food consumption, and pregnancy efficiency and outcome indices (pregnancy rate and numbers of implantations, resorptions and fetuses). Fetal endpoints assessed included sex distribution, body weight, and external, visceral and skeletal abnormalities.

Clinical signs (staining of the fur in the anogenital area) and decreased body weight gain during days 6-20 occurred in maternal rats at the mid and high dose. Actual weight gain was 4.5% higher, 1.8% lower and 26% lower than controls at the low, mid, and high dose, respectively. Adjusted weight gain (excluding uterus) was 5.5, 22.2 and 69.4% lower than controls at the low, mid, and high dose, respectively. The decreases in actual and adjusted body weight gains were statistically significant ($p<0.01$) in the high dose group and apparently related to increased resorptions. Based on decreased body weight gain and anogenital staining during

gestation, the LOAEL for maternal toxicity is 9 mg/kg-day and the NOAEL is 5 mg/kg-day.

Indications of developmental toxicity were observed in all dose groups. Effects included dose-related increased incidences of fetal ossification variations, particularly asymmetric sternebrae, rudimentary structures and 14th rib pair. Percentages of fetuses with asymmetric sternebrae #2, #3 and #4 ranged from 55.9-79.0% in treated rats vs. 34.7% in controls, 39.5-90.5% vs. 31.4% and 58.2-93.4% vs. 44.6%, respectively. Percentages of exposed fetuses with unilateral rudimentary structures, bilateral rudimentary structures and 14th rib pair ranged from 10.7-19.9% vs. 8.3% in controls, 23.7-39.4% vs. 8.3% and 2.3-18.2% vs. 0%, respectively. Increased incidences of other ossification variations (asymmetric sternebrae #1 and #5, cervical unilateral, and bilateral ossifications, unossified caudal vertebrae) and some skeletal malformations (scrambled sternebrae and cleft palate) were observed at the high dose. Evaluation of these data is complicated by lack of statistical analysis and litter incidences, however, percentages of fetuses with at least one skeletal ossification variation were significantly ($p<0.01$) increased at the mid and high dose. Other effects occurred at the high dose, including significantly decreased percentage of fetuses to implants (86.8% compared to 94.7% in controls, $p<0.01$), increased percentage of resorptions (13.2% compared to 5.3% in controls, $p<0.01$) and decreased fetal weight (16% lower than controls in both sexes, $p<0.01$). Due to increases in fetal skeletal ossification variations that were evident at the lowest tested dose and dose-related, this study identifies a LOAEL of 5 mg/kg-day for developmental toxicity.

Postnatal developmental toxicity was evaluated in Long-Evans rats that were pre- or postnatally exposed to tributyltin oxide (purity 97%) in corn oil by gavage (Crofton et al., 1989). Rats were administered doses of 0, 2.5, 5, or 10 mg/kg-day (15-16 rats/group) or 0, 12 or 16 mg/kg-day (18 rats/group) on days 6-20 of gestation. Endpoints assessed included maternal body weight, implantation sites, litter indices (number, size and weight) and external malformations. Additionally, offspring from the rats exposed to 0-10 mg/kg-day were evaluated for postnatal toxic signs, survival, body and brain weights, developmental landmarks, motor activity and acoustic startle response through day 110.

Effects observed included vaginal bleeding in 60 and 75% of the rats administered 12 and 16 mg/kg-day, respectively. Maternal body weight gain was significantly reduced at 10 and 12 mg/kg-day and body weight was decreased at 16 mg/kg-day. One dam in each of the 10, 12 and 16 mg/kg-day groups died during the study. Litter size and pup body weight (at postnatal day 1 and 3) were significantly reduced at 10, 12 and 16 mg/kg-day. Litter sizes on postnatal day 1 were 50, 73 and 96% lower than control values at 10, 12, and 16 mg/kg-day, respectively. Pup survival on days 1-3 also was decreased in these groups. There were no significant changes in litter size or neonatal pup weight in the groups treated with 2.5 or 5 mg/kg-day. No clear treatment-related malformations were observed. Cleft palate was found in 3% (2/71) of 12 mg/kg-day offspring born dead, however, no malformations occurred in live or dead offspring in the other dose or control groups. Postnatal mortality was increased (14%) on day 21 at 10 mg/kg-day, and body weight gain was decreased on postnatal day 5 (but not at days 1, 3, 10, 15, or 19) at 5 mg/kg-day and on postnatal days 1, 3, 5, 10, 15, and 19 at 10 mg/kg-day. There was a

significant delay in age of vaginal opening in 10 mg/kg-day offspring (sexual maturity in males was not altered). There was an apparent transient decrease in motor activity on postnatal day 14 at all doses. Motor activity was approximately 60% lower than in controls in the 2.5, 5 and 10 mg/kg-day groups on postnatal day 14, but not on days 13 or 15 to 21. The apparent transient decrease at postnatal day 14 is not considered compound related. Motor activity was significantly reduced on postnatal days 47 and 62 at 10 mg/kg-day but not at lower doses. No effects on acoustic startle response were observed in the prenatally exposed rats. Whole brain, cerebellum and hippocampus weights were significantly reduced following exposure to 10 mg/kg-day (measured on postnatal day 110).

In a companion study, survival, body and brain weight, developmental landmarks, motor activity, and acoustic startle response were assessed in the offspring of previously unexposed rats that were treated with a single oral dose of 0, 40, 50 or 60 mg/kg tributyltin oxide on postnatal day 5 and sacrificed on day 64. Mortality was increased in rats treated with 50 or 60 mg/kg (32%), and body weight was 25% lower than controls at all dosages (40-60 mg/kg) by day 10. Body weight remained reduced on postnatal day 30, but recovered by postnatal day 62 at 40 and 50 mg/kg (still decreased at 60 mg/kg). No changes in motor activity were observed. Amplitude of response in the acoustic startle test was decreased in all groups (40-60 mg/kg) on day 22, but this effect did not persist to day 47 or 62 and was not accompanied by significant alterations in latency to onset or number of responses. Whole brain and cerebellum weights were significantly reduced at 60 mg/kg (measured on postnatal day 64).

Based on decreased body weight gain the NOAEL and LOAEL for maternal toxicity are 5 and 10 mg/kg-day, respectively. The LOAEL for developmental toxicity is 10 mg/kg-day. The effects observed at this dose include reduced litter size, decreased pup survival on postnatal days 1 and 3, increased postnatal mortality, decreased weight gain, delay in vaginal opening, and reduced motor activity. The NOAEL for developmental toxicity is 5 mg/kg-day.

Mice

Groups of 8 Swiss albino mice were treated with 0, 5, 20 or 40 mg/kg-day doses of tributyltin oxide (purity >96%) in vegetable oil by gavage on gestation days 6-15 (Baroncelli et al., 1990). The dams were sacrificed on gestation day 17. Maternal toxicity endpoints included clinical signs, survival, body weight and relative organ weight and gross pathology of brain, kidneys, liver and spleen. Developmental toxicity endpoints included numbers of implantations, live and dead fetuses and resorptions; placental and fetal body weights; and gross external abnormalities. Visceral or skeletal examinations of fetuses were not performed.

No maternal deaths were observed. Maternal body weight and body weight gain were approximately 21% and 50% lower than control values, respectively, on gestation day 17 at the high dose. Weight loss was rapid during the first days of exposure. Other effects at the high dose included piloerection, lethargy, hunched posture and vaginal bleeding. Relative spleen weight showed a dose-related decrease compared to controls (approximately 20-40%, $p<0.05$) in all dose groups. The toxicological significance of the change in spleen weight is unclear as histology and

other pertinent endpoints were not evaluated and there were no macroscopic changes in the spleen. Based on decreased body weight gain and clinical signs, the NOAEL and LOAEL for maternal toxicity are 20 and 40 mg/kg-day, respectively.

Indications of developmental toxicity occurred only in the high dose group. Of the 8 dams, 5 had totally resorbed litters, 3 had vaginal bleeding on gestation days 8-9 and 3 had undersized fetuses (gestation day 12-13 size on day 17). Fetal body weight was approximately 21% lower than controls in the high dose group. Dose-related increased placental weight (approximately 11, 21 and 25% at 5, 20 and 40 mg/kg-day, respectively, $p<0.05$ all doses) and decreased fetal/placental weight ratio were observed, however, the toxicological significance of increased placental weight is unclear. Based on increased resorptions and decreased body weight the NOAEL and LOAEL for developmental toxicity in mice are 20 and 40 mg/kg-day, respectively.

Groups of 118, 12, 10, 22, 20, 12 and 6 mated NMRI mice were treated with 0, 1.2, 3.5, 5.8, 11.7, 23.4 or 35 mg/kg-day tributyltin oxide in olive oil by gavage on gestation days 6-15 (Davis et al., 1987). Animals were sacrificed on gestation day 18. Maternal endpoints included pregnancy rate, survival and body weight. Developmental toxicity endpoints included implantations, resorptions, live fetuses, fetal weight and external, visceral and skeletal abnormalities.

Slight maternal toxicity, indicated by reduced body weight gain (not quantified), was observed at 11.7 mg/kg-day and higher dosages. Fetal effects also occurred at these maternotoxic dosages, including dose-related increased frequency of cleft palate. Percentages of fetuses with cleft palate were 0.7, 0.8, 3, 2, 7, 24 and 48% at 0, 1.2, 3.5, 5.8, 11.7, 23.4 and 35 mg/kg-day, respectively. Because 11 out of a total of 14 cleft palate-affected fetuses were clustered in one of 18 affected litters (15 litters were not affected), cleft palate occurs spontaneously in NMRI mice, and cleft palate can be induced non-specifically (e.g., by stress or malnutrition), the investigators concluded that the effect is likely secondary to maternal toxicity rather than a direct teratogenic effect of tributyltin oxide. Effects observed at 23.4 and 35 mg/kg-day included reduced average fetal body weight (8 and 20% lower than controls, respectively), increased number of fetuses with minor skeletal abnormalities (28 and 29% compared to 0.5% in controls) (e.g., fusion of bases of os occipitalis) and skeletal variations (43 and 43% compared to 10% in controls) (e.g., irregular ossification of sternebrae centers). Resorption rate was increased at 35 mg/kg-day (58.8% vs. 8.3-15.7% in control and other groups; number of resorptions/litter and percentage of litters with resorptions also were increased). In an accompanying experiment, no embryonic damage (assessed using electron microscopy) was found in mice 26 and 48 hours after treatment with a single 30 or 110 mg/kg dose of tributyltin oxide on gestation day 10. Based on reduced body weight gain in dams and increased cleft palate in fetuses, the LOAEL for maternal and developmental toxicity is 11.7 mg/kg-day. The maternal and developmental NOAEL is 5.8 mg/kg-day.

Pregnant Swiss mice were treated with 0, 5, 10, 20, or 30 mg/kg body weight on gestational days 6-15 (Baroncelli et al. 1995). At birth litters were normalized to eight pups and postnatal

evaluation of pup growth rate and behavioral observations of dams were conducted. Dam weight gain was not impaired during the exposure period (gd 6-15). Dam weight gain was impaired at 10, 20, and 30 mg/kg (15%, 13%, and 20%, respectively) between gd 16 and 18. Maternal weight gain between gd 6 and postnatal day 1 decreased in all dose groups (18%, 18%, 34%, and 53%, respectively). A high incidence of early parturitions was observed in all dose groups (19.2%, 12.0% , 8.3%, and 14.3%, respectively, versus 0% in controls). There was also a change in delayed parturitions (0%, 16.0%, 27.8%, and 0%, respectively, versus 5.9% in controls). There was no correlation in early or delayed parturitions with fetal mass. At birth, only the 20 and 30 mg/kg dose groups showed reduced litter size and reduced pup weight. Only the highest dose showed a decrease in number of pups per litter. All the treated dams showed a significant increase in resorptions. The number of pups per implantation site was 90.4%, 88.4%, 80.6%, and 88.5%, respectively, versus 96.8% in controls. Body weight gain was reduced in pups during the first week of life at doses of 10 and 20 mg/kg (17% and 21%, respectively), but not at doses of 5 and 30 mg/kg. Maternal weight gain during the lactation period was reduced at doses of 20 and 30 mg/kg (data were imprecisely reported). Postnatal death rate and growth rate of treated pups were affected by altered maternal behavior. Pups, apparently viable and with normal weight, were found often scattered throughout the cage with signs of wounds, and the percentage of dams that has not built a nest increased in the 10, 20, and 30 mg/kg dose groups. Total absence of parental care was noted in many litters, and many infanticidal events were reported. Based on the reduction in maternal weight gain from gd 6 to pnd 1, the increase in early parturitions, and the increased number of resorptions, this study established a LOAEL of 5 mg/kg-day (the lowest dose tested) for maternal toxicity in mice.

The effect of in utero TBTO exposure on hematological parameters in neonates, pups during nursing, and dams in the same period were investigated in Swiss mice (Karrer et al., 1995, a companion study to Baroncelli et al., 1995). The dams were gavaged at doses of 0, 5, 10, or 20 mg/kg body weight on gestational days 6-15. At birth litters were culled to eight pups. Analysis of blood was conducted on excess pups. On post natal days 7, 14, and 21 the entire litters were sacrificed and blood of dams and pups was analyzed. In dams and pups no significant differences were found in blood composition, or in spleen or thymus weight at any dose. In neonates the only effect noted was a statistically significant increase in mean corpuscular volume at all doses (9%, 9%, and 7% at 5, 10, and 20 mg/kg-day, respectively). The effect did not become more severe with increasing dose and was not observed in pups at any time point. Accordingly, this change is not considered biologically significant. This study establishes a NOAEL of 20 mg/kg-day (the highest dose tested) for effects on blood composition in dams, neonates, and pups.

Data with mouse limb buds in culture show that a concentration of TBTO as low as 0.1 µg/mL (50 nM) causes profound malformations of the skeletal elements of the limb (Barrach and Neubert, 1986; Krowke et al., 1986). No suggestion of these types of malformations, however, have been observed in in vivo studies by this same research group (Davis et al., 1985).

4.4 Other Studies

4.4.1 Immunotoxicity

A large number of studies have been conducted showing that TBTO causes depression of immune functions dependent on the thymus. The studies following are grouped according to length of exposure. The chronic study conduced by Vos et al. (1990) shows effects on thymus-dependent immune responses at a dose lower than any other toxic effect. Accordingly this study is used to establish the NOAEL/LOAEL, Benchmark Dose, and Reference Dose.

Immunotoxicity was evaluated in four separate experiments in which groups of 10 male and 10 female weanling Sprague-Dawley rats (four to five weeks old at initiation of treatment) each were fed tributyltin oxide (purity 96.5%) in concentrations of 0, 0.5, 2, 5 or 50 mg/kg diet for at least 28 days (Verdier et al., 1991). The authors stated that this dietary concentration of 5 mg/kg was equivalent to a dose of 0.5 mg/kg body weight-day. The doses for the study are 0.05, 0.2, 0.5 and 5 mg/kg-day. Clinical signs, body weight and food and water consumption were evaluated in all animals throughout the study. Hematology (8 standard indices) and serum chemistry (blood urea nitrogen, creatinine, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) were assessed in 10 rats/sex/dose after 4 weeks of treatment. Necropsies also were performed after 4 weeks and included evaluation of absolute relative organ weight (brain, liver, spleen, thymus and iliolumbar lymph nodes) and histology (iliolumbar/mesenteric lymph nodes and thymus, including thickness of thymic cortex and numbers of primary and secondary follicles in mesenteric lymph nodes) on 10 rats/sex/dose, and total cell count and cell viability of splenic and thymic cells in 5 rats/sex/dose. Immunotoxicity assays were performed on 10 rats/sex/dose after 34-36 days of exposure and included splenic plaque-forming cell response to sheep erythrocytes, delayed-type hypersensitivity against bovine serum albumin and splenic clearance of Listeria monocytogenes.

No treatment-related effects occurred at doses of 0.05, 0.2, and 0.5 mg/kg-day. Effects observed in males at 5 mg/kg-day included slightly and inconsistently reduced body weight gain accompanied by slightly reduced food and water consumption (not quantified), decreased absolute liver weight (not quantified), and 30% decreased relative thymus weight. Clearance of L. monocytogenes was moderately suppressed at 5 mg/kg-day [16% ($p<0.05$) increase in males and 18% ($p<0.01$) increase in females in the number of bacteria per spleen]. The splenic plaque-forming cell response was significantly ($p<0.05$) increased at 0.2 and 5 mg/kg-day in males (42 and 37% higher than controls). This change, however, is not considered compound-related as there was no consistent changes with increasing dose and all values remained in the range of historical controls. Based on the reduced thymus weight and reduced clearance of L. monocytogenes, the LOAEL is 5 mg/kg-day and the NOAEL is 0.5 mg/kg-day.

Immunotoxicity was evaluated in weanling SPF-derived Wistar rats fed tributyltin oxide (purity 95.3%) in dietary concentrations of 0, 5, 20, 80 or 320 ppm for 4 weeks (10 males and 10 females per dose); 0, 20 or 80 ppm for 6 weeks (8-10 males per dose); or 0, 80 or 320 ppm for 3-42 days (4-8 males per dose) (Vos et al., 1984; Krajnc et al., 1984). The dietary concentrations of 5, 20, 80 and 320 ppm provided estimated doses of 0.5, 2, 7 and 30 mg/kg-day (U.S. EPA,

1988). The 4-week study was a dose range-finding experiment which evaluated clinical signs; food and water consumption; hematology, serum chemistry (including IgG and IgM) and urinalysis values; organ weights; and gross and histopathology (thymus, spleen, mesenteric lymph nodes, liver, thyroid and adrenals). The main objective of the 6-week study was evaluation of immune and endocrine function. Immunologic endpoints included mitogenic responses of thymus and spleen cells to phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM) or E. coli lipopolysaccharide (LPS); numbers of viable nucleated splenic lymphocytes (subpopulations of T and B cells determined by cell surface marker analysis); delayed-type skin hypersensitivity reaction to ovalbumin and tuberculin; resistance to oral infection by Trichinella spiralis larvae; IgG, IgM and/or IgE responses to sheep red blood cells, ovalbumin, T. spiralis and tetanus toxoid; splenic clearance of Listeria monocytogenes, phagocytizing and killing capacity of spleen and peritoneal macrophages, natural cell-mediated cytotoxicity of spleen and peritoneal cells, and susceptibility to endotoxin from E. coli LPS. Endocrine function was assessed by measurement of serum concentrations of thyroxin, thyroid stimulation hormone (TSH), insulin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and corticosterone. Hematology, serum iron, serum isocitrate dehydrogenase activity, and histology of thyroid and pituitary also were evaluated in the 6-week study. In the 3-42-day study, serum IgM and IgG concentrations were measured up to exposure day 42 and number and viability of thymus, spleen and bone marrow cells were assessed up to exposure day 20.

Changes observed in the 4-week study included significantly reduced IgG levels in males at 7 mg/kg-day and both sexes at 30 mg/kg-day (39% and 61-70% lower than controls, respectively), increased IgM levels in both sexes at 7 and 30 mg/kg-day (32-45% and 51-123% higher), reduced leukocyte count in males at 7 mg/kg-day and both sexes at 30 mg/kg-day (15% and 39-43% lower than controls, respectively). Other effects included dose-related, slightly increased serum alanine aminotransferase activity at 2 (males only), 7 and 30 mg/kg-day; slightly increased aspartate aminotransferase activity at 7 (females only) and 30 mg/kg-day; decreased relative thymus weight at 7 and 30 mg/kg-day and reduced serum insulin, serum glucose, liver glycogen and weight gain at 30 mg/kg-day. Food and water intake were reduced (approximately 50% lower than controls) and emaciation was apparent at 30 mg/kg-day. Lymph nodes showed evidence of hemorrhage (erythrocyte rosettes) in all exposure groups that was dose-related in incidence and severity; at 0.5 mg/kg-day, 7/10 males and 2/10 females had few to moderate rosettes compared to 1/10 in male and 0/10 in female controls. Other histopathologic changes in the 4-week study included slight and marked atrophy in thymic cortex (caused by lymphocyte depletion) at 7 mg/kg-day (2/10 males) and 30 mg/kg-day (9/10 males, 10/10 females); slight and slight-to-marked splenic atrophy at 7 mg/kg-day (1/10 males, 2/10 females) and 30 mg/kg-day (9/10 males, 10/10 females); and slight and slight-to-marked centrilobular hepatocyte atrophy accompanied by decreased glycogen at 7 mg/kg-day (0/10 males, 3/10 females) and 30 mg/kg-day (9/10 males, 10/10 females), respectively. Hepatic multifocal necrosis (parenchyma) and bile duct hyperplasia also occurred at 30 mg/kg-day.

In the 6-week study, immunity was suppressed at 2 and 7 mg/kg-day as shown by significantly decreased delayed-type hypersensitivity reactions to ovalbumin (43 and 55% lower

than controls after 24 hours), decreased resistance to T. spiralis infection (counts of larvae in muscle were 43 and 167% higher than controls; decreased expulsion of adult worms from small intestine, inflammatory reaction in parasitized muscle, and reduced serum IgE titers); suppressed response of thymocytes to stimulation with PHA and PWM; reduced numbers of TSH- and LH-immunoreactive pituitary cells; impaired splenic clearance of L. monocytogenes; and reduced activity of peritoneal cytotoxic (adherent) macrophages. Hematocrit and insulin levels also were reduced at 2 and 7 mg/kg-day. Other effects found at 7 mg/kg-day in the 6-week study included decreased delayed-type hypersensitivity reactions to tuberculin, reduced number of splenic T-cells, suppressed response of thymocytes to Con A stimulation and response of spleen cells to Con A, PHA and LPS stimulation; reduced IgG titers to sheep red blood cells; reduced natural killer cell activity in spleen; decreased serum iron, thyroxin and TSH; decreased absolute and relative thyroid weight; flattened epithelial lining in thyroid follicles; and increased LH and serum isocitrate dehydrogenase activity.

Effects observed in the 3-42-day study included significantly decreased serum IgM concentrations at 7 and 30 mg/kg-day after 42 and 28 days, respectively, and decreased IgG at 30 mg/kg-day after 28 days. After 20 days exposure, significant decreases were found in thymus, spleen and bone marrow cell counts and body weight at 7 and 30 mg/kg-day, and viability of thymus and spleen cells at 30 mg/kg-day.

Based on the hemorrhagic changes in lymph nodes in the 4-week study, the LOAEL is 0.5 mg/kg-day. A NOAEL was not identified.

Immunotoxicity was evaluated in groups of 8, 4 and 8 male Wistar rats fed diets containing 0, 5 or 25 ppm pure tributyltin oxide, respectively, or 0, 5 or 25 ppm commercial tributyltin oxide (80% pure containing various unspecified solvents and/or dispersants), respectively (Bressa et al., 1991). Half of the rats in the control and 25 ppm groups were treated for 1 week, and the remaining rats in these groups as well as the rats in the 5 ppm groups were treated for 4 weeks. Based on reported average tin consumption, the 5 and 25 ppm dietary levels of pure oxide provided dosages of 0.4 and 1.4 mg tributyltin oxide/kg-day, respectively, and that the 5 and 25 ppm commercial oxide diets provided dosages of 0.3 and 1.7 mg tributyltin oxide/kg-day. Body weight and food consumption were assessed throughout the study. Rats were sacrificed following the last exposure and gross pathology was evaluated in major organs and liver, spleen, thymus and brain were weighed. Histological examinations were performed on the tissues that were weighed as well as on mesenteric lymph nodes.

After one week of treatment, rats exposed to pure tributyltin oxide at 1.4 mg/kg-day, showed significantly increased relative liver weight (42%, absolute weight not affected), histological changes indicative of atrophy and lymphocyte depletion in the thymus cortex, and a decrease in thymus-dependent lymphocytes in the spleen. Thymus weight was not provided for this time-point. After 4 weeks exposure to 1.4 mg/kg-day, body weight gain, food consumption and relative and absolute thymus weights were significantly reduced, however, normal thymic histology was almost completely restored and no other treatment-related changes in organ weight

or histology were found. Following 4 weeks exposure to pure tributyltin oxide at 0.4 and 1.4 mg/kg-day (2/4 and 8/8 rats, respectively) or commercial tributyltin oxide at 0.3 and 1.7 mg/kg-day (2/4 and 8/8 rats, respectively), lymph nodes were markedly hemorrhagic and partially atrophic. Based on lymph node hemorrhage, this study identifies LOAELs of 0.4 mg/kg-day for pure tributyltin oxide and 0.3 mg/kg-day for commercial grade tributyltin oxide. A NOAEL was not established.

Effects of TBTO exposure on resistance to cytomegalovirus were investigated in male Wistar rats that were fed TBTO (purity 95.3%) at 0, 20, or 80 mg/kg diet for six weeks (Garssen et al. 1995). The treated diet provided approximate doses of 0, 2, or 8 mg/kg body weight-day (USEPA, 1988). After six weeks of treatment, rats were inoculated (i.p.) with 10E+5 plaque forming units of cytomegalovirus. Exposure to TBTO in the diet continued during the infection period. At 15, 17, or 20 days after inoculation, virus titers were determined in five rats in the salivary gland, lungs, and spleen by plaque assay. There was an significant increase ($P<0.05$) in virus titers at both doses in salivary gland at 15 and 17 days, but not at 20 days post infection. There was a significant increase ($P<0.01$) in virus titers in the lungs only at 15 days post infection and only at the lowest dose. There was a significant increase ($P<0.05$) in virus titer in the spleen in the high dose at 17 days, but not at 15 or 20 days, post infection. This study identifies a LOAEL of 2 mg/kg-day, the lowest dose tested.

Van Loveren et al. (1990) measured the effect of TBTO on natural killer activity in the rat lung. TBTO (purity 95.3%) was added to the diet of weanling Wistar rats (number not specified) at a concentration of 0, 20, or 80 mg/kg. Estimated doses were 0, 2, or 8 mg/kg body weight-day (USEPA, 1988). After six weeks of dosing, rats were sacrificed and body weight and the weight of the thymus, spleen, mesenteric lymph nodes, liver, and kidneys were determined. Lymphoid cell suspensions were obtained after enzymatic dispersion of lungs and purification over nylon wool columns. Natural killer cell activity was measured using a four hour release assay using ^{51}Cr-labeled YAC lymphoma target cells.

At 8 mg/kg-day there was a depression of body weight (93% of control), spleen weight (89% of control), and thymus weight (80% of control). There was a significant ($P<0.05$) decrease in natural killer cell activity when measured by specific release of ^{51}Cr per culture at an effector to target cell ratio of 100 at both doses, but not at cell ratios of 25 and 50. Because there was a significant increase in the number of cells isolated per lung at the lower dose, when the data were expressed as specific release per lung, there was no significant effect at any cell ratio at either dose. There was, however, a significant ($P<0.05$ by variance analysis) overall trend for a decrease in natural killer cell activity with increasing TBTO exposure. Based on the decreased thymus weight, this study establishes a LOAEL of 8 mg/kg-day and a NOAEL of 2 mg/kg-day.

Effects of tributyltin oxide exposure on resistance to virus- and bacteria-induced pneumonia were evaluated in weanling F344 rats that were fed 0 or 150 ppm tributyltin oxide (purity 96%) in the diet for up to 18 weeks (Carthew et al., 1992). The treated diet provided an approximate dosage of 16 mg/kg-day (U.S. EPA, 1988). After 6 weeks of exposure groups of 8

rats (tributyltin oxide-exposed or unexposed males or females) were intranasally infected with pneumonia virus of mice (PVM). Four rats from each group were killed 7 or 10 days after infection for histologic evaluation of any lesions due to persistence of the virus. Other groups of tributyltin oxide-exposed or unexposed rats (8/sex) were intranasally infected with Mycoplasma pulmonis after 6 weeks. A one-week period was used for the bacteria to establish as a nasopharyngeal commensal, after which the rats were infected with PVM. Pulmonary histology and recovery and immunochemical demonstration of M. pulmonis was assessed in 4 rats/group at 1 and 3 months after PVM infection. For all groups of treated rats, the chemical exposure was maintained throughout the periods of exposure to either microorganism until the time of sacrifice. Body weight, thymus weight and liver histology were the only non-pulmonary endpoints reported to have been assessed (groups of 4 rats/sex evaluated), however, it is not indicated whether these rats were exposed to PVM or PVM in conjunction with mycoplasma.

No statistically significant increase in the extent or persistence of PVM-induced lung lesions indicative of chronic infection (e.g., inflammation, focal necrosis) was found in the tributyltin oxide-exposed rats. Evaluation of the rats infected with M. pulmonis showed that susceptibility to secondary mycoplasma pneumonia also was not increased by tributyltin oxide exposure. Effects observed in tributyltin oxide-exposed rats included reduced body weight gain (27 and 16% lower than unexposed controls in males and females, respectively), reduced relative thymus weights (28 and 22.5% lower than unexposed controls in males and females, respectively), and increased incidence of cholangitis with severe biliary retention due to obstruction of the extrahepatic bile duct (33 and 66% prevalence in males and females, respectively). These effects identify a LOAEL of 16 mg/kg-day in rats, the only dose tested.

In a subchronic immunotoxicity study (Vos et al., 1990, a companion to the chronic study summarized below), aged (1-year-old) male Wistar rats were exposed to the same diets used in the principal study for 5 months. Based on the authors statement from the chronic study (see below), estimated compound intake was 0, 0.025, 0.25 or 2.5 mg/kg-day. Endpoints were the same as some of those evaluated in the chronic study, including body weight (12 rats/group), absolute thymus and spleen weights (12 rats/group), resistance to infection by T. spiralis larvae (5-12 rats/group) and L. monocytogenes bacteria (6 rats/group), and natural cell-mediated cytotoxicity of spleen cells (numbers of rats evaluated not reported).

Compound-related effects occurred only in the high dose group and consisted of significantly decreased thymus weight (39% lower than controls, $p<0.01$), impaired resistance to T. spiralis [indicated by increased recovery of adult worms from the small intestine (780% higher than controls, $p<0.01$) and number of larvae in muscle (80% higher, $p<0.001$)], impaired resistance to L. monocytogenes (indicated by approximately 300% increased splenic bacterial count, $p<0.05$). This study identifies a subchronic LOAEL of 2.5 mg/kg-day and NOAEL of 0.25 mg/kg-day for immunotoxicity in aged rats.

Subchronic and chronic immunotoxicity studies were conducted in which weanling SPF-derived Riv:TOX Wistar rats were fed bis(tri-n-butyltin) oxide (tributyltin oxide, purity 95.3%) in

concentrations of 0, 0.5, 5 or 50 ppm. Male rats (females not tested) were evaluated following exposure to TBTO for up to 18 months (Vos et al., 1990; Krajnc et al., 1987). The authors reported the 5 ppm dietary concentration to be equivalent to a dose of 0.25 mg/kg-day, indicating that estimated test doses were 0.025, 0.25 and 2.5 mg/kg-day. Body weight, absolute thymus weight and absolute spleen weight were measured in groups of 18, 12 and 12 rats, respectively, following exposure for 4.5 months. Immunologic function studies for specific and nonspecific resistance were performed in 9-12 rats per group after 4-6 or 15-17 months of exposure. Antigen-specific functional assays evaluated IgM and IgG responses to sheep red blood cells (immunized after 16 months); IgM and IgG responses to ovalbumin and delayed-type hypersensitivity (24-, 48- and 72-hour) responses to ovalbumin and Mycobacterium tuberculosis (immunized after 6 or 15 months exposure); resistance to oral infection by Trichinella spiralis larvae (infected after 5.5 or 16.5 months). Nonspecific resistance was assessed by splenic clearance of i.v. injected Listeria monocytogenes bacteria (after 5 or 17 months exposure), and natural cell-mediated cytotoxicity of spleen cells (after 4.5 or 16 months exposure) and peritoneal cells (after 4.5 months exposure only) using a four-hour ^{51}Cr-release assay with YAC-lymphoma target cells. Non-specific endpoints included the numbers of viable nucleated thymus and spleen cells, and responses of thymus and spleen cells to T-cell and/or B-cell mitogens (phytohemagglutinin, concanavalin A, pokeweed mitogen and/or E. coli lipopolysaccharide) after exposure for 4.5 months (thymus and spleen) or 16 months (spleen only); and numbers of viable nucleated mesenteric lymph node cells with cell surface marker analysis (after 6 and 18 months exposure; low dose group not tested in this assay).

No significant effects were observed in the IgM or IgG responses to sheep red blood cells, the IgM or IgG responses to Trichinella spiralis, the IgM or IgG responses to ovalbumin, or the delayed-type hypersensitivity responses to ovalbumin and mycobacterium tuberculosis.

Thymus weight was significantly reduced in the high dose group (17% lower than controls, $p<0.05$), although the response of thymocytes to T-cell mitogens was unaltered. No significant alterations in spleen weight, response of spleen cells to T- and B-cell mitogens or body weight were found at any dose. Statistically significant changes occurred in the percentage of mesenteric lymph node T-lymphocytes in the high dose group (20% lower than controls after 18 months exposure) and B-lymphocytes in the mid dose group (60% higher than controls after 18 months) and in the high dose group (48% higher than controls after 18 months), however, the absolute number of T-lymphocytes and B-lymphocytes per lymph node were not significantly altered. The low dose group was not tested with these assays. The B-cell increase was an increase in the percent of B-cells but the interpretation of these data is equivocal because they are counter-intuitive when viewed in context with the other effects, especially the IgE titers.

In vivo clearance of injected L. monocytogenes was impaired in rats exposed to the high dose for 17 months, as shown by approximately seven-fold increased number of viable bacteria per spleen, indicating that macrophage function was reduced. Resistance to infection by T. spiralis was suppressed in rats exposed to the mid or high dose, as shown by significantly reduced serum IgE titers (50 and 47% lower than controls after 16.5 months exposure), increased

numbers of larvae in muscle 42 days after infection (56% and 306% higher than controls after 16.5 months), and moderately reduced inflammatory reaction around cysts in parasitized musculature (qualitative assessment only).

There was no significant reduction in the activity of natural killer cells isolated from the peritoneum following exposure of weanling or aged (1-year old) rats to TBTO for 4.5 months. Also there was no significant reduction in the activity of natural killer cells isolated from the spleen following exposure of weanling rats for 4.5 months. In contrast, the activity of natural killer cells isolated from the spleen was suppressed when weanling rats were exposed to all doses of TBTO for 16 months (31, 25 and 36% lower than controls, respectively, at an effector to target cell ratio of 100, and 32, 18, and 30% lower, respectively, at an effector to target cell ratio of 50). Based on these data, the effect did not progress significantly with dose. The authors considered these data equivocal in this experiment. Because there was no clear treatment related effect, EPA will not use the suppression of natural killer cell activity from this study to estimate the reference dose.

Essentially identical results on the immune system were observed following 4.5 or 16.5 months of exposure. Based on the depression of IgE titers and increase in T. spiralis larvae in muscle following 16.5 months of exposure, the LOAEL for immunotoxicity is 0.25 mg/kg-day. The NOAEL is 0.025 mg/kg-day.

Developmental Immunotoxicity

Effects of prenatally administered tributyltin oxide on the developing immune system of mice were evaluated in a study reported as an abstract (Buckiova et al., 1992). Unspecified numbers of pregnant ICR mice were treated with 0.1 mg/kg-day of tributyltin oxide in Tween 80:ethanol:saline (1:2:97) by gavage on gestation days 4-17 or 11-17. The females were allowed to deliver and humoral and cell-mediated immune responses in offspring were assessed 4 and 8 weeks after birth (types of assays were incompletely reported). Other endpoints included embryolethality, postnatal mortality and postnatal growth.

Effects in the exposed offspring included suppressed primary antibody responses to sheep red blood cells, ovalalbumin and lipopolysaccharide, and increased number of leukocytes. Suppressed delayed-type hypersensitivity to sheep red blood cells and unspecified alterations in polyclonal proliferative responses of thymocytes and splenocytes were also observed; the severity of these effects was greater in the mice exposed on gestation days 11-17 than from gestation day 4. This study identifies a LOAEL of 0.1 mg/kg-day (the only dose tested) for developmental immunotoxicity. The significance of this value, however, is unclear because of deficiencies in reporting information on experimental design and results (e.g., quantitative data, numbers of animals, compound purity, etc.).

A study comparing immunotoxic effects in pre-weanlings and adult rats shows that some responses of the developing immune system are more sensitive to TBTO (Smialowicz et al.,

1989). Adult (9 weeks old) male Fischer rats or pre-weanling rats (3-24 days old) were dosed by oral gavage three times per week for a total of 10 doses. The adults were dosed with 5, 10, or 20 mg/kg per dose; the pre-weanlings were dosed with 2.5, 5, or 10 mg/kg per dose. Reductions in mitogen responses were observed in adults at 10 and 20 mg/kg and in pre-weanlings at 5 and 10 mg/kg. The mixed lymphocyte reaction was suppressed in adults at 20 mg/kg and in pre-weanlings at 10 mg/kg. Finally, natural killer cell activity was suppressed only in pre-weanlings at 10 mg/kg.

4.4.2 Neurotoxicity

Triethyltin and trimethyltin compounds have been shown to cause severe neurotoxicity (for a summary, see Boyer, 1989). Triethyltin causes interstitial edema throughout the white matter in the spinal cord and various regions of the brain, less marked damage occurs in the peripheral nervous system. Trimethyltin also causes severe and permanent damage to the central nervous system. In this case, however, the effect is neuronal necrosis, rather than edema. TBTO, in contrast, causes no severe neurological signs or morphological or histopathological changes in brain tissue. In a four week study, a dietary concentration of 320 ppm (equivalent to 30 mg/kg-day) rats exhibited ptosis or enophthalmia and slight ataxia (Krajnc et al., 1984). One chronic study in dogs also gave a slight suggestion of neurotoxicity (atactic gait and apathy). As noted above, however, this study is significantly flawed.

Crofton et al. (1989) measured brain weight and motor activity in developmental studies (see Section 4.3.). There was some suggestion of neurotoxicity at exposures in excess of 10 mg/kg-day, but no reported effects at 5 mg/kg-day.

Although the potential for neurotoxicity has not been completely investigated with focused studies, there is no suggestion that neurotoxicity is a likely critical or co-critical effect.

4.4.3 Genotoxicity

The genetic effects of TBTO were evaluated in multiple in vivo and in vitro short-term tests (Davis et al., 1987). The preponderance of the data show that TBTO is not genotoxic in short-term tests using a wide variety of genetic endpoints. At cytotoxic concentrations, TBTO was mutagenic in one bacterial strain, clastogenic in Chinese hamster ovary cells in vitro, and produced micronuclei in mouse bone marrow cells in vitro.

TBTO was not mutagenic in the rec assay in B. subtilis, did not induce reverse mutations in K. pneumoniae, did not produce point mutations in S. typhimurium stains TA1530, TA1535, TA1538, TA97, TA98, or TA100 in the presence of absence of a rat liver activation system. TBTO was mutagenic in S. typhimurium stain TA 100 in fluctuation test, but only in the presence of rat liver S9 (Arochlor-induced). TBTO did not induce gene mutations in S. pombe, mitotic gene conversions in S. cerevisiae, nor sister-chromatic exchange in Chinese hamster ovary cells in the presence or absence of rat or mouse liver S9. Structural chromosomal aberrations,

endoreduplicated and polyploid cells were induced in Chinese hamster ovary cells. TBTO did not induce gene mutations in V79 Chinese hamster cells or in mouse lymphoma cells. TBTO did not induce recessive lethal mutations in adult male D. melanogaster, either by feeding or injection. Doses of 0.37 or 0.74 mM did not increase the number of X-linked recessive mutations. An increased number of micronuclei was observed in polychromatic erythrocytes of male BALB/c mice 48 hours after a single oral dose of TBTO (60 mg/kg bw). A lower dose (30 mg/kg bw) was ineffective. Neither dose induced micronuclei 30 hours after treatment.

One report demonstrates that TBTO and triphenyltin chloride (TPTC) are co-clastogens in a whole mammalian system (Yamada and Sasaki, 1993). The frequency of micronuclei induced by mitomycin C in mouse peripheral reticulocytes was enhanced approximately 50% when 50 mg/kg TBTO and 100 mg/kg TPTC were given orally to mice. No effect was observed when the chemicals were administered separately.

4.5 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action

A large number of studies have been conducted showing that TBTO causes depression of immune functions dependent on the thymus. These effects occur at doses lower than doses that cause other toxicity. See the table below. Accordingly, the critical effect for TBTO is immunotoxicity. See Section 3 for a discussion of potential modes of action.

Toxicity	Species	Study Length	Endpoint	LOAEL mg/kg-day	NOAEL mg/kg-day	Ref.
General						
	Monkey	22 weeks	Decreased leukocytes	0.14	-	Karrer et al. 1992
	Dog	12 months	-	-	-	Schuh 1992
	Rat	24 months	Chronic toxicity	2.1	0.19	Wester et al. 1987, 1988, 1990
	Mouse	18 months	Decreased survival	0.7 (FEL)	-	Daly 1992
Reproductive						
	Rat	2 gen.	Parental Repro. Develop.	2.95 - 3.43	0.29 4.42 0.34	Schroeder 1990
Developmental						

	Rat	gd 6-19	Maternal Develop.	9 5	5 -	Schroeder 1981
	Rat	gd 6-20	Maternal Develop.	10 10	5 5	Crofton et al. 1989
	Mouse	gd 6-15	Maternal Develop.	40 40	20 20	Baroncelli et al. 1990
	Mouse	gd 6-15	Maternal Develop.	11.7 11.7	5.8 5.8	Davis et al. 1987
	Mouse	gd 6-15	Maternal	5	-	Baroncelli et al. 1995
	Mouse	gd 6-15	Develop.	-	20	Karrer et al. 1995
Immune System						
	Rat	28 days	Thymus dependent immunity	5	0.5	Verdier et al. 1991
	Rat	4 weeks	Lymph node hemorrhage	0.5	-	Vos et al. 1984; Krajnc et al. 1984
	Rat	1 week; 4 weeks	Lymph node hemorrhage	0.4	-	Bressa et al. 1991
	Rat	6 weeks	Virus titers	2	-	Garssen et al. 1995
	Rat	6 weeks	Reduced thymus weight	8	2	Van Loveren et al. 1990
	Rat	18 weeks	Reduced thymus weight	16	-	Carthew et al. 1992
	Rat, aged	5 months	Thymus dependent immunity	2.5	0.25	Vos et al. 1990
	Rat, weanling	18 months	Thymus dependent immunity	0.25	0.025	Vos et al. 1990

Developmental Immune System						
	Mouse	gd 4-17	Humoral and cell mediated immunity	0.1	-	Buckiova et al. 1992 (abstract)
	Rat	10 doses to pre-weanlings	Depressed mitogen response	5	2.5	Smialowicz et al. 1989

4.6 Weight of Evidence Evaluation and Cancer Classification

There are no data in humans concerning development of cancer following exposure to TBTO. Cancer bioassays following oral exposure have been conducted in rats and mice. The bioassay in rats shows increases in benign pituitary tumors, in pheochromocytomas, and in parathyroid tumors at the highest dose tested. The significance of these tumors, which normally occur in this strain of rat with variable incidence, is unclear. The bioassay in mice showed no increase in tumors at any site. A large number of genetic toxicity studies show that TBTO is not genotoxic. There are no structure-activity relationships suggesting that TBTO might be a carcinogen. Because of the questionable data from the bioassay in rats, EPA assigns TBTO to category D (under the 1986 cancer guidelines) or to the "cannot be determined" category (under the 1996 proposed cancer guidelines).

4.7 Other Hazard Identification Issues

4.7.1 Possible Childhood Susceptibility

There is some evidence that a child might be more sensitive to the toxic effects of TBTO. For example, Smialowicz et al. (1989) showed that pre-weanling rats were more sensitive than adult rats. In addition, the principal study (Vos et al., 1990) showed that immunotoxic effects were observed when weanling rats were dosed for 4.5 or 16.5 months. A companion study (Vos et al., 1990) showed that these effects were absent or occurred at a higher dose when adults rats (1 year old) were dosed for 5 months. As the reference dose is based on the effects observed when weanlings were dosed for the remainder of their lives, any potential childhood sensitivity is already accounted for.

4.7.2 Possible Gender Differences

The principal study (Vos et al., 1990) only tested male animals. Other studies, however, show no evidence of gender differences in the toxic responses to TBTO.

5.0 Dose Response Assessments

5.1 Oral Reference Dose (RfD)

5.1.1 Choice of Principal Study and Critical Effect

The principal study is the chronic study on immunotoxicity in rats (Vos et al., 1990). This study shows that TBTO causes toxicity to several components of the thymus dependent immune system. The dose required to cause immunotoxicity is lower than the dose required to cause toxicity to other organ systems.

5.1.2 Methods of Analysis

The data were analyzed using the NOAEL/LOAEL approach and the Benchmark dose approach. Standard uncertainty and modifying factors were then applied.

DESIGNATION OF CRITICAL EFFECT, LOAEL, AND NOAEL:

Based on the study of Vos et al. (1990), the critical effect is immunosuppression (reduced IgE titers and increase in T. spiralis larvae in muscle). The LOAEL is 0.25 mg/kg-day and the NOAEL is 0.025 mg/kg-day. These values were based on the authors' report that 5 ppm in the diet is equivalent to 0.25 mg/kg bw-day.

DERIVATION OF A BENCHMARK DOSE (BMD):

Benchmark dose analyses for continuous data were conducted using the polynomial mean response regression model (THC, I.C.F. Kaiser, 1990a) and the Weibull power mean response regression model (THCW, I.C.F. Kaiser, 1990b). A 10% relative change (treated-control/control) was chosen as the benchmark response (BMR). The BMD10 (the lower 95% confidence bound on the dose corresponding to the BMR) was calculated for the IgE titer, T. spiralis larvae in muscle by digestion, and T. spiralis larvae in muscle by histology (Vos et al., 1990). See Appendix A. The BMD10 of 0.03 mg/kg-day was used to estimate the Reference Dose.

5.1.3 Oral Reference Dose Derivation

The reference dose of 3E-4 mg/kg-day was estimated from the BMD10 of 0.03 mg/kg-day for immunosuppression and an uncertainty factor (UF) of 100 and a modifying factor (MF) of 1. Uncertainty factors of 10 each were applied for uncertainty associated with extrapolating from a laboratory animal species to humans and to protect sensitive humans.

5.2 Inhalation Reference Concentration

Adequate data are not available to derive an RfC as the requirement for the minimum data base (i.e. a 90-day inhalation bioassay) has not been met. The inhalation studies that are available

document irritation to the respiratory system. There are no pharmacokinetic studies available to conduct a route-to-route extrapolation for extrarespiratory effects. TBTO might cause immunosuppression following chronic exposure by inhalation.

5.3 Cancer Assessment

Because of the questionable data from the bioassay in rats, EPA assigns TBTO to category D (under the 1986 cancer guidelines) or to the "cannot be determined" category (under the 1996 proposed cancer guidelines). See also Section 4.5.

6.0 Major Conclusions in Characterization of Hazard and Dose-Response

6.1 Hazard Identification

No human data are available to characterize the toxicity of TBTO. A wealth of data from laboratory animals, however, is available. These data adequately characterize the noncancer toxicity from oral exposure to TBTO. EPA has high confidence in this assessment. The species studied include monkey, dog, rat, and mouse. In addition there is a two-generation reproduction study and several developmental studies in rats and mice. The principal study and a variety of supporting studies convincingly demonstrate that the critical effect for TBTO is immunotoxicity. Some evidence indicates that young animals are more sensitive than adults to the immunotoxic effects.

Limitations in the principal study include somewhat limited sizes of the test groups, lack of testing of females, and exposure for only 18 months. The chronic study in dogs is fatally flawed. Other limitations include lack of a demonstrated NOAEL is some studies, particularly a developmental immunotoxicity study (available only as an abstract) claiming a LOAEL only four-fold higher that the NOAEL established by the principal study. The potential for neurotoxicity has not been completely studied. These limitations, however, are not sufficient to require an uncertainty factor for data base limitations.

Animals are regularly exposed to a variety of organisms that, under certain circumstances, cause infection. In mammals, physical and chemical barriers, in conjunction with other forms of nonspecific immunity, prevent some types of infections. In other cases, the host responds to specific antigens associated with the infectious agent or its products. It is well established that immunosuppressed humans are less resistant to infection, and that the type of infections developed depend on the affected arm of the immune system (e.g., decreased T-cell, accessory cell, or antibody response). Resistance to infection is thus a hallmark of a normally-functioning immune system; as such, many immunotoxicologists believe that challenge with an infectious agent or transplantable tumor cells following chemical exposure presents the best summation of host immunocompetence, provided that an appropriate (i.e., matched to the suspected immunologic defect) challenge test is used. Studies used to set the RfD for TBTO included infection with the parasitic nematode Trichinella spiralis because a defect in cell-mediated

immunity was suspected based on previously observed thymic atrophy in exposed rats. In this infection, adult parasites are found in the small intestine; gravid female parasites release living larvae which migrate to host muscle via the blood and lymph circulatory systems. The "goal" of the host is to limit the number of migrating larvae since this phase of the life cycle causes the greatest damage. The host attacks the parasite in three ways: (1) a T-lymphocyte response which eliminates adults from the intestine; (2) a T-cell dependent antibody response which limits production of larvae by female parasites; and (3) a combined response of antibodies (including IgE) and accessory cells (macrophages, eosinophils, and basophils) which destroy a portion of the migrating larvae. A significant decrease in any one of these responses, or the cumulative effects of more minor decreases in more than one protective mechanism, can lead to a greater number of larvae encysted in host muscles, as was observed in the principal study supporting the oral RfD for TBTO. Table 9 of Vos et al. (1990) also indicates that exposure to TBTO can suppress elimination of adult parasites. Although this occurred at an exposure level of 50 mg/kg of feed in aged rats, elevated larvae counts were also only observed in aged rats at 50 mg/kg of feed. While aged rats appear to be less susceptible (in terms of applied effective dose) to TBTO-mediated suppression of resistance to infection, the data do suggest that delayed expulsion of adult parasites may have contributed to or was responsible for the elevated numbers of larvae observed in younger rats exposed to 5 mg/kg of feed. Although this is speculation, the data presented by Vos et al. (1990) do not provide evidence that the increased larvae burdens in exposed rats are attributable solely to suppression of the IgE response. Because resistance to a variety of other infectious agents has a strong T-cell component, possible adverse effects of TBTO exposure on resistance to other organisms can not be ruled out unless additional experiments are done.

Insufficient data are available to determine the critical effect for TBTO following exposure by inhalation.

The potential human hazard for carcinogenicity for TBTO cannot be determined. A bioassay in mice showed no excess tumors. A bioassay in rats, however, showed some tumors in endocrine organs (pituitary, adrenal medulla, and parathyroid). The study in rats is inconclusive because of increased mortality at the high dose, reduced statistical power because of the dose spacing, and the high and variable background rates for the tumors observed. A large number of genetic toxicity studies show that TBTO is not genotoxic.

6.2 Dose-Response

The quantitative estimate of human risk from chronic exposure to TBTO is based on laboratory animal studies because no appropriate human data exist.

The human dose that is likely to be without appreciable risk of deleterious noncancer effects following a lifetime of oral exposure (the RfD) is 3E-4 mg/kg-day. The overall confidence in this value is high. The RfD is 1/100th of the lower 95% confidence bound on the benchmark response (10% relative response) for immunotoxic effects in rats dosed orally with TBTO for 18 months. The total uncertainty factor of 100 includes 10-fold for extrapolation from laboratory

animals to people and 10-fold to protect sensitive humans. EPA considers that any additional uncertainty factor for data base limitations is not needed.

No appropriate data are available to calculate a reference concentration (RfC) or cancer slope factor.

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8.0 Appendices

Appendix A. Benchmark Dose Analysis of data from Vos et al. (1990)

A. COMPUTATIONAL MODELS

EPA used only commercially available software for the computation. EPA used the polynomial mean response regression model (THC, I.C.F. Kaiser, 1990a) and the Weibull power mean response regression model (THWC, I.C.F. Kaiser, 1990b).

THC $F(d) = q_0 + SIGN \times \left(q_1 (d-d_0) + ... + q_k (d-d_0)^k \right)$

THWC $F(d) = q_0 + SIGN \times q_1 (d-d_0)^{q2}$

where:
d = dose
F(d) = average response at dose d
q_0 , q_1 , q_2 , k = estimated parameters
SIGN = input indicating an increasing or decreasing dose-response function

For THC, the degree of the polynomial was set to the number of dose groups minus one, the corrected sum of squares (CSS) for each group = (N-1) x (standard deviation)^2 , the response type was relative (F(d) - F(0)) / F(0)), and no threshold was estimated. For THWC, the setting were the same save that the lower limit of q_2 was set at 1. Although lower values of q_2 may produce a better fit to the data (i.e. lower SS_f), the shapes of dose-response curves generated from the lower values often lack a reasonable biological motivation.

B. DATA

EPA modeled the IgE titer, T. spiralis larvae in muscle by digestion, and T. spiralis larvae in muscle by histology.

C. MODEL FIT

EPA judged model fit by comparison of a test statistic (F') with F distribution at specified degrees of freedom (df_f ,df_e; numerator, denominator). When F' equals or exceeds the appropriate value in the F distribution tables at 0.01, EPA concludes that the model did not fit the data.

$$F' = (SS_f / df_f) / MS_e)$$

where:
SS_f = sum of squares lack of fit (generated by THC)
MS_e= pooled mean square pure error (generated by THC)

df_f = dose groups - parameters fit by THC
df_e = degrees of freedom generated by THC

D. RESULTS

Data Modeled	THC BMD10 (ppm)	Fit (F')
IgE titer (all exposure groups)	6.41	2.08
IgE titer (omitting highest exposure)	0.68	0.246
T. spiralis larvae by digestion	1.17	0.406
T. spiralis larvae by histology	1.09	0.932

E. DISCUSSION

To apply the benchmark dose methodology, EPA must specify a percent of change in the assay (the benchmark response, BMR) that is considered biologically significant and adverse. Although varying degrees of concordance have been established between changes in immune function assays and alterations in host resistance (Luster et al., 1993), there is no generally accepted percent of change in functional endpoints that is taken as predictive of an adverse outcome in the host resistance (Immunotoxicology Technical Committee, 1995). For this assessment EPA has chosen a BMR of 10% (with a 95% confidence limit). EPA bases this decision on its assessment of the analytical methodology (the measured value and its variability) and the slope of the exposure-response relationship in the region of interest. EPA concluded that using a relative change of 5% would be unreasonable because of the variability in results among animals. For example, the range of the standard deviation for the IgE titer is 43 to 124% of the measured value; the range of the standard deviation for T. spiralis larvae in muscle is 24 to 75% of the measured value. EPA concluded that using a relative change of 20% would be equally unreasonable given the steep slope of the exposure-response relationship in the range of interest and the demonstrated correlation between the exposure causing the decrease in IgE titer and the depression in host resistance as shown by the T. spiralis larvae in muscle. EPA's use of a relative change of 10% in this case, however, does not mean that a relative change of 9% is without risk and a relative change of 11% represents an unacceptable risk or that EPA will always use a BMR of 10% for immunological endpoints in the future.

As shown in the table above, there is adequate fit of the mathematical model to the reported data for each endpoint modeled. The polynomial model and the Weibull model gave identical results for these data because the polynomial model used only two parameters [Q(0) and Q(1)] to fit the model. In such a case, the equations for the two models are identical. EPA, therefore, did not report the results of the Weibull model.

The IgE titer data following 15-16.5 months of exposure shows a plateau at the mid and high dose (1.9 ± 1.6 and 2.0 ± 2.1 at 5 and 50 ppm, respectively). When fitting the polynomial model to these data, the computer program decreases the control value and increases the response at 5 ppm to fit a line to all four data points (see observed and predicted values in table 1, following). This operation essentially obviates using the observed data in the primary exposure range of interest (0 to 5 ppm). For this reason EPA conducted an additional analysis omitting the data at 50 ppm. This data censoring is an accepted procedure of achieving a better fit to the observed data and to achieve better correlation with the underlying biological phenomenon (U. S. EPA. 1995c). Omitting the data from 50 ppm leaves three data points, two of which give a non-zero response, but only one of which is statistically different from control ($P<0.01$). These data still meet the minimum criteria for application of the methodology. Using the censored data set, the polynomial model gives a much better fit to the observed data in the exposure range of interest (compare observed and predicted values in table 2).

Because the data on IgE titer provide a measure of the primary biological response (the depressed IgE titer is an indicator of weakened host resistance) and the better fit to the observed data in the exposure range of interest using the control, low, and mid exposure groups, EPA will use the BMD of 0.68 ppm (equivalent to 0.034 mg/kg-day, rounded to 0.03 mg/kg-day) to estimate the Reference Dose.

Table 1. IgE titers (Table 3, Vos et al., 1990)

GROUP	DOSE	NUMBER OF ANIMALS	CORRECTED SUM OF SQUARES	MEAN VALUE FOR OBSERVATIONS
1	.00000	9	32.000	3.8000
2	.50000	9	28.880	3.2000
3	5.0000	9	20.480	1.9000
4	50.000	9	35.280	2.0000

PREDICTED AND OBSERVED MEAN RESPONSES

LEVEL	DOSE	OBSERVED	PREDICTED
1	.00000	3.8000	2.9666
2	.50000	3.2000	2.9558
3	5.0000	1.9000	2.8584
4	50.000	2.0000	1.8849

SUM OF SQUARES LACK-OF-FIT ==> 15.174
POOLED MEAN SQUARE PURE ERROR ==> 3.6450
DEGREES OF FREEDOM ==> 32

MAXIMUM LIKELIHOOD ESTIMATES OF PROBABILITY FUNCTION COEFFICIENTS

POLYNOMIAL MEAN RESPONSE MODEL

F(DOSE) = Q(0) + SIGN * (Q(1) * (DOSE - D0) + Q(2) * (DOSE - D0)**2 + ... + Q(3) * (DOSE - D0)**3)

Q(0) = 2.9666118041
Q(1) = 2.16338818983E-02
Q(2) = .00000000000
Q(3) = .00000000000
THRESHOLD (D0) = .00000000000
SIGN = -1.0

MAXIMUM VALUE OF THE LOG-LIKELIHOOD ==> -23.2293163007
CALCULATIONS ARE BASED UPON RELATIVE RESPONSE

THC LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RESPONSE

RESPONSE	MLE DOSE	LOWER BOUND ON DOSE	CONF. LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.1000	13.71	6.4065	95.0%	Q(0) = 3.0766
				Q(1) = 4.80234E-02
				Q(2) = .00000
				Q(3) = .00000
				THRESHOLD D(0) = .00000
				SIGN = -1.0

Table 2. IgE titers (Table 3, Vos et al., 1990)

GROUP	DOSE	NUMBER OF ANIMALS	CORRECTED SUM OF SQUARES	MEAN VALUE FOR OBSERVATIONS
1	.00000	9	32.000	3.800
2	.50000	9	28.880	3.200
3	5.0000	9	20.480	1.900

PREDICTED AND OBSERVED MEAN RESPONSES

LEVEL	DOSE	OBSERVED	PREDICTED
1	.00000	3.800	3.5855
2	.50000	3.200	3.4154
3	5.0000	1.900	1.8849

SUM OF SQUARES LACK-OF-FIT ==> 0.83388
POOLED MEAN SQUARE PURE ERROR ==> 3.3900
DEGREES OF FREEDOM ==> 24

MAXIMUM LIKELIHOOD ESTIMATES OF PROBABILITY FUNCTION COEFFICIENTS

POLYNOMIAL MEAN RESPONSE MODEL

F(DOSE) = Q(0) + SIGN * (Q(1) * (DOSE - D0) + Q(2) * (DOSE - D0)**2)

Q(0) = 3.5854868518
Q(1) = .34010954467
Q(2) = .00000000000
THRESHOLD (D0) = .00000000000
SIGN = -1.0

MAXIMUM VALUE OF THE LOG-LIKELIHOOD ==> -14.7779035170

CALCULATIONS ARE BASED UPON RELATIVE RESPONSE

THC LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RESPONSE

RESPONSE	MLE DOSE	LOWER BOUND ON DOSE	CONF. LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.1000	1.054	0.68190	95.0%	Q(0) = 3.7764
				Q(1) = .55380
				Q(2) = .00000
			THRESHOLD	D(0) = .00000
				SIGN = -1.0

Table 3. T. spiralis larvae by digestion (Table 4, Vos et al., 1990)

GROUP	DOSE	# OF ANIMALS	CORRECTED SUM OF SQUARES	MEAN VALUE FOR OBSERVATIONS
1	.00000	9	2048.0	34.000
2	.50000	9	2048.0	33.000
3	5.0000	9	5408.0	53.000
4	50.000	9	17672.	138.00

PREDICTED AND OBSERVED MEAN RESPONSES

LEVEL	DOSE	OBSERVED	PREDICTED
1	.00000	34.000	34.259
2	.50000	33.000	35.318
3	5.0000	53.000	44.853
4	50.000	138.00	140.21

SUM OF SQUARES LACK-OF-FIT ==> 690.11
POOLED MEAN SQUARE PURE ERROR ==> 849.25
DEGREES OF FREEDOM ==> 32

MAXIMUM LIKELIHOOD ESTIMATES OF PROBABILITY FUNCTION COEFFICIENTS

POLYNOMIAL MEAN RESPONSE MODEL

F(DOSE) = Q(0) + SIGN * (Q(1) * (DOSE - D0) + Q(2) * (DOSE - D0)**2 + ... + Q(3) * (DOSE - D0)**3)

Q(0) = 34.258561315
Q(1) = 2.1189762133
Q(2) = .00000000000
Q(3) = .00000000000
THRESHOLD (D0) = .00000000000
SIGN = 1.0

MAXIMUM VALUE OF THE LOG-LIKELIHOOD ==> -112.349631263
CALCULATIONS ARE BASED UPON RELATIVE RESPONSE

THC LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RESPONSE

RESPONSE	MLE DOSE	LOWER BOUND ON DOSE	CONF. LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.1000	1.617	1.1683	95.0%	Q(0)= 29.974
				Q(1)= 2.5657
				Q(2)= .00000
				Q(3)= .00000
				THRESHOLD D(0)= .00000
				SIGN = 1.0

Table 4. T. spiralis larvae by histology (Table 4, Vos et al., 1990)

GROUP	DOSE	NUMBER OF ANIMALS	CORRECTED SUM OF SQUARES	MEAN VALUE FOR OBSERVATIONS
1	.00000	9	5832.0	36.000
2	.50000	9	2592.0	39.000
3	5.0000	9	9248.0	55.000
4	50.000	9	59168.	145.00

PREDICTED AND OBSERVED MEAN RESPONSES

LEVEL	DOSE	OBSERVED	PREDICTED
1	.00000	36.000	38.287
2	.50000	39.000	39.389
3	5.0000	55.000	49.303
4	50.000	145.00	148.45

SUM OF SQUARES LACK-OF-FIT ==> 447.65
POOLED MEAN SQUARE PURE ERROR ==> 2401.3
DEGREES OF FREEDOM ==> 32

MAXIMUM LIKELIHOOD ESTIMATES OF PROBABILITY FUNCTION COEFFICIENTS

POLYNOMIAL MEAN RESPONSE MODEL

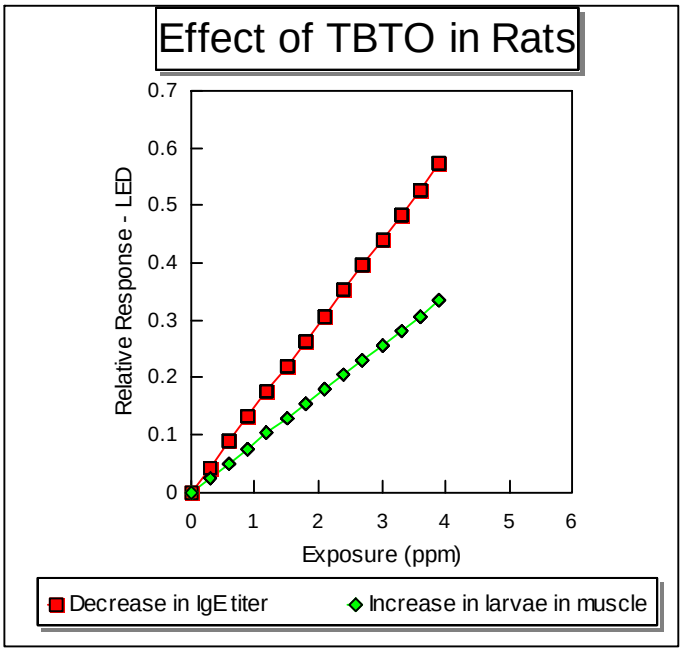
F(DOSE) = Q(0) + SIGN * (Q(1) * (DOSE - D0) + Q(2) * (DOSE - D0)**2 + ... + Q(3) * (DOSE - D0)**3)

Q(0) = 38.287078072
Q(1) = 2.2032704078
Q(2) = .00000000000
Q(3) = .00000000000
THRESHOLD (D0) = .00000000000
SIGN = 1.0

MAXIMUM VALUE OF THE LOG-LIKELIHOOD ==> -125.568750151
CALCULATIONS ARE BASED UPON RELATIVE RESPONSE

THC LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RESPONSE

RESPONSE	MLE DOSE	LOWER BOUND ON DOSE	CONF. LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
.1000	1.738	1.0861	95.0%	Q(0) = 33.189
				Q(1) = 3.0559
				Q(2) = .00000
				Q(3) = .00000
				THRESHOLD D(0) = .00000
				SIGN = 1.0


Effect of TBTO in Rats
Relative Response - LED
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0.6
0.5
0.4
0.3
0.2
0.1
0
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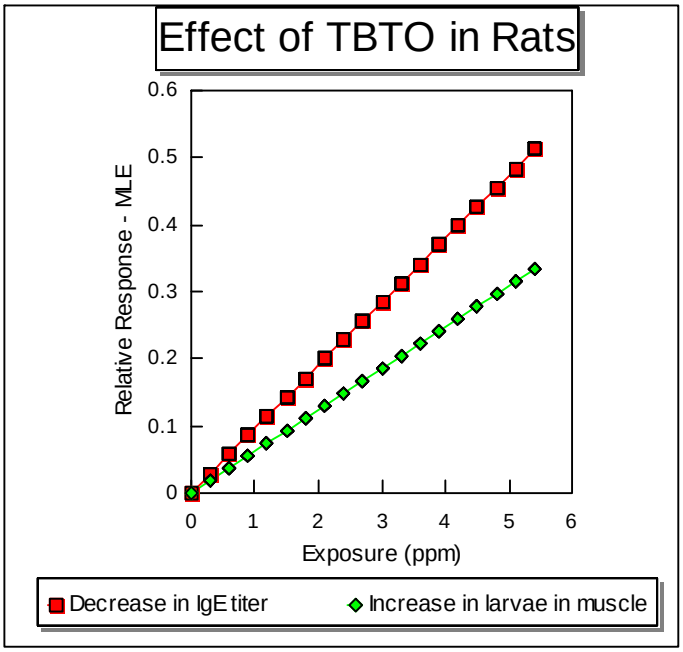
Exposure (ppm)
Decrease in IgE titer
Increase in larvae in muscle


Effect of TBTO in Rats
Relative Response - MLE
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6
Exposure (ppm)
Decrease in IgE titer
Increase in larvae in muscle

Appendix B. Summary of Comments from External Peer Reviewers.

Each of the external peer reviewers agreed that the document adequately summarized the toxicological data on TBTO. Each reviewer recommended acceptance of the document with revision. The suggestions for minor revisions, accepted by EPA, are not discussed further. The more substantive comments, and EPA's resolution of the issue, are summarized below.

1. One reviewer was "surprised" that specific mention was not made of the apparent lack of neurotoxic effects given the well known neurotoxicity of trimethyltin and triethyltin.

   None of the general toxicity studies suggested that neurotoxicity might be the critical effect. One study in dogs gave a slight suggestion of neurotoxicity (atactic gait and apathy). However, as noted in the document, this study was significantly flawed. One developmental study investigated some neurological endpoints. No focused studies on neurotoxicity have been published. The only relevant information, therefore, is the lack of clinical signs of neurotoxicity and the lack of histopathological changes in studies in which nervous tissue was examined. Based on this limited information, EPA does not believe that an extensive discussion of the lack of neurotoxicity is warranted. EPA, however, added a some material in Section 4.4. on the apparent lack of significant neurotoxicity.

2. Several reviewers requested adding references relating to the possible mechanism of toxicity (inhibition of ATP synthesis in mitochondria and apoptosis, programmed cell death, in the thymus) and relating to in vitro studies on developmental toxicity.

   EPA agrees that including this material will improve the document. EPA modified section 3 and section 4.3 to incorporate this material.

3. One reviewer requested adding an additional reference on the role of age on the immunotoxic effects. (R. J. Smialowicz, M. M. Riddle, R. R. Rogers, R. W. Luebke, and C. B. Copeland. 1989. Immunotoxicity of tributyltin oxide in rats exposed as adults or pre-weanlings. Toxicol. 57:97-111.)

   EPA originally excluded this reference because of the short duration of dosing (10 total doses) and because the doses used exceeded those used for subchronic and chronic studies focused on immunotoxicity. EPA agrees, however, to include this reference in section 4.4.1. (Developmental Immunotoxicity) as it does relate to potential toxicity to an important subgroup of the population.

4. One reviewer requested adding a reference to the first observation that TBTO induced thymus weight reduction. (N. Funahashi, I. Iwasaki, and G. Ide. 1980. Acta Pathol. Japan. 30:955-966.)

   EPA agrees that this observation is of historical importance but declines to include a

discussion of the results in this document. The effects were observed following a single oral dose of 100 mg/kg or by gavage (3, 6, or 12 mg/kg) during three and six months. These doses are far in excess of those used in the focused immunotoxicity tests used to establish the critical effect. In addition, the full study was published in a Japanese journal and a translation is not presently available.

5. One reviewer questioned the correctness of the conversion from TBTO in the diet to the dose in mg/kg body weight-day in the Vos et al. (1990) study (the principal study). The reviewer cited the difference in dose in mg/kg body weight-day between the Vos et al. (1990) study and the Verdier et al. (1991) study even though the concentration of TBTO in the diet was the same.

   EPA relied on the information in each publication for the doses in mg/kg body weight-day. Neither publication provided the detail required to confirm the calculation or to determine the variability in the estimate. As noted by the reviewer, the researchers used different strains of rats with different body weights. In addition, the rats were of different ages. Verdier et al. dosed young Sprague-Dawley rats (4-5 weeks old) for 28 days. Vos et al. dosed Wistar rats for 18 months. Given the decline in food consumption with age, it is logical that the average dose in the chronic study would be lower even though the concentration of TBTO in the diet was the same.

6. One reviewer questioned whether the lowest dose tested by Vos et al. (1990), selected by EPA as the NOAEL, was the NOAEL or an unrecognized effect level. The reviewer cited a number of reasons for questioning EPA's conclusion. These included several changes observed at the lowest dose but which did not reach statistical significance, the changes in natural killer cell activity, the potential for other more sensitive effects within the immune system that were not measured, effects in other studies in which a NOAEL was not established, and some in vitro studies suggesting the potential for effects at doses comparable or lower than the lowest dose in Vos et al. Based on these considerations, the reviewer suggested it was "premature and probably inaccurate" to consider 0.5 mg/kg diet (0.025 mg/kg body weight-day) as a NOAEL in the absence of further studies. In lieu of such studies, the reviewer suggested using an additional uncertainty factor.

   EPA rejects the suggestion of using an additional uncertainty factor in deriving the reference dose. As noted by the reviewer, the Benchmark Dose methodology, used to derive the critical dose, uses a statistical approach to compensate for the number of animals tested and the biological variability among animals. For the reason stated in the document, EPA concluded that changes in the natural killer cell activity would not be used to derive the reference dose. Although it is possible that some other changes in the immune system occur at the lowest dose, no definitive data establish this fact. In such a case, it has been EPA's standard practice not to assign an additional uncertainty factor when the major toxicological endpoints have been adequately evaluated. Finally, without pharmacokinetic data it is impossible to relate in vitro to in vivo doses.

If new data demonstrate effects at or below a dose of 0.025 mg/kg-day, EPA will reevaluate the reference dose for TBTO.

7. This reviewer also requested a discussion of the quantitative effect on the reference dose should a lower NOAEL be assigned.

   Because the Benchmark Dose methodology was used to determine the critical dose (LED_{10} with 95% confidence) using data on IgE titer, the same reference dose (3E-4 mg/kg-day) would be derived whether the lowest dose is assigned a NOAEL or LOAEL. On the other hand, if a different benchmark response had been selected (LED_{05} with 95% confidence), then the reference dose would decrease by a factor of two.

   If a different critical effect had been used to derive the reference dose, such as the decrease in natural killer cell activity in spleen, then EPA would have derived a lower reference dose. Because of the lack of a dose-response relationship for this effect, application of the Benchmark Dose method would not have yielded a meaningful result. If EPA assigned the LOAEL for this endpoint as 0.025 mg/kg-day, EPA would apply a total uncertainty factor of 1,000. The reference dose would have been 3E-5 mg/kg-day (after rounding to one significant digit).

   If a new study showed effects at a dose lower than 0.025 mg/kg-day and the number of animals tested, the number of doses, the biological variability, and dose-response slope were comparable to the Vos et al. (1990) study, then the derived reference dose would decrease linearly with the decline in NOAEL or benchmark response (i.e., a two-fold decrease in NOAEL or benchmark response would result in a two-fold decrease in the reference dose.

8. One reviewer requested more discussion of the relevance of the immunological endpoints to a human health risk assessment.

   It has been EPA's standard practice to conclude that a biologically significant effect in laboratory animals is relevant to a human health risk assessment, unless there is some convincing rationale to exclude the effect. EPA concludes that the immunotoxic endpoints reported by Vos et al. (1990) are biologically significant and indicate a potential hazard to people. EPA has augmented section 6 (Major Conclusions in Characterization of Hazard and Dose-Response) to make that conclusion more apparent.