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Provisional Peer Reviewed Toxicity Values for

Mono-, Di- and Tri- Butyltin Compounds

(Various CASRN)

Derivation of Subchronic and Chronic Oral RfDs

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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	**provisional inhalation unit risk**
p-OSF	**provisional oral slope factor**
p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	**Provisional Peer Reviewed Toxicity Value**
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

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PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR MONO-, DI- AND TRI- BUTYLTIN COMPOUNDS (Various CASRN) Derivation of a Subchronic and Chronic Oral RfD

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

No RfDs for monobutyltin, dibutyltin or tributyltin compounds other than tributyltin oxide are available on IRIS (U.S. EPA, 2006), the HEAST (U.S. EPA, 1997a) or the Drinking Water Standards and Health Advisories (U.S. EPA, 2002). An RfD for tributyltin oxide [bis(tri-n-butyltin)oxide; CASRN 56-35-9] is available on IRIS (U.S. EPA, 1997b, 2006). This RfD of 3E-4 mg/kg-day is based on a NOAEL of 0.025 mg/kg-day and LOAEL of 0.25 mg/kg-day for immunosuppression in rats exposed via the diet for 18 months (Vos et al., 1990). The RfD was estimated from a BMDL of 0.03 mg/kg-day for immunosuppression and an uncertainty factor of 100. ATSDR (2003) established a chronic-duration oral MRL of 3E-4 mg/kg-day for tributyltin oxide based on the NOAEL of 0.025 for immunosuppression in rats (Vos et al.,1990). ATSDR (2003) also established an intermediate-duration oral MRL of 5E-3 mg/kg-day for dibutyltin

dichloride based on a LOAEL of 5 mg/kg-day for immunological effects in rats exposed via the diet for six weeks (Seinen et al., 1977a).

The CARA database (U.S. EPA, 1991, 1994) does not list any documents covering organotin compounds. A NIOSH (1976) Criteria Document for organotin compounds, Poison Information Monographs on dibutyltin dichloride (WHO, 1993) and tributyl tin compounds (WHO, 1994), an Environmental Health Criteria document on tributyl tin compounds (WHO, 1990) and a review of the biological activity of organotin compounds (Snoeij et al., 1987) were consulted for relevant information. In addition, the NTP (2003a) background document for testing of methyltin and butyltin compounds, the management status documents for monobutyltin trichloride (NTP, 2003b) and dibutyltin diacetate (NTP, 2003c) and the health and safety report for dibutyltin acetate (NTP, 2003d) were also consulted. IARC (2003) has not reviewed butyltin compounds. In addition to the above, several documents on tributyltin oxide, which is not a subject of this issue paper because it already has an RfD on IRIS, were consulted for relevant information: the IRIS document (U.S. EPA, 2006) and Toxicological Review (U.S. EPA, 1997b) and a Concise International Chemical Assessment Document (WHO, 1999).

Computer literature searches of TOXLIT (1965-1992), TOXLINE (1965-1992), CHEM ID, RTECS (through August, 1992), and TSCATS databases were conducted for monobutyltin oxide and dibutyltin oxide in August, 1992. An update literature search of TOXLINE, MEDLINE, EMIC, HSDB, DART, and RTECS was performed in March, 1995 for dibutyltin and tributyltin compounds. More recently, update literature searches were conducted on monobutyltin, dibutyltin and tributyltin compounds for the period from 1994 to August 2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. An additional literature search was conducted through September 2004 which produced no new data.

Monosubstituted organotins have had limited application as stabilizers in PVC films. Dialkylorganotin compounds such as dibutyltin are used in the chemical industry as heat stabilizers in the production of PVC, curing agents for silicon rubber, and catalysts in the production of polyurethane. Tributyltin compounds are used mainly for their biocidal properties as molluscicides, fungicides, insecticides and miticides. Tetrasubstituted organotin compounds are mainly used as intermediates in the preparation of other organotin compounds (Boyer, 1989; Bulten and Meinema, 1991; Magos, 1986; Nicklin and Robson, 1988; NIOSH, 1976; WHO, 1980).

Toxicity of organotin compounds is somewhat determined by the nature and number of groups bound to tin (Bulten and Meinema, 1991). In general, toxicity decreases as the number of linear carbons increases, such that triethyltin is more toxic than trioctyltin (Magos, 1986; NIOSH,

1976). Also, toxicity decreases as the number of substitutions decrease, for example, triethyltin chloride is more toxic than monoethyltin trichloride (Magos, 1986; WHO, 1980).

Table 1 gives the butyltin compound molecular weights and associated factors for converting exposures (mg/kg-day) from the administered compound to the relevant butyltin moiety on which each RfD is based.

Table 1. Molecular weights and dose-conversion factors for butyltin compounds

Compound	MW	moiety conversion factor [a]	fraction Sn [b]
butyltin moiety	175.74	–	0.675
monobutyltin trichloride	282.10	0.623	0.421
dibutyltin moiety	232.79	–	0.510
dibutyltin dichloride	303.70	0.767	0.391
dibutyltin oxide	248.79	0.936	0.477
dibutyltin diacetate	350.83	0.664	0.339
tributyltin moiety	289.83	–	0.410
tributyltin chloride	325.28	0.891	0.362
tributyltin oxide	305.83	0.948	0.388

[a] MW of corresponding moiety divided by MW of compound
[b] for conversion to or from tin mass (dose expressed in terms of Sn in some studies)

REVIEW OF THE PERTINENT LITERATURE

Human Studies

No information was located regarding the effects of butyltin compounds in humans following oral exposure. Early reports summarized by WHO (1980) and Boyer (1989) provide information regarding the dermal effects of some of these compounds. Dermal lesions caused by

di- and tributyltin compounds in laboratory workers and process workers handling these compounds were described as typical acute skin burns developing 1 to 8 hours after exposure. This could be prevented by washing the skin immediately after exposure. A single dermal application of undiluted dibutyltin maleate, dibutyltin oxide, dibutyltin dilaurate, dibutyltin diacetate, or tetrabutyltin on the back of the hands of volunteers did not cause irritation. However, within 8 hours of a single application of dibutyltin dichloride, tributyltin chloride, tributyltin acetate, tributyltin laurate, or bis(tributyltin) oxide, the exposed area of the skin reddened. Follicular inflammation and pruritus accompanied this effect. Irritant contact dermatitis was reported in painters that came in contact with a paint containing 0.6% bis(tributyltin)oxide. The effect was characterized by severe itching, redness, swelling and blistering, and confined to the areas of the skin directly exposed to the paint.

Animal Studies

Monobutyltin Compounds

Information on the oral toxicity of monobutyltin compounds in animals comes from developmental toxicity studies in rats exposed by gavage to monobutyltin trichloride (CASRN 1118-46-3) (Noda et al., 1992a; Ema et al., 1995a; Ema and Harazono, 2001); synonyms for this compound are butyltin trichloride and n-butyltin trichloride. No subchronic or chronic duration studies were located.

Noda et al. (1992a) exposed groups of 13-16 pregnant Wistar rats to 0, 50, 100, 200, or 400 mg/kg-day monobutyltin trichloride by gavage in olive oil on gestational days 7-17. These doses are equivalent to 30.2, 60.3, 120.6 and 241.2 mg monobutyltin/kg-day, respectively. Body weights, food consumption and clinical signs were recorded daily. The dams were killed on gestational day 20. Endpoints examined at termination included the weight of the maternal thymus, the number of corpora lutea, the numbers of living and dead fetuses, the number of resorptions, fetal weight, fetal sex, and external malformations; half of the fetuses in each litter were examined for skeletal and half for visceral anomalies. No significant alterations in maternal body weight gain, food consumption or thymus weights were observed. In the offspring, no consistent alterations in the incidence of external, visceral, or skeletal alterations were observed. This study identifies a NOAEL of 400 mg/kg-day for developmental or maternal effects of monobutyltin trichloride in rats (equivalent to 248 mg monbutyltin/kg-day); a LOAEL was not identified.

