Tributyltin oxide (TBTO); CASRN 56-35-9

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR TBTO

File First On-Line 08/22/1988

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	09/01/1997
Inhalation RfC (I.B.)	qualitative discussion	09/01/1997
Carcinogenicity Assessment (II.)	yes	09/01/1997

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9
Last Revised — 09/01/1997

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. 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 effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is

essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

NOTE: ****SEE BENCHMARK DOSE IN DERIVATION OF A BENCHMARK DOSE. Discussion can be found in the Discussion of Principal and Supporting Studies Section. The BMD10 is 0.03 mg/kg-day.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Immunosuppression **18-Month Immuno-toxicity Study in Rats** **Vos et al., 1990**	Benchmark Dose: BMD10 = 0.03 mg/kg-day (0.68 ppm diet)	100	1	3E-4 mg/kg-day

*Conversion Factors and Assumptions — 1 ppm = 0.05 mg/kg-day (rat food consumption given by authors)

I.A.2. Principal and Supporting Studies (Oral RfD)

Vos, J.G., A. DeKlerk, E.I. Krajnc, V. Van Loveren, and J. Rozing. 1990. Immunotoxicity of bis(tri-n-butyltin)oxide in the rat: Effects on thymus- dependent immunity and on nonspecific resistance following long-term exposure in young versus aged rats. Toxicol. Appl. Pharmacol. 105: 144-155.

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 (TBTO), purity 95.3%] in concentrations of 0, 0.5, 5.0 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/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), and 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 4-hour 51Cr-release assay with YAC-lymphoma target cells. Nonspecific 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 altered significantly. 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 the 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 the increase in T. spiralis larvae in muscle following 16.5 months of exposure, the LOAEL for immunotoxicity is 0.25 mg/kg-day (5 ppm diet). The NOAEL is 0.025 mg/kg-day (0.5 ppm diet).

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).

To apply the benchmark dose methodology, EPA must specify a percent of change in the assay (the 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, and 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 that 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.

There was 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.

The IgE titer data following 15-16.5 months of exposure show a plateau at the mid- and high dose [1.9 (SD 1.6) and 2.0 (SD 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. 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 of achieving better correlation with the underlying biological phenomenon (U.S. EPA, 1995). 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 the 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.

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 a better fit to the observed data in the exposure range of interest, using the control and low- and mid-exposure groups, EPA used 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.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — 100. Factors of 10 each are applied for uncertainty associated with extrapolating from a laboratory animal species to humans and to protect sensitive humans.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

No information was located regarding toxicity of TBTO in humans following oral exposure. Human data summarized by Boyer (1989) suggest that tributyltin oxide is a potent nonallergenic dermal irritant.

IMMUNOTOXICITY: A large number of studies have been conducted showing that TBTO causes depression of immune functions dependent on the thymus. The chronic study conducted by Vos et al. (1990) is the principal study. Results of a subchronic study and a developmental immunotoxicity study are summarized below. Shorter term studies are summarized in the toxicological review of TBTO.

In a subchronic immunotoxicity study (Vos et al., 1990, a companion to the principal study), 1-year-old male Wistar rats were exposed to the same diets used in the principal study for 5 months. Based on data from the chronic study, estimated compound intake was 0, 0.025, 0.25 or 2.50 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 a subchronic NOAEL of 0.25 mg/kg-day for immunotoxicity in aged rats.

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 TBTO 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 also were observed; the severity of these effects was greater in the mice exposed on gestation days 11-17 than those exposed on gestation days 4-17. 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.).

GENERAL TOXICITY: MONKEYS - Effects of TBTO (purity 96%) on hematology and serum chemistry were assessed in groups of three and four 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 TBTO 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 beagles were treated with TBTO [purity 95.9% (Batch 1) or 97.4% (Batch 2)] in arachis oil by gavage in dosages of 0, 0.2, 1.0, or 5.0 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 because of deficiencies with respect to study conduct and reporting. Study deficiencies are six-fold: (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 absence 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 pretest 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 TBTO for 2 years (Wester et al., 1990, 1988, 1987). Based on estimates of average body weight and food consumption from reported data, ingested dosages are approximately 0.019, 0.19 or 2.10 mg/kg-day in males and 0.025, 0.25 or 2.50 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, and 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 in 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 increased significantly ($p < 0.05$, 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 with 69-71% in controls and other treated groups) and 12 months (80% compared with 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 weeks 90 and 96, respectively. At termination, survival in females in the high-dose group was 54% vs. 74% in controls, and survival in males in the high-dose group was 40% vs. 60% in controls. Body weight gain was significantly reduced (p values not reported) in high-dose males and females after weeks 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 - TBTO (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 and low-, mid-, and high-dose groups, 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% vs. 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 and low-, mid- and high-dose groups, respectively ($p < 0.05$, except for the low-dose group). No information on causes 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 nonneoplastic 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 with 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.

REPRODUCTIVE TOXICITY: A two-generation reproduction study was conducted in which groups of 30 male and 30 female Crl:CD(SD)BR rats (F0 generation) were fed TBTO (purity 97.1%) in dietary concentrations of 0, 0.5, 5.0 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, the respective mean compound intakes during the premating period were 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 examination 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%, respectively) and significantly ($p < 0.01$) lower than controls in F1 males at the high dose (38 and 31%, respectively) and F1 females at the high dose (28 and 26%, respectively). 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 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 absence 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 TOXICITY: RATS - Groups of 24 mated female CD Sprague- Dawley rats were treated with TBTO (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 Nos. 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% in controls, and 58.2-93.4% vs. 44.6% in controls, 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% in controls, and 2.3-18.2% vs. 0% in controls, respectively. Increased incidences of other ossification variations (asymmetric sternebrae Nos. 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 a significantly decreased percentage of fetuses to implants (86.8% compared with 94.7% in controls, $p < 0.01$), increased percentage of resorptions (13.2% compared with 5.3% in controls, $p < 0.01$) and decreased fetal weight (16% lower than controls in both sexes, $p < 0.01$). Because of 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 TBTO (purity 97%) in corn oil by gavage (Crofton et al., 1989). Rats were administered doses of 0, 2.5, 5.0 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 days 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 the 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 on 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, 5.0, 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 reduced significantly 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 TBTO 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 TBTO (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; relative organ weight; gross pathology of the 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 with 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 days 12-13, sized 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 35.0 mg/kg-day TBTO 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, 3.0, 2.0, 7.0, 24.0 and 48.0% at 0, 1.2, 3.5, 5.8, 11.7, 23.4 and 35.0 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 nonspecifically (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 TBTO. Effects observed at 23.4 and 35.0 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%, respectively, compared with 0.5% in controls) (e.g., fusion of bases of os occipitalis) and skeletal variations (43 and 43%, respectively, compared with 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 the control and other groups; the 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 TBTO 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 (gestation days 6-15). Dam weight gain was impaired at 10, 20 and 30 mg/kg (15, 13 and 20%, respectively) between gestation days 16 and 18. Maternal weight gain between gestation day 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, vs. 0% in controls). There was also a change in delayed parturitions (0, 16.0, 27.8 and 0%, respectively, vs. 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 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, vs. 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, often were found scattered throughout the cage with signs of wounds, and the percentage of dams that had 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 gestation day 6 to postnatal day 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 during 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 postnatal 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.

For more detail on other Hazard Identification Issues, exit to the toxicological review, Section 4.7 (PDF).

I.A.5. Confidence in the Oral RfD

Study — High
Database — High
RfD — High

Limitations of the principal study include somewhat minimal test group sizes, lack of testing of females, and exposure for only 18 months. Confidence in the principal study is high, however, because it generally is well-designed and comprehensively assessed the immunotoxic potential of TBTO through the use of a number of assays. When the principal study is evaluated in conjunction with the supporting parallel chronic toxicity study, it is clear that the immune system was the most sensitive target. Additionally, there are a number of other chronic and subchronic studies supporting immunotoxicity as a critical effect. The oral database for TBTO includes chronic toxicity studies in three species, developmental toxicity studies in two species, and a multigeneration reproduction study in one species. Confidence in the database is high-to-medium because of limitations in some of these studies, particularly insufficient assessment of immunotoxicity in the chronic studies in mice and dogs, deficiencies precluding identifying a NOAEL or LOAEL in the study in dogs, lack of a NOAEL in a developmental study showing skeletal ossification variations, and a developmental immunotoxicity study (reported only as an abstract) that claims a LOAEL only four-fold higher than the NOAEL established by the principal study. The limitations in the database are not sufficient to warrant an additional uncertainty factor. Reflecting high confidence in the principal study and high-to-medium confidence in the database, confidence in the RfD is high-to-medium.

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 because of previously observed thymic atrophy in exposed rats. In this infection, adult parasites are found in the small intestine; gravid female parasites release living larvae that migrate to host muscle via the blood and lymph circulatory systems. The "goal" of the host is to limit the number of migrating larvae because this phase of the life cycle causes the greatest damage. The host attacks the parasite in three ways: (1) a T-lymphocyte response that eliminates adults from the intestine, (2) a T- cell-dependent antibody response that limits production of larvae by female parasites; and (3) a combined response of antibodies (including IgE) and accessory cells (macrophages, eosinophils, and basophils) that destroys 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 also were observed only in aged rats at 50 mg/kg of feed. Although 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 cannot be ruled out unless additional experiments are conducted.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 1997

This assessment was peer reviewed by external scientists (U.S. EPA, 1994). Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of there comments is included as an appendix to the Toxicological Review of Tributyltin Oxide (U.S. EPA, 1997). ***To review this appendix, exit to the toxicological review, Appendix B, Summary of and Response to External Peer Review Comments (PDF)***.

Other EPA Documentation — None

Agency Consensus Review Date -- 07/02/1997

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Tributyltin oxide conducted in September 2002 identified one or more significant new studies. IRIS users may request the references for those studies from the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9

The health effects data for tributyltin oxide (TBTO) were reviewed by EPA and determined to be inadequate for derivation of an inhalation RfC.

The database lacks the minimum criteria for derivation of an RfC (i.e., a 90-day inhalation bioassay).

There are several case reports claiming irritation of the respiratory tract following acute inhalation exposure of people to TBTO (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.

Schweinfurth and Gunzel (1987) summarized the results of several short- term inhalation studies in laboratory animals. After a single 4-hour exposure of rats to aerosols of TBTO, signs of irritation (nasal discharge, lung edema, and congestion of pulmonary circulation) and enteritis were observed. The LC50 was 77 mg/cu.m (total particles) or 65 mg/cu.m (particles with a diameter < 10 um). In guinea pigs exposed to aerosols of TBTO in olive oil at 200 mg/cu.m and above, death occurred within 1 hour of exposure. Ten male and 10 female rats were exposed to almost-saturated vapors of TBTO without a single death occurring during the

7-hour exposure or the following 14-day observation period. Only minor clinical signs (slight nasal discharge immediately following 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/cu.m, 5 days/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 (also 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.

There are no pharmacokinetic data for conducting a route-to-route extrapolation for extrarespiratory effects. TBTO might cause immunosuppression following chronic exposure by inhalation.

Anonymous. 1991. Acute effect of indoor exposure to paint containing bis(tributyltin)oxide -- Wisconsin. Morb. Mortal. Wkly. Rep. 40: 280-281.

Hay, A. and C. R. Singer. 1991. Wood preservatives, solvents, and thrombocytopenic purpura (letter). Lancet. 338: 766.

Schweinfurth, H. A. and P. Gunzel. 1987. The tributyltins: Mammalian toxicity and risk evaluation for humans. Oceans '87: The Ocean "an international workplace." Proceedings of the International Organotin Symposium. 4: 1421-1429.

Shelton, D., B. Urch, and S. M. Tarlo. 1992. Occupational asthma induced by a carpet fungicide -- Tributyltin oxide. J. Allergy Clin. Immunol. 90(2): 274-275.

U.S. EPA. 1994. Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Signed by Administrator Carol Browner, June 7.

U.S. EPA. 1997. Toxicological Review of Tributyltin Oxide. Contact EPA's IRIS Hotline [(202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address)] or the IRIS Web site at www.epa.gov/iris.

Agency Consensus Review Date -- 07/02/1997

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfC for Tributyltin oxide conducted in September 2002 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

EPA Contacts:

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address).

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9
Last Revised — 09/01/1997

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The 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 ug/L drinking water or risk per ug/cu.m 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. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Classification — D, not classifiable as to human carcinogenicity

Basis — There are no data in humans concerning development of cancer following exposure to tributyltin oxide (TBTO). Cancer bioassays following oral exposure have been conducted in rats and mice. The bioassay in rats shows increases in benign pituitary tumors, pheochromocytomas, and 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 (U.S. EPA, 1987) or to the "cannot be determined" category [U.S. EPA, 1996 (proposed)].

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on other Hazard Identification Issues, exit to the toxicological review, Section 4.7 (PDF).

II.A.2. Human Carcinogenicity Data

None

II.A.3. Animal Carcinogenicity Data

RATS - In a carcinogenicity/chronic toxicity study, groups of 60 male and 60 female rats were exposed to dietary TBTO for 2 years (Wester et al., 1990, 1988, 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, respectively. Food consumption in males was slightly increased in all dose groups throughout the study (p value 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% vs. 74% in controls; survival in males in the high-dose group was 40% vs. 60% in controls. Body weight gain was reduced (p values not reported) in the high- dose males and females after weeks 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 also were 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	Total Pituitary Tumors for Groups of 50 Rats	
ppm diet	**Female**	**Male**
0	22	34
0.5	32*	39*
5.0	22	29
50	35**	43***

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

Concentration of TBTO	Total Pheochromocytomas for Groups of 50 Rats	
ppm diet	**Female**	**Male**
0	3	16
0.5	3	13
5.0	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	Number of Adenomas/ Number of Parathyroids Examined	
ppm diet	**Female**	**Male**
0	0/64	0/39
0.5	0/44	2/50
5.0	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%, respectively; the reported background occurrence of pheochromocytomas in females was 10 and 12% and, in males, was 26 and 44%, respectively. 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 thyroid-stimulating lutenizing, or follicle-stimulating hormones; insulin; total thyroxine (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 in the mid-dose group. Although the pituitary tumors were 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.

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, respectively. 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 groups, 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 groups, respectively ($p < 0.05$ except for the low-dose group). No information on the causes 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.

II.A.4. Supporting Data for Carcinogenicity

The genetic effects 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 recombinant 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 or absence of a rat liver activation system. TBTO was mutagenic in the S. typhimurium stain TA100 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.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

Not available.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Not available.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 1997

This assessment was peer reviewed by external scientists (U.S. EPA, 1994). Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to U.S. EPA, 1997. ***To review this appendix, exit to the toxicological review, Appendix B, Summary of and Response to External Peer Review Comments (PDF)***.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Consensus Review Date -- 07/02/1997

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for Tributyltin oxide conducted in September 2002 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address).

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9

VI.A. Oral RfD References

Baroncelli, S., D. Karrer, and P.G. Turillazzi. 1990. Embryotoxic evaluation of bis(tri-n-butyltin)oxide (TBTO) in mice. Toxicol. Lett. 50: 257-262.

Baroncelli, S., D. Karrer, and P. G. Turillazzi. 1995. Oral bis(tri-n- butyltin)oxide in pregnant mice. I. Potential influence of maternal behavior on postnatal mortality. J. Toxicol. Environ. Health. 46: 355-367.

Boyer, I.J. 1989. Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and to experimental animals. Toxicology. 55: 253-298.

Buckiova, D., M. Dostal, and V. Hofmannova. 1992. Embryotoxicity of organotins. Reprod. Toxicol. 6: 178-179 [Abstract].

Crofton, K.M., K.F. Dean, V.M. Boncek et al. 1989. Prenatal or postnatal exposure to bis(tri-n-butyltin)oxide in the rat: Postnatal evaluation of teratology and behavior. Toxicol. Appl. Pharmacol. 97: 113-123.

Davis, A., R. Barale, G. Brun et al. 1987. Evaluation of the genetic and embryotoxic effects of bis(tri-n-butyltin)oxide (TBTO), a broad-spectrum pesticide, in multiple in vivo and in vitro short-term tests. Mutat. Res. 188: 65-95.

Daly, I.W. 1992. An eighteen month oncogenicity feeding study in mice with bis(tri-n-butyltin)oxide (TBTO). Unpublished report by Bio/dynamics, Inc. prepared for TBTO Consortium. MRID No. 422650-01. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

I.C.F. Kaiser. 1990a. THC: A computer program to compute a reference dose from continuous animal toxicity data using the benchmark dose method. K.S. Crump Division, Ruston, LA.

I.C.F. Kaiser. 1990b. THCW: A computer program to compute a reference dose from continuous animal toxicity data using the benchmark dose method. K.S. Crump Division, Ruston, LA.

Immunotoxicology Technical Committee. 1995. ILSI Health and Environmental Science Institute. 1995. Immunotoxicity testing and risk assessment: Summary of a 1994 workshop. Fd. Chem Toxic. 33: 887-894.

Karrer, D., S. Baroncelli, L. Ciaralli, and P.G. Turillazzi. 1992. Effect of subchronic bis(tri-n-butyltin)oxide (TBTO) oral administration on haematological parameters in monkeys: A preliminary report. Fd. Chem. Toxic. 30(8): 715-718.

Karrer, D., S. Baroncelli, and P. G. Turillazzi. 1995. Oral bis(tri-n- butyltin)oxide in pregnant mice. II. Alterations in hematological parameters. J. Toxicol. Environ. Health. 46: 369-377.

Krajnc, E.I., J.G. Vos, P.W. Wester, J.G. Loeber, and C.A. van der Heijden. 1987. Toxicity of bis(tri-n-butyltin)oxide (TBTO) in rats. Unpublished report submitted to the U.S. EPA Office of Toxic Substances with cover letter dated 5/18/87. Document Control Number: FYI-OTS-0687-0550 Sequence A.

Luster, M. I., C. Portier, D. G. Pait et al. 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. Fund. Appl. Toxicol. 21: 71-82.

Schroeder, R.E. 1981. A teratology study in rats with bis(tri-n- butyltin)oxide. Unpublished report by Bio/dynamics, Inc., prepared for Elf Atochem. MRID No. 00137158, 92172005, 92172016. HED Doc. No. 003914, 004691, 010916. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

Schroeder, R.E. 1990. A two-generation reproduction study in rats with bis(tri-n-butyltin)oxide. Unpublished report by Bio/dynamics, Inc., prepared for Schering AG and M&T Chemicals, Inc. MRID No. 416938-01. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

Schuh, W. 1992. One year chronic oral toxicity study in beagle dogs. Unpublished report by Schering AG Laboratories prepared for Elf Atochem North America, Inc., Aceto Chemicals and Schering Berlin Polymers. MRID No. 425498. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

U.S. EPA. 1994. Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Signed by Administrator Carol Browner, June 7.

U.S. EPA. 1995. Use of the Benchmark Dose Approach in Health Risk Assessment. February. EPA/630/R-94/007. NTIS PB95-213765.

U.S. EPA. 1997. Toxicological Review on Tributyltin Oxide. Contact EPA's IRIS Hotline [(202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address)] or the IRIS Web site at www.epa.gov/iris.

Vos, J.G., A. DeKlerk, E.I. Krajnc, V. Van Loveren, and J. Rozing. 1990. Immunotoxicity of bis(tri-n-butyltin)oxide in the rat: Effects on thymus- dependent immunity and on nonspecific resistance following long-term exposure in young versus aged rats. Toxicol. Appl. Pharmacol. 105: 144-155.

Wester, P.W., E.I. Krajnc, and C.A. van der Heijden. 1987. Chronic toxicity and carcinogenicity study with bis(tri-n-butyltin)oxide (TBTO) in rats. Unpublished report submitted to the U.S. EPA Office of Toxic Substances with cover letter dated 5/18/87. Document Control Number: FYI-OTS-0687-0550 Sequence A.

Wester, P.W., E.I. Krajnc, F.X.R. van Leeuwen et al. 1988. Two year feeding study in rats with bis(tri-n-butyltin)oxide (TBTO). Unpublished report from the National Institute of Public Health and Environmental Hygiene, Bilthoven, Netherlands.

Wester, P.W., E.I. Krajnc, F.X.R. van Leeuwen et al. 1990. Chronic toxicity and carcinogenicity of bis(tri-n-butyltin)oxide (TBTO) in the rat. Fd. Chem. Toxic. 28: 179-196.

VI.B. Inhalation RfC References

Anonymous. 1991. Acute effect of indoor exposure to paint containing bis(tributyltin)oxide -- Wisconsin. Morb. Mortal. Wkly. Rep. 40: 280-281.

Hay, A. and C. R. Singer. 1991. Wood preservatives, solvents, and thrombocytopenic purpura (letter). Lancet. 338: 766.

Schweinfurth, H. A. and P. Gunzel. 1987. The tributyltins: Mammalian toxicity and risk evaluation for humans. Oceans '87: The Ocean "an international workplace." Proceedings of the International Organotin Symposium. 4: 1421-1429.

Shelton, D., B. Urch, and S. M. Tarlo. 1992. Occupational asthma induced by a carpet fungicide -- Tributyltin oxide. J. Allergy Clin. Immunol. 90(2): 274-275.

U.S. EPA. 1994. Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Signed by Administrator Carol Browner, June 7.

U.S. EPA. 1997. Toxicological Review of Tributyltin Oxide. Contact EPA's IRIS Hotline [(202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address)] or the IRIS Web site at www.epa.gov/iris.

VI.C. Carcinogenicity Assessment References

Daly, I.W. 1992. An eighteen month oncogenicity feeding study in mice with bis(tri-n-butyltin)oxide (TBTO). Unpublished report by Bio/dynamics, Inc. prepared for TBTO Consortium. MRID No. 422650-01. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

Davis, A., R. Barale, G. Brun et al. 1987. Evaluation of the genetic and embryotoxic effects of bis(tri-n-butyltin)oxide (TBTO), a broad-spectrum pesticide, in multiple in vivo and in vitro short-term tests. Mutat. Res. 188: 65-95.

Kroes, R., J. M. Garbis-Berkyens, T. deVries, and J. H. J. van Nellesrooy. 1981. Histopathological profile of a Wistar rat stock including a survey of the literature. J. Geront. 36(3): 259-279.

U.S. EPA. 1987. Risk Assessment Guidelines of 1986. EPA/600/8-87/045, August. Prepared by the Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA. 1994. Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Signed by Administrator Carol Browner, June 7.

U.S. EPA. 1996. (New Proposed) Guidelines for Carcinogen Risk Assessment, 1996. These guidelines are available currently only as a draft.

U.S. EPA. 1997. Toxicological Review of Tributyltin Oxide. Contact EPA's IRIS Hotline [(202)566-1676 (phone), (202)566-1749 (FAX), or hotline.iris@epa.gov (internet address), or the IRIS Web site at www.epa.gov/iris.

Wester, P. W., C. A. van der Heijden, A. Bisschop, and G. J. van Esch. 1985. Carcinogenicity study with epichlorohydrin (CEP) by gavage in rats. Toxicology. 36: 325-339.

Wester, P.W., E.I. Krajnc, and C.A. van der Heijden. 1987. Chronic toxicity and carcinogenicity study with bis(tri-n-butyltin)oxide (TBTO) in rats. Unpublished report submitted to the U.S. EPA Office of Toxic Substances with cover letter dated 5/18/87. Document Control Number: FYI-OTS-0687-0550 Sequence A.

Wester, P.W., E.I. Krajnc, F.X.R. van Leeuwen et al. 1988. Two year feeding study in rats with bis(tri-n-butyltin)oxide (TBTO). Unpublished report from the National Institute of Public Health and Environmental Hygiene, Bilthoven, Netherlands.

Wester, P.W., E.I. Krajnc, F.X.R. van Leeuwen et al. 1990. Chronic toxicity and carcinogenicity of bis(tri-n-butyltin)oxide (TBTO) in the rat. Fd. Chem. Toxic. 28: 179-196.

Yamada, H. and Y. F. Sasaki. 1993. Organotins are co-clastogens in a whole mammalian system. Mutat. Res. 301: 195-200.

VII. Revision History

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9

Date	Section	Description
08/22/1988	I.A.	RfD summary on-line
09/01/1997	I.A.	RfD summary replaced; RfD changed
09/01/1997	I.B.	Inhalation RfC discussion on-line
09/01/1997	II.	Carcinogenicity assessment on-line
12/03/2002	I.A.6., I.B., II.D.2.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Tributyltin oxide (TBTO)
CASRN — 56-35-9
Last Revised — 08/22/1988

- 56-35-9
- BIOMET TBTO
- BIS-(TRI-n-BUTYLCIN)OXID
- BIS(TRIBUTYLOXIDE) of TIN
- BIS(TRIBUTYLSTANNIUM) OXIDE
- BIS(TRIBUTYLSTANNYL)OXIDE
- BIS(TRIBUTYLTIN)OXIDE
- BIS(TRI-n-BUTYLZINN)-OXYD
- BTO

- BUTINOX
- C-Sn-9
- DISTANNOXANE, HEXABUTYL-
- ENT 24,979
- HEXABUTYLDISTANNIOXAN
- HEXABUTYLDISTANNOXANE
- HEXABUTYLDITIN
- KYSLICNIK TRI-n-BUTYLCINICITY
- L.S. 3394
- OTBE
- OXYBIS(TRIBUTYLTIN)
- OXYDE DE TRIBUTYLETAIN
- STANNANE, TRI-n-BUTYL-, OXIDE
- TBOT
- TBTO
- TIN, BIS(TRIBUTYL)-, OXIDE
- TIN, OXYBIS(TRIBUTYL-
- Tributyltin Oxide