Higher doses were tested by Ema et al. (1995a). In this study, groups of 6-11 pregnant female Wistar rats were given monobutyltin trichloride at doses of 0, 1000, 1500 or 2000 mg/kg-day by gavage in olive oil on gestational days (GD) 7 and 8. Maternal body weight was monitored up to GD 20; results were reported for the periods GD 0-7, 7-9, 9-20 and 0-20. At termination on GD 20, uteri were examined for the numbers of resorptions and dead and live

fetuses. All live fetuses were sexed, weighed and examined for external malformations, including those of the oral cavity; two thirds of the fetuses were examined for skeletal malformations and the rest for visceral malformations. Maternal mortality was significantly increased at ≥1500 mg/kg-day (0/10, 0/10, 5/11 and 6/6 for the control and low-to-high dose groups, respectively). Most deaths occurred within two days of administration; necropsy of all dead rats revealed hemorrhages in the stomach. A loss in maternal body weight was observed in all treated groups for the period GD 7-9, which includes the two days of treatment; body weight gain was significantly reduced in these groups for the remainder of the study. Total litter resorption occurred in 1/6 litters at 1500 mg/kg-day. Also at 1500 mg/kg, fetal body weights were significantly reduced (male and female). The incidence of malformations was not increased at any dose. This study identifies a LOAEL for monobutyltin trichloride of 1000 mg/kg (equivalent to 620 mg monobutyltin/kg-day) for maternal toxicity (reduced body weight on days of treatment and reduced body weight gain overall); a maternal NOAEL was not identified. The NOAEL for fetal toxicity of butyltin trichloride administered to rats on GD 7 and 8 was 1000 mg/kg-day and the LOAEL was 1500 mg/kg-day (equivalent to 930 mg monobutyltin/kg-day) for reduced body weight in male and female offspring.

Ema and Harazono (2001) evaluated the effect of monobutyltin trichloride (purity 95%) administered by gavage in olive oil on early pregnancy in rats. Groups of 16 pregnant female Wistar rats received butyltin trichloride at doses of 0, 56, 226, or 903 mg/kg-day on gestational days (GD) 0-3 or 4-7. Maternal body weight and food consumption were recorded daily; results for these parameters were reported for the periods GD 0-4 and GD 4-20 for dams treated on GD 0-3 and for the periods GD 0-4, GD 4-8 and GD 8-20 for dams treated on GD 4-7. Dams were sacrificed on GD 20 and examined for corpora lutea and the numbers of resorptions and dead and live fetuses in the uterus. Live fetuses were examined for sex, weight and external malformations. Similar results were observed for the two treatment schedules. No maternal mortality or other clinical sign of toxicity was observed. In dams that received 903 mg/kg-day, food consumption and body weight gain were significantly reduced only for the reporting period that included days of treatment, that is, GD 0-4 for dams treated on GD 0-3, and GD 4-8 for dams treated on GD 4-7; in the latter group a slight body weight loss was observed. The only treatment-related developmental effect was a significantly lower body weight in female fetuses at the 903 mg/kg-day dose on either schedule. This study identifies a NOAEL of 226 mg/kg-day and a LOAEL of 903 mg/kg-day for maternal effects (reduced food consumption and body weight gain) and fetal toxicity (reduced female body weight) for butyltin trichloride administered to rats on GD 0-3 or GD 4-7. The maternal and fetal NOAELs and LOAELs are equivalent to 140 and 560 mg monobutyltin/kg-day.

Dibutyltin Compounds

Several studies have examined the toxicity of dibutyltin compounds in rodents following subchronic (Seinen et al., 1977a,b; Bartalini, 1959; Barnes and Stoner, 1958; Gaunt et al., 1968)

and chronic (NCI, 1979) administration. Noda et al. (1992a, 1992b, 1993) and Ema et al. (1991, 1992) have studied the developmental toxicity of several dibutyltin compounds in rats.

Seinen et al. (1977a) administered dibutyltin dichloride (CASRN 683-18-1; purity >98%) in the diet at levels of 0, 50, or 150 ppm to weanling Wistar-derived rats (10/sex/dose level) for 2 weeks. The dietary levels correspond to 0, 5, and 15 mg/kg-day, respectively, using the standard food consumption rate of 10% of body weight per day for young rats. At termination, animals were necropsied. Body weights and the weights of the thymus, spleen, popliteal lymph node, liver, kidneys, and adrenals were recorded; the same organs were examined for histopathology. Six rats in the 150 ppm group died during the second week. Significant and dose-related changes in males and females included decreased body weight and decreased relative weights of spleen, thymus, and popliteal lymph nodes. Relative liver weight was increased in 150 ppm males only. Necropsy revealed a marked reduction in thymus size in all treated rats. In addition, rats that died early and two male and two female survivors in the 150 ppm group had thickened and dilated bile ducts and yellowish discolored livers. No liver histopathology was seen at 50 ppm, but severe proliferation of bile duct epithelial cells and bile ductules, associated with pericholangiolitis and periportal fibrosis was observed in 150 ppm rats. Depletion of lymphocytes in the thymic cortex was observed at 50 ppm and was nearly complete at 150 ppm; lymphocyte depletion was also observed in thymus-dependent areas of the spleen (periarteriolar lymphocyte sheets) and popliteal lymph node (paracortical areas). This study identifies a LOAEL of 50 ppm for immunotoxicity (depletion of thymic cortex lymphocytes) in rats exposed to di-n-butyltin dichloride for two weeks (equivalent to 3.84 mg dibutyltin/kg-day), with hepatotoxicity at higher doses. A NOAEL was not identified.

Seinen et al. (1977b) conducted a series of experiments evaluating immune system functions in weanling Wistar rats (groups of 5-10 of one sex) fed dibutyltin dichloride in the diet at concentrations of 0, 50, or 150 ppm for 4-6 weeks. Using the standard food consupmtion factor of 10% of body weight per day for young rats, these dietary concentrations correspond to approximate doses of 0, 5, and 15 mg/kg-day. Thymus-dependent cellular immunity was evaluated in an allograft rejection experiment; two donor tail-skin grafts (from Wistar-derived WAG x B F_1 hybrid rats) were transferred to groups of 5-9 seven-week-old inbred male WAG rats that were dietarily exposed to dibutyltin dichloride for 6 weeks (after weaning at week 3 to week 9). Terminal body weights were significantly reduced (by 28.5% compared to controls) and allograft (i.e., from other animals) rejection times were significantly increased by 1.3 days, indicating impaired thymus-dependent cellular immunity, in the 150 ppm group; dose-related changes were also observed in the 50 ppm group, but were not statistically significant. A second experiment evaluated thymus-dependent humoral immunity; groups of 7-8 female rats were immunized with sheep red blood cells five days before the end of a 4-week dietary exposure. In these rats, a statistically significant decrease in body weight gain (-6.6% compared to controls) was observed in the 150 ppm group. Statistically significant immunological effects in females included reductions in the number of spleen cells and the number of antibody-producing cells per

whole spleen in the 50 and 150 ppm groups and in the hemagglutination titer in the 150 ppm group. A third experiment evaluated thymus-independent humoral immunity; groups of 10 male rats were immunized *i.v.* with *E. coli* 38 days after dietary exposure. No significant alterations in hemagglutinating antibody titers were observed; however, 4/10 rats in the 150 ppm group died (authors noted that deaths were probably the result of endotoxin shock). Considering the three experiments, the most sensitive LOAEL for dibutyltin dichloride was 50 ppm (equivalent to 3.84 mg dibutyltin/kg-day) for reduced thymus-dependent humoral immunity (reduction in the numbers of spleen cells and antibody-producing cells per spleen) in female rats exposed in the diet for 4 weeks. A NOAEL was not identified.

Seinen et al. (1977b) also examined the allograft rejection process in groups of 5-10 neonatal Wistar rat pups that received gavage doses of 0, 1, or 3 mg/kg-day dibutyltin dichloride in arachis oil three times per week, for nine weeks, beginning the second day after birth. Significant dose-related decreases in terminal body weights (10-12% lower than controls) were observed in the pups treated with 1 or 3 mg/kg-day dibutyltin dichloride. Allograft rejection times were significantly prolonged by 0.6 and 2.6 days, respectively, in the 1 and 3 mg/kg-day groups. It is conceivable that a generalized stress in these animals, as evidenced by the body weight loss, contributed to the immunotoxicity. However, a direct effect of dibutyltin on immune suppression is highly likely given its well established effect on cell-mediated immune response. The lowest gavage dose of 1 mg/kg-day dibutyltin dichloride, administered 3 days/week, was a LOAEL for reduced body weight and suppressed thymus-dependent cellular immunity (delayed allograft rejection) in the pups exposed for nine weeks after birth; averaged over a week, the LOAEL for dibutyltin dichloride during the postnatal period was 0.43 mg/kg-day (equivalent to a daily dose of 0.33 mg dibutyltin/kg-day). The study did not identify a NOAEL.

Bartalini (1959) orally administered 2.5 mg dibutyltin oxide/kg-day to 7 rats for 60 days (the study was published in Italian with an English summary). Weight gain was not affected by treatment, and the animals showed no signs of toxicity throughout the study. Hematologic testing at sacrifice revealed a slight increase in red blood cell count. Histological examination was limited to the liver and kidneys. Liver changes were described as mild, and consisted of signs of nuclear hypertrophy and cytoplasmic vacuolation. Changes in the kidneys were restricted to the renal tubules and were indicative of a degenerative process followed by signs of regeneration. The lack of comparison of the incidences of hepatic and renal lesions to a control group precludes identifying a NOAEL or LOAEL for this study.

Gaunt et al. (1968) fed groups of CFE rats (16/sex/group) diets containing 0, 10, 20, 40, or 80 ppm dibutyltin dichloride for 90 days. From data on body weight and food consumption, Gaunt et al. (1968) estimated that these dietary levels provided an average of 0, 0.5, 1.0, 2.0, or 4.0 mg dibutyltin dichloride/kg-day. Endpoints examined included body weight and food consumption, hematological parameters, clinical chemistry (AST, ALT, amylase), urinalysis, and

gross (with special reference to the bile duct and pancreas) and microscopic examination of major organs and tissues. Effects were limited to a slight reduction in body weight gain at 4.0 mg/kg-day (<9%), and a slight but significant decrease in blood hemoglobin concentration at 4.0 mg/kg-day in females at week 6 and in males at week 13. Since the observed changes were minor, it appears that the 4.0 mg/kg-day dibutyltin dichloride dose (equivalent to 3 mg dibutyltin/kg-day) represents a NOAEL in this study.

In a study conducted by Barnes and Stoner (1958), albino rats (6/group, sex distribution not specified) were administered dibutyltin dichloride in the diet at levels of 0, 20, 50, 75, or 100 ppm for up to 6 months. These levels correspond to 0, 1.0, 2.5, 3.8, or 5 mg/kg-day dibutyltin dichloride, using a food consumption factor of 0.05 for adult rats. The results are described with few details and can be summarized as follows: doses of 50 ppm or greater significantly reduced weight gain and food intake. After 6 months of treatment, 7 out of 10 rats in the 50 ppm group had bile duct damage characterized by thickening and dilation of the duct, and fibrosis of the pancreas. Mortality was reported in the 100 ppm group during the first 4 weeks, but survivors exhibited no clinical signs of toxicity. At sacrifice (6 months), all the surviving rats in the 100 ppm group had some bile duct damage. The LOAEL for body weight and bile duct changes is 50 ppm dibutyltin dichloride and the NOAEL is 20 ppm (equivalent to 1.9 and 0.75 mg dibutyltin/kg-day, respectively).

NCI (1979) conducted chronic feeding bioassays with dibutyltin diacetate in rodents. Fischer 344 rats (50/sex/group) were given a basal diet containing 66.5 or 133 ppm time-weighted average dietary levels of dibutyltin diacetate for 78 weeks followed by a 26-week observation period on the basal diet; control rats (20/sex) received the basal diet throughout the study. Based on a food consumption factor of 0.05, it can be estimated that the rats were dosed with 3.3 or 6.7 mg/kg-day. Animals were inspected twice daily for clinical signs of toxicity. Body weights were recorded weekly for the first six weeks, every two weeks for the next twelve weeks and at monthly intervals thereafter. Food consumption data were collected only at monthly intervals from 20 percent of the animals in each group. All animals found dead or euthanized prematurely or killed at 104 weeks were necropsied; microscopic examinations were conducted on gross lesions and all major tissues and organs. Most tissue samples were lost for 17/50 high-dose female rats. Survival at 104 weeks was 85, 78, and 52% for males, and 74, 84, and 64% for females in the control, low- and high-dose groups, respectively; the decrease was statistically significant for high-dose males. Dose-related depression of body weight gain compared to controls was observed in male rats throughout the experiment; mean body weights were lower in high-dose female rats for most of the study, but not significantly. No other clinical signs were recorded. Calculi of the bile ducts were observed in 0/20 control, 0/42 low-dose and 10/50 high-dose males, and in 0/19 control, 1/49 low-dose and 3/33 high-dose females. There was a significant dose-related increase in the incidence of pneumonia in male rats, possibly suggesting a compromised immune system. However, no histological alterations were observed in the spleen, thymus or lymph nodes. The average low dose of 3.3 mg/kg-day (equivalent to 2.3

mg dibutyltin/kg-day) is a NOAEL and 6.7 mg/kg-day (equivalent to 4.4 mg dibutyltin/kg-day) is an FEL for accelerated mortality, decreased body weight gain and increased bile duct calculi in males rats.

B6C3F1 mice (50/sex/species/group) were exposed to 76 or 152 ppm time-weighted average dietary levels of dibutyltin diacetate for 78 weeks followed by a 26-week observation period; control mice (20/sex) received the basal diet throughout the study (NCI, 1979). Based on a food consumption factor of 0.1 for adult mice, it can be estimated the male mice were dosed with 7.6 or 15.2 mg/kg-day. Mice were analyzed as described above for rats. Survival was 95, 96, and 86% for male mice and 95, 90, and 58% for female mice in the control, low-, and high-dose groups, respectively. The decreased survival in high-dose female mice was statistically significant. Body weight gain was suppressed in high-dose female mice after week 60, but not in other groups. No other clinical signs were recorded. Degenerative and necrotic changes in the liver were absent in controls and were dose-related in treated mice; however, the incidence of these lesions was low, less than 10% at the high dose. The average low dose of 7.6 mg/kg-day (equivalent to 5.0 mg dibutyltin/kg-day) is a NOAEL and 15.2 mg/kg-day (equivalent to 10.1 mg dibutyltin/kg-day) is a FEL for accelerated mortality and reduced body weight gain in female mice exposed to dibutyltin diacetate.

Seinen et al. (1977a,b) also conducted dietary assays in mice. Weanling male Swiss mice (8-10 per group) were exposed for four weeks to 0, 50, or 150 ppm dietary concentrations of dibutyltin dichloride (0, 7.5, or 23 mg/kg-day, estimated using a food consumption factor of 0.15 for young mice). Body weights and the weights of the thymus, spleen, and liver were recorded. No clinical signs, body weight effects, or effects on thymus or spleen weights were observed in treated mice. No significant alteration in the antibody response to sheep red blood cells was observed. Thus, the NOAEL for immunotoxicity of dibutyltin dichloride is 23 mg/kg-day in mice exposed for 4 weeks (equivalent to 17.6 mg dibutyltin/kg-day).

A number of developmental toxicity studies have been conducted in rats exposed to dibutyltin compounds. Studies using standard exposure periods - on or close to gestational days 6-15 (GD 6-15) - have been conducted in rats exposed by gavage to dibutyltin diacetate (Noda et al., 1992a) or dibutyltin dichloride (Ema et al., 1991; Farr et al., 2001). Follow-up experiments were conducted to identify vulnerable periods during gestation for exposure to dibutyltin diacetate (Noda et al., 1992b) or dibutyltin dichloride (Ema et al., 1992, 1995a, 1996; Ema and Harazono, 2000). Noda et al. (1993) compared the developmental toxicity of several dibutyltin compounds administered at a selected dibutyltin dose on GD 8. Seinen et al. (1977a) evaluated immunotoxicity in rat pups exposed to dibutyltin dichloride during gestation and lactation and subsequently for several weeks via the diet. As presented in detail below, the standard developmental toxicity studies conducted by Noda et al. (1992a) and Farr et al. (2001) provide evidence that teratogenicity of dibutyltin, primarily external and skeletal malformations, occurs only at doses that are toxic to dams. Conversely, Ema et al. (1991) reported malformations at the

maternal no-effect level; however, as this study did not investigate changes in the maternal thymus, the maternal no-effect level may have been overestimated. The Noda et al. (1992a) study identifies a NOAEL and LOAEL of 1.7 and 5.0 mg/kg-day for dibutyltin diacetate, and the Ema et al. (1991) study identifies a NOAEL and LOAEL of 2.5 and 5.0 mg/kg-day for dibutyltin dichloride.

Noda et al. (1992a) administered by gavage 0, 1.7, 5.0, 10.0, or 15.0 mg/kg-day di-n-butyltin diacetate in olive oil to groups of 13-16 pregnant Wistar rats on gestational days 7-17. The dams were killed on gestational day 20. A significant decrease in maternal body weight was observed in the 15.0 mg/kg-day group (without a significant alteration in food intake) and decreased thymus weights were observed in the 5.0, 10.0, and 15.0 mg/kg-day groups. In the 15 mg/kg-day group, a significant decrease in the number of dams with living fetuses, an increase in the number of dams with total resorptions, and an increase in the incidence of dead or resorbed fetuses in the early stage were observed. Decreases in male and female fetal body weights were observed in the 10.0 and 15.0 mg/kg-day groups. Significant increases in the number of fetuses and litters with skeletal malformations (primarily anomaly of mandibular fixation, fused ribs and fused thoracic vertebral arches) and external malformations (primarily cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia, and anury or vestigial tail) were observed in the 10.0 and 15.0 mg/kg-day groups. In the 5.0, 10.0, and 15.0 mg/kg-day groups, dose-related significant increases in the number of fetuses and litters with skeletal variations (primarily cervical ribs) were observed. No visceral alterations were observed. Thus, this study identifies a NOAEL of 1.7 mg/kg-day and LOAEL of 5.0 mg/kg-day for developmental effects and maternal toxicity in rats exposed to di-n-butyltin diacetate (equivalent to 1.1 and 3.3 mg dibutyltin/kg-day, respectively).

Follow-up studies by these researchers showed that the critical period of exposure for production of these effects by dibutyltin diacetate was early in organogenesis (GD7-9) (Noda et al., 1992b) and that similar effects were produced by equimolar doses of other dibutyltin compounds (dibutyltin maleate, dibutyltin dilaurate, dibutyltin oxide, and dibutyltin dichloride) (Noda et al., 1993).

Dibutyltin dichloride was used in another series of studies. Ema et al. (1991) administered by gavage 0, 2.5, 5.0, 7.5, or 10.0 mg/kg-day dibutyltin dichloride in olive oil on gestational days 7-15 to groups of 12 pregnant Wistar rats. In the 7.5 and 10.0 mg/kg-day groups, significant increases in maternal deaths, decreases in body weight gain, and decreases in food consumption were observed. The mean time to death was 8 and 6 days after the start of dosing in the 7.5 and 10.0 mg/kg-day groups, respectively. The cause of death was not reported, but it was noted that hemorrhage in the stomach was observed in most of the dams dying early. Significant increases in the number of resorbed or dead fetuses per litter and the number of post-implantation losses per litter were observed in the 7.5 mg/kg-day group. Decreases in male and female fetal body weights and placental weights were observed in the 5.0, 7.5, and 10.0 mg/kg-

day groups. An increase in the number of litters with external malformations (primarily cleft mandible and ankyloglossia) and skeletal malformations (primarily mandibular defects, fusion and/or absence of cervical vertebral arches, and fusion of ribs) were observed in the 5.0, 7.5, and 10.0 mg/kg-day offspring. No significant alterations in the incidence of visceral malformation were observed in these groups. No external, skeletal, or visceral malformations were observed in the control or 2.5 mg/kg-day groups. This study identifies a NOAEL of 2.5 mg/kg-day and LOAEL of 5.0 mg/kg-day (equivalent to 1.9 and 3.8 mg dibutyltin/kg-day) for developmental effects and a NOAEL of 5.0 mg/kg-day and FEL of 7.5 mg/kg-day (equivalent to 3.8 and 5.7 mg dibutyltin/kg-day) for maternal effects following administration of dibutyltin dichloride on GD 7-15.

Other studies by these researchers investigated the effect of dosing with dibutyltin dichloride at different times during gestation. Early organogenesis (GD7-8) was identified as the most sensitive period for induction of post-implantation loss and teratogenesis (Ema et al., 1992, 1995a, 1996). Exposure earlier in gestation produced an increase in preimplantation loss (Ema and Harazono, 2000).

Farr et al. (2001) also evaluated developmental toxicity of dibutyltin dichloride administered to pregnant female Wistar rats (25/group) by gavage in olive oil at doses of 0, 1, 2.5, 5 or 10 mg/kg-day on GD 6-15. Maternal endpoints included food consumption and body weight gain (both reported for the period GD 6-16), thymus weight, number of pregnant females, number of females with total litter loss and the number of females with viable fetuses. Pregnancy outcomes were evaluated on GD 20 for corpora lutea, implantations, viable fetuses, early and late resorptions, dead fetuses, and fetal weight; all fetuses were examined for external malformations and then equally divided into two groups for examination of visceral or skeletal malformations. The only maternal effects were statistically significant reductions in food consumption, body weight gain, and thymus weight following treatment at 10 mg/kg-day. No statistically significant effects were noted in litter/fetal parameters although, at 10 mg/kg-day, there was a slight increase in the number of single defects/malformations (4 affected fetuses/262 from 3/20 litters vs 1/269 control fetuses). The investigators considered one malformation, ankyloglossia, to be significant because of its rarity and because other studies on dibutyltin dichloride had reported increased incidences of this defect at higher doses. This study identifies a NOAEL of 5 mg/kg-day and a LOAEL of 10 mg/kg-day (equivalent to 3.8 and 7.7 mg dibutyltin/kg-day, respectively) for maternal toxicity (reductions in food consumption, body weight gain, and thymus weight) and teratogenicity (slightly increased incidence of defects, including one instance of ankyloglossia) in rats exposed to dibutyltin dichloride.

Seinen et al. (1977a) evaluated immune function in rats exposed pre- and post-natally to dibutyltin dichloride (purity >98%) by dietary exposure. Groups of 5-6 pregnant Wistar rats were fed diets containing 0, 50, or 150 ppm dibutyltin dichloride throughout pregnancy (starting on gestational day 2) and lactation; after weaning, exposure of offspring continued through the

diet up to postnatal day 39. Using the U.S. EPA (1988) reference values for body weights and food consumption in Wistar rats and the reported body weights for offspring at termination, the calculated doses for dams during gestation were 0, 5 and 15 mg/kg-day and the doses for offspring were 0, 5 or 16 mg/kg-day. In the exposed offspring, significant decreases were observed in body weight (in males at ≥50 ppm and females at 150 ppm), the number of spleen cells, and the number of antibody-producing cells per 1 million spleen cells (in males at 150 ppm and females at ≥50 ppm) and per whole spleen. A significant decrease in hemagglutination titer was also observed in both sexes at 150 ppm. The 50 ppm dietary level (equivalent to 5 mg/kg-day dibutyltin dichloride or 3.8 mg dibutyltin/kg-day) was a LOAEL for suppressed thymus-dependent humoral immunity in rats exposed both prenatally and postnatally (via milk and diet) to dibutyltin dichloride. A NOAEL was not identified.

Tributyltin Compounds

Oral (dietary) toxicity studies for tributyltin compounds other than tributyltin oxide have been conducted in rats: a subchronic study on tributyltin acetate (Barnes and Stoner, 1958), and short-term immunotoxicity assays (Bressa et al., 1991; Snoeij et al., 1985), a two-generation reproductive toxicity assay (Ogata et al., 2001; Omura et al., 2001), and developmental toxicity assays on tributyltin chloride (Ema et al., 1995a, 1995b, 1996; Harazono et al., 1996).

In a study conducted by Snoeij et al. (1985), groups of 10 male weanling Wistar rats were fed a diet containing 0, 15, 50, or 150 ppm tributyltin chloride (purity >98%) in the diet for 2 weeks. These dietary concentrations correspond to doses of approximately 0, 1.5, 5, or 15 mg/kg-day using a food intake factor of 0.1 for young rats. Body and brain weights were significantly decreased at 50 ppm; in this group food intake was reduced by about 25%. Relative spleen weight was significantly reduced at ≥2 mg/kg-day, and relative thymus weight was significantly reduced at ≥7 mg/kg-day (61% reduction at 21 mg/kg-day). Relative liver weight was increased at ≥7 mg/kg-day. Severe and dose-related (≥7 mg/kg-day) lymphocyte depletion was observed in cell suspensions prepared from thymus glands. The spleen showed no lymphocyte depletion or extramedullary hematopoiesis. There was a dose-related (≥7 mg/kg-day) decrease in the amount of iron per spleen, but the iron concentration was not altered. A dose-related increase in the number of erythrocytes situated as rosettes around mononuclear cells was observed in the splenic medullary sinuses. No morphological changes were noticed in the livers. Snoeji et al. (1985) also exposed a group of 6 male weanling rats to 0 or 100 ppm tributyltin trichloride (0 or 10 mg/kg-day, calculated in same manner as 2 week study) in the diet for 4 weeks. A decrease in body weight and a decrease in relative thymus weight were observed. A severe reduction in lymphocyte density was observed in the thymic cortex. The authors noted that lymphocyte depletion was more severe in the rats treated for 4 weeks as compared to those treated for 2 weeks. After 4 weeks on the tributyltin chloride diets, the rats were fed the basal diet for 1-8 weeks. Thymus weight was similar to control values after 1 week, although body weights remained depressed for 3 weeks. When 100 ppm tributyltin chloride was fed to

adrenalectomized rats for 13 days, thymic atrophy was observed, suggesting that this effect was not due to stress. The lowest dietary level of 15 ppm in the 2-week study, equivalent to a dose of 1.5 mg/kg-day for tributyltin chloride (equivalent to 1.3 mg tributyltin/kg-day), was a LOAEL for reduced relative spleen weight. A NOAEL was not identified.

Bressa et al. (1991) examined the immunotoxic effects of bis(tributyltin)oxide (TBTO) and tributyltin chloride in rats. Groups of 4 male Wistar rats (0.190-0.200 kg) were fed diets containing 5 ppm pure bis(tributyltin)oxide, commercial bis(tributyltin)oxide (80% pure), or tributyltin chloride; groups of 8 rats were fed 25 ppm of each chemical, and a group of 8 animals served as control. Treatment lasted 4 weeks. From the average tin consumption, provided by the authors, it can be estimated that the 5 and 25 ppm dietary levels of pure oxide provided doses of 0.432 and 1.46 mg bis(tributyltin) oxide/kg-day, respectively; the 5 and 25 ppm commercial oxide provided doses of 0.332 and 1.74 mg bis(tributyltin)oxide/kg-day; and, the 5 and 25 ppm salt provided doses of 0.403 and 1.70 mg tributyltin chloride. In terms of tributyltin, alone, these doses correspond to 0.410 and 1.39 mg/kg-day for the 5 ppm and 25 ppm pure TBTO exposures respectively, 0.315 and 1.65 mg/kg-day for the commercial TBTO exposures, respectively, and 0.359 and 1.52 mg/kg-day for the 5 ppm and 25 ppm tributyltin chloride exposures, respectively. A group of control and 25 ppm rats were sacrificed after 1 week of treatment. At necropsy, major organs were examined, and liver, spleen, thymus, and brain were weighed and processed for histological examination. Mesenteric lymph nodes were also examined. Rats treated with 25 ppm of pure bis(tributyltin)oxide or tributyltin chloride ate significantly less than controls and gained weight at a significantly reduced rate. After 1 week of exposure to 25 ppm pure bis(tri-n-butyltin)oxide, relative, but not absolute liver weight was significantly increased; other organs were not affected. Thymus weight was not provided for this time-point. Histologically, there were changes indicative of atrophy and lymphocyte depletion in the thymus; the spleen showed a decrease in thymus-dependent lymphocytes. No effects were observed in the kidneys. After 4 weeks of treatment, the only effect on organ weights noticed was a significant decrease in relative and absolute thymus weight at 25 ppm pure bis(tributyltin)oxide and tributyltin chloride. No histological alterations were noticed in the liver. All rats exposed to tributyltin chloride or to 25 ppm of either pure or commercial oxide had markedly hemorrhagic lymph nodes; this same effect was seen in half of the rats treated with 5 ppm of either oxide. Partial atrophy was evident in these nodes, whereas the appearance of the thymus was almost completely normal. No histopathological alterations were detected in the liver, kidneys, or spleen. This study identifies a LOAEL of 0.315 for hemorrhage and partial atrophy of lymph nodes in rats There was no NOAEL.

Barnes and Stoner (1958) investigated the effects of tributyltin acetate in rats. Groups of 12 rats (sex distribution was not specified) were given tributyltin acetate in the diet at levels of 0, 25, 50, or 100 ppm for three months. These levels correspond to approximately 0, 2.5, 5.0, or 10 mg/kg-day tributyltin acetate, respectively, based on a food consumption rate of 0.1. Graphic data presented in the report show that dose levels $\geq$50 ppm greatly decreased body weight gain in

a dose-related manner (≈9% decrease in weight gain at 50 ppm and ≈26% at 25 ppm), but a statistical analysis of the data was not reported. Food consumption data were not reported. Without giving further details, the investigators indicated that animals in the 50 ppm group showed slightly impaired health. Two rats died at 100 ppm during the first three weeks, but the survivors appeared healthy at sacrifice. At necropsy, 4 out of 11 rats in the 100 ppm group showed some degree of bile duct injury. No bile duct lesions were seen in other groups. In addition, the authors stated that the water content in the spinal cord, but not in the brain, was significantly increased ($p<0.01$) in rats treated with the 100 ppm level; the toxicological significance of this finding is unclear. Based on the limited information provided in this study, it appears that the 50 ppm (5.0 mg/kg-day) level represents a NOAEL for tributyltin acetate. The LOAEL, which is also a frank effect level, is 100 ppm (10 mg/kg-day) for bile duct injury and death in rats exposed to tributyltin acetate. The NOAEL and LOAEL/FEL values are equivalent to 4.2 and 8.4 mg tributyltin/kg-day.

A two-generation reproductive toxicity study was conducted in Wistar rats exposed to tributyltin chloride (>95% purity) in feed; methods and results for the parental generation and for female offspring were published by Ogata et al. (2001), whereas methods and results for male offspring were published by Omura et al. (2001). Groups of male and female Wistar rats were mated over a 4-day period and females with confirmed vaginal plugs (10-12/group) were given diets containing 0.03 (control), 5, 25, or 125 ppm of tributyltin chloride from gestational day 0 (GD0) until weaning of the F_1 generation (postnatal day 22, PND 22). The authors estimated doses as 0, 0.4, 2.0, or 10 mg/kg-day. Maternal body weights were recorded on GD 0, 7, and 14, and food consumption was measured between GD 7 and 8 and GD 14 and 15. During the lactational period, body weights were recorded on PND 7, 14, and 21, and food consumption was measured between PND7 and 8 and PND 14 and 14. F_0 generation rats were euthanized at weaning on PND22. On the day of birth of F_1 rats (PND 0), the live and dead offspring were counted, sexed, and examined for gross malformations. On PND 1, litters were culled to 4 males and 4 females. The body weights of F_1 rats were recorded on PND 1, 7, and 21, the anogenital distance was recorded on PND 1 and 4, and eye opening was examined from PND 14. F_1 rats were weaned on PND 22, at which time they received the same parental diet. Body weights and mean food consumption were recorded weekly for F_1 rats. Vaginal opening was examined from PND30 and the estrous cycle was evaluated during PND71-92. A 14-day cohabitation period of F_1 mating pairs started on PND 92; females that did not mate were euthanized. Procedures for the F_1 generation (10-14 per group) during gestation and lactation were the same as for the F_0 generation. On the first day of the estrous stage from PND 148, F_1 rats were killed, a blood sample was taken for measurement of serum estradiol and testosterone, and the uterus and ovaries were weighed. Procedures for the F_2 offspring were identical except that these rats were not mated.

Ogata et al. (2001) reported that for both the F_0 and F_1 parental generations, treatment with tributyltin chloride had no significant adverse effect on maternal survival, food

consumption, the fertility index, the number of litters, or the mean duration of gestation; there were also no effects in the F_1 and F_2 pups on the neonatal sex ratio, or at termination on ovarian or uterine histology or serum levels of 17β-estradiol or testosterone. The following statistically significant changes compared to controls were observed. In both generations, treatment at 125 ppm significantly reduced maternal weight gain, the total number of pups per litter, the live birth index, and pup body weights per litter on PND1. The weights of F_1 and F_2 female pups were significantly reduced at ≥25 ppm during lactation and at 125 ppm after weaning up to PND92; the effect at 25 ppm appears to be minimal and transient (observed only at PND 14 and PND 21) and therefore, not biologically significant. The day of eye opening was not affected in F_1 pups, but was significantly delayed in F_2 pups exposed at 125 ppm. There was a dose-related trend for increasing anogenital distances (measured on PND 1 and PND 4) in treated female pups, but only the 125 ppm group showed statistically significant increases (ranging from 10 to 15%) in both the F_1 and F_2 generations. The statistically significant increases at 5 ppm and 25 ppm in F_1 pups were judged not to be biologically relevant, as they were minimal (ca. 7%), and because no effect was noted in the F_2 generation. The age of vaginal opening was delayed by 6 days in F_1 and F_2 pups exposed at 125 ppm. Estrous cycle effects at 125 ppm included a significantly reduced duration (F_2 only) and a significantly reduced percentage of normal cycles in both F_1 and F_2 rats. At 125 ppm, uterine weights relative to body weight were significantly elevated only in F_2 rats and ovarian relative weights were significantly reduced only in F_1 rats. In this study, the 25 ppm dietary level of tributyltin chloride, equivalent to 2 mg/kg-day, is a NOAEL and 125 ppm, equivalent to 10 mg/kg-day is a LOAEL for significant effects noted in both generations: reductions in maternal weight gain, number of live pups per litter, and pup body weights (at birth, during lactation and after weaning), and effects on sexual development (delays in vaginal opening and increases in anogenital distances and the frequency of abnormal estrous cycles). The NOAEL and LOAEL are equivalent to 1.8 and 8.9 mg tributyltin/kg-day.

Omura et al. (2001) reported the results for F_1 and F_2 male rats exposed to tributyltin chloride in the two-generation reproductive toxicity study; see Ogata et al. (2001) for initial description of parental treatments. Dietary exposure levels were 0, 5, 25, or 125 ppm, reported as equivalent to daily doses of 0, 0.4, 2, or 10 mg/kg-day. Body weights of F_1 pups were recorded on postnatal days (PND) 1, 4, 14, and 21. Anogenital distances for male pups were recorded on PND 1 and 4, eye opening was examined from PND 14 and the descent of the testes was examined from PND 20. Beginning at weaning on PND 22, body weights and food consumption of F_1 male were recorded weekly. On PND 91, F_1 males cohabited with F_1 females for 14 days. F_1 males were sacrificed on PND 119, at which time venous blood was collected for the analysis of testosterone, 17-β-estradiol and luteinizing hormone (LH). Absolute weights of the testes and epididymis, and weights of the ventral prostate and seminal vesicles (without fluid) relative to 100 g body weight were recorded; the testes were evaluated for histopathology. Spermatids from the testes and sperm from the cauda epididymis were counted; mature sperm were evaluated for motility and structural aberrations. F_2 males were treated as the F_1 generation except that single males from each litter underwent terminal sacrifice and analysis on PND 91 and were not mated.

The results of exposure to tributyltin chloride were similar in F_1 and F_2 male rats (Omura et al., 2001). Body weight gains were significantly reduced during lactation (PND 1-21) and weaning (PND 22-91) at 125 ppm in both generations (70-80% for F_1 and 65-71% for F_2 males compared to controls); weight gains were also significantly reduced at PND 14 and PND 21 in F_1 males exposed at 25 ppm. Treatment had no significant effect on food consumption (PND 22-91), anogenital distance (AGD), or on the day of testis descent in either generation, or on fertility or copulation indices in F_1 adults. The opening of the eyes was delayed in F_1 males exposed at $\geq$25 ppm and in F_2 males exposed at 125 ppm; at 125 ppm, the delay was 0.6 days for F_1 rats and 1.2 days for F_2 rats. Note that for the following parameters under discussion, F_1 males were exposed up to PND 119, whereas F_2 males were exposed up to PND 91; despite the shorter duration of exposure, compound-related changes in male reproductive parameters tended to be more severe in the F_2 generation than in the F_1 generation. Treatment at 125 ppm resulted in biologically and statistically significant reductions in organ weights; for the F_1 and F_2 generations, respectively, the absolute weights were reduced in the testes by 16 and 21% and in the epididymis by 12 and 20%. Relative weights of the ventral prostate were significantly reduced in F_1 males by 16% at 125 ppm and in F_2 males by 16% at 25 ppm and 31% at 125 ppm; treatment had no effect on relative weights of the seminal vesicles. The testicular and epididymal weight effects at 5 ppm in F_1 rats were judged not to be of biological significance because the effects were minimal (less than 5%) and were not observed in the F_2 males. Statistically significant effects on sperm parameters included lowered testicular spermatid counts (by 20% at 125 ppm in F_1 males and by 11% at 25 ppm and 23% at 125 ppm in F_2 males), reduced epididymal sperm counts (by 24% at 125 ppm in F_2 males only), and increased incidence of sperm abnormalities (tailless sperm at 125 ppm in F_1 males only); sperm motility was not affected by treatment. The authors did not regard occasional testicular histopathological findings (vacuolization of and spermatid retention in seminiferous epithelium and germ cell degeneration) in F_1 males to be abnormal because of the low frequency (data not reported). In F_2 rats, the frequencies of minimal changes were high in some treated rats and thus were categorized as abnormal by the authors, but the overall number of affected animals was low and not statistically different from controls. Statistically significant changes in serum levels were observed at 125 ppm: elevations in testosterone and decreases in 17-β-estradiol in both generations and increased LH in F_2 males only. In this study, the lowest dietary level of 5 ppm tributyltin chloride (a dose 0.4 mg/kg-day) was a NOAEL and 25 ppm (a dose of 2 mg/kg-day) was a LOAEL for decreased relative weight of the ventral prostate and lowered testicular spermatid counts in F_2 rats. The NOAEL and LOAEL are equivalent to 0.36 and 1.8 mg tributyltin/kg-day, respectively.

Harazono et al. (1996) evaluated the effects of tributyltin chloride (96% purity) on early gestation in rats. Groups of 10-14 inseminated female Wistar rats received tributyltin by gavage in olive oil at doses of 0, 8.1, 12.2, or 16.3 mg/kg-day on GD 0-7. Maternal body weight, food consumption and clinical signs of toxicity were recorded daily. At termination on GD 20, live fetuses were sexed, weighed, and inspected for external and oral malformations; two-thirds of the live fetuses were examined for skeletal malformations and the rest for visceral malformations.

Treatment had no effect on maternal mortality, but resulted in dose-related increases in the incidences (not reported) of clinical signs of toxicity (sluggishness, bloody stain around the eyes and diarrhea). For the reporting period GD 0-8, which included days of dosing, food consumption was severely reduced compared to control values: by 41, 67, and 73% in the low-to-high dose groups. Food consumption was normal in all treated groups for the reporting period after dosing (GD 8-20). Statistically significant reductions in maternal body weight occurred at ≥12.2 mg/kg-day during GD 0-8; maternal body weight gain at 8.1 mg/kg-day was lower than in controls, but the difference was not statistically significant. Maternal body weight gain excluding the uterus was not affected by treatment. Treatment with tributyltin chloride resulted in a dose-related increase in percent pregnancy failure that was statistically significant at ≥12.2 mg/kg-day: 0, 18.2, 71.4, and 76.9% for the control and low-to-high dose groups. There were no dose-related effects on the number of corpora lutea, the number of implantations, preimplantation or postimplantation losses per litter, the sex ratio or body weights of live fetuses, or the incidences of malformations. For treatment with tributyltin chloride on GD 0-7, the NOAEL for maternal toxicity was 8.1 mg/kg-day and the LOAEL was 12.2 mg/kg-day (equivalent to 7.2 and 10.9 mg tributyltin/kg-day respectively) for reduced feed consumption and absence of implantation. The highest dose of tributyltin chloride, 16.3 mg/kg-day (equivalent to 14.6 mg tributyltin per kg-day), was a NOAEL for fetal toxicity in successful pregnancies.

As part of a comparative developmental toxicity study on mono-, di- and tributyltin compounds, groups of 6-11 pregnant female Wistar rats were given tributyltin chloride at doses of 0, 40, or 80 mg/kg-day by gavage in olive oil on gestational days (GD) 7 and 8 (Ema et al., 1995a). Maternal body weight was monitored up to GD 20; results were reported for the periods GD 0-7, 7-9, 9-20, and 0-20. At termination on GD 20, uteri were examined for the numbers of resorptions and dead and live fetuses. All live fetuses were sexed, weighed, and examined for external malformations, including those of the oral cavity; two thirds of the fetuses were examined for skeletal malformations and the rest for visceral malformations. Treatment with tributyltin chloride had no effect on maternal survival. Dose-related reductions in maternal body weight were reported for the period GD 7-9, resulting in significant reductions in body weight gain in both dosed groups compared to controls at termination. Treatment at ≥40 mg/kg-day caused a significant dose-related increase in the percentage of postimplantation loss per litter. Treatment at 80 mg/kg-day significantly increased the number of litters totally resorbed and reduced the number of live fetuses per litter. Tributyltin chloride did not significantly increase the incidences of external, skeletal, or visceral malformations in rats at doses that were maternally toxic. In this study, 40 mg/kg-day (equivalent to 35.8 mg tributyltin/kg-day) was a LOAEL for maternal toxicity (reduced body weight gain) and fetal toxicity (increased postimplantation loss per litter) following exposure to tributyltin chloride.

Ema et al. (1995b) also exposed groups of 11-14 pregnant Wistar rats to tributyltin chloride (96% purity) by gavage in olive oil at doses of 25 or 50 mg/kg-day on GD 7-9, doses of 50 or 100 mg/kg-day on GD 10-12 or doses of 25, 50, or 100 mg/kg-day on GD 13-15. A control

group received olive oil on GD 7-15. Analysis was as described for Ema et al. (1995a) except that results for maternal body weight were reported for the periods GD 0-7, GD 7-10, GD 10-13, GD 13-16, GD 16-20, and GD 0-20. There was no treatment-related maternal mortality. Maternal weight gain was significantly reduced for the period of dosing for all treated groups and reduced overall (GD 0-20) for treatment at ≥25 mg/kg-day on GD 7-9 or at 100 mg/kg-day on GD 10-12 or GD 13-15. Treatment at ≥25 mg/kg-day on GD 7-9 resulted in significant reductions in the numbers of live fetuses per litter and increases in post-implantation losses per litter and total litter resorptions, but had no effect on the incidence of malformations. Treatment with tributyltin chloride at ≥50 mg/kg-day on GD 10-12 resulted in significantly lower body weight for female fetuses. Treatment at 100 mg/kg-day on GD 10-12 significantly reduced the numbers of live fetuses per litter and increased post-implantation losses per litter; at this dose, male and fetal body weights were reduced and the incidence of fetuses or litters with malformations (cleft palate) was increased. Effects observed following treatment on GD 13-15 included significantly reduced fetal body weights in both sexes at 100 mg/kg-day and increased external malformations (cleft palate) at ≥25 mg/kg-day. The lowest dose of 25 mg/kg-day for tributyltin chloride (equivalent to 22.4 mg tributyltin/kg-day) was a LOAEL for maternal toxicity (reduced body weight gain following treatment on GD 7-9), fetal toxicity (increased death and total litter losses after treatment on GD 7-9) and teratogenicity (external malformations, primarily cleft palate, after treatment on GD 13-15).

In another comparative developmental toxicity study, Ema et al. (1996) exposed groups of 10-11 pregnant Wistar rats to 0, 50, or 100 mg/kg-day of tributyltin chloride by gavage in olive oil on gestational days (GD) 13-15. The protocol was the same as for the study by Ema et al. (1995a) except that maternal body weights were reported for the periods GD 0-13, GD 13-16, GD 16-20, and GD 0-20. One of the high dose rats died, but the cause of death was not reported. Maternal body weight gain was significantly reduced in both treated groups. Treatment had no effect on numbers of litters or implantations, the fetal sex ratio, or the incidences of skeletal or visceral malformations. Treatment at 100 mg/kg-day significantly reduced body weights of male and female fetuses and treatment at ≥50 significantly increased the incidence of fetuses or litters with external malformations, primarily cleft palate. The low dose of 50 mg/kg-day for tributyltin chloride (equivalent to 44.7 mg tributyltin/kg-day) was a LOAEL for maternal toxicity (reduced body weight gain) and teratogenicity.

Other Studies

An acute exposure study conducted by Snoeij et al. (1988) compared the toxicity of monobutyltin trichloride, dibutyltin dichloride, and tributyltin chloride in rats. Significant decreases in thymus weight were observed at ≥5 mg/kg dibutyltin dichloride and at ≥10 mg/kg tributyltin chloride, but no changes in thymus weight were observed at doses of 10-180 mg/kg monobutyltin trichloride. These results suggest that the dose-responses for immunological

effects observed following exposure to dibutyltin or tributyltin compounds may not apply to monobutyltin compounds.

Data summarized by Boyer (1989) indicate that alkyltin compounds are metabolized mainly in the liver by the P-450 monooxygenase system. Tributyltin acetate was metabolized by isolated rat microsomes to form alpha-, beta-, gamma- and delta-hydroxybutyldibutyltin derivatives, as well as 1-butanol and 1-butane. Further oxidation of the gamma-hydroxy compound yielded the corresponding ketone. Tetrabutyltin incubated with the liver microsome fraction produced tributyltin derivatives; similarly, dibutyltin diacetate produced butyltin derivatives. Several of the metabolites that were produced *in vitro* were also detected in the liver and feces of mice exposed to tributyltin acetate or dibutyltin diacetate by gavage. In rats, an initial increase in tributyltin observed in the liver after exposure to tributyltin fluoride by gavage was followed by an increase in dibutyltin, monobutyltin and inorganic tin. Biliary excretion represents the main route of excretion of butyltin compounds.

In a comparative *in vitro* toxicity experiment, Seinen et al. (1977b) exposed primary cultures of human (children), rat, mouse or guinea pig thymocytes to dibutyltin dichloride at concentrations of 0, 0.5, 0.5, 5 or 50 μg/mL for up to 24 hours. The compound had no effect on the cell counts or viability of thymocytes from mice or guinea pigs. Cell counts were reduced for human thymocytes exposed at ≥0.5 mg/mL and cell counts and viability of rat thymocytes were reduced following exposure at ≥0.05 mg/mL. These data suggest that rats are a more appropriate animal model than mice or guinea pigs for the immunotoxicity of dibutyltin dichloride in humans.

Measured and estimated chemical properties of three tributyltin compounds are compared in Table 2. Tributyltin oxide is different from the acetate or chloride in having a larger molecular size (because of its bis configuration) and the lowest solubility in water. However, the Log Kow values suggest that the ability of the three compounds to cross biological membranes may be similar.

Table 2. Comparison of Chemical Properties of Some Tributyltin Compounds[a]

	Tributyltin Acetate	Tributyltin Chloride	Tributyltin Oxide
CAS No.	56-36-0	1461-22-9	56-35-9
Molecular Weight	349.1	325.51	596.12
Log Kow	3.24 (estimate)	4.76 (experimental) 4.70 (estimated)	3.84 (experimental) 4.05 (estimated)
Water Solubility at 25 degrees C (mg/L)	10.78 (estimated)	0.7478 (estimated)	0.08958 (estimated)

[a] Based on EPIWIN version 3.11

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR BUTYLTIN COMPOUNDS

Monobutyltin Compounds

No data are available for the oral toxicity of monobutyltin compounds in humans and no subchronic- or chronic-duration oral studies are available for animals. Three developmental toxicity studies are available for Wistar rats exposed to monobutyltin chloride by gavage (Noda et al., 1992a; Ema et al., 1995a; Ema and Harazono, 2001). The results of these studies are summarized in Table 3. No teratogenicity was reported for monobutyltin trichloride and the compound appears not to be a specific developmental toxicant, because fetal effects only occurred at doses that were toxic to dams. These data are insufficient to derive an RfD for monobutyltin compounds because sensitive targets have not been identified.

Table 3. Developmental Toxicity in Wistar Rats Exposed to Monobutyltin Trichloride by Oral Gavage

Study	Treatment Days	NOAEL / LOAEL (mg monobutyltin/kg-day)	Effect
Noda et al. (1992a)	GD 7-17	Maternal: 248 / ND Fetal: 248 / ND	none
Ema et al. (1995a)	GD 7-8	Maternal: ND / 620 Fetal: 620 / 930	reduced body weight gain in dams and fetuses
Ema and Harazono (2001)	GD 0-3 or GD 4-7	Maternal: 140 / 560 Fetal: 140 / 560	reduced feed consumption and body weight gain in dams and female fetuses

ND = not determined

Dibutyltin Compounds

Table 4 summarizes NOAEL and LOAEL values (expressed as mg dibutyltin per kg body weight per day) for the toxicity studies most relevant to risk assessment for dibutyltin.

No data are available for the oral toxicity of dibutyltin compounds in humans. The oral toxicity database includes chronic-duration (primarily carcinogenicity) studies in rats and mice, subchronic studies in rats, short-term studies in rats and mice, and developmental studies in rats. Based on the available data, the most sensitive target of dibutyltin compounds is the immune system. Thymic atrophy, as evidenced by a decrease in thymus weight, was observed in weanling rats exposed to ≥5.4 mg dibutyltin/kg-day as dibutyltin dichloride in the diet for two weeks (Seinen et al., 1977b) and in pregnant rats given gavage doses of 7.7 mg dibutyltin/kg-day as dibutyltin dichloride on GD 6-15 (Farr et al., 2001) or ≥3.3 mg dibutyltin/kg-day as dibutyltin

Table 4. Oral Toxicity of Dibutyltin Compounds in Rodents

Reference	Exposure Conditions (days/vehicle/compound)	NOAEL / LOAEL (mg dibutyltin/kg-day)	Effect
Rats- Systemic toxicity studies			
Seinen et al. (1977a)	2 weeks/feed/dibutyltin dichloride	ND / 5.4	Reduced thymus weight and size; depletion of thymic lymphocytes
Seinen et al. (1977b)	4-6 weeks/feed/dibutyltin dichloride	ND / 3.8	Reduced thymus-dependent humoral immunity (to sheep RBCs)
Seinen et al. (1977b)	9 weeks/arachis oil gavage/ dibutyltin dichloride	ND / 0.33	Reduced body weight gain and thymus-dependent cellular immunity (delayed allograft rejection)
Gaunt et al. (1968)	90 days/feed/dibutyltin dichloride	3 / ND	No systemic toxicity
Barnes and Stoner (1958)	6 months/feed/dibutyltin dichloride	1.5 / 3.0	Reduced body weight gain; histopathology of bile duct
NCI (1979)	2 years/feed/dibutyltin diacetate	3.5 / 7.0	Reduced body weight gain in both sexes; reduced survival and increased bile calculi in males
Mice- Systemic toxicity studies			
Seinen et al. (1977a,b)	4 weeks/feed/dibutyltin dichloride	24.5 / ND	No systemic or immunotoxic effects
NCI (1979)	2 years/feed/dibutyltin diacetate	8.9 / 17.8	Reduced survival and body weight gain in females
Rats- Developmental toxicity studies			
Noda et al. (1992a)	GD 7-17/olive oil gavage/ dibutyltin diacetate	maternal: 1.1 / 3.3 fetal: 1.1 / 3.3	Reduced thymus weight in dams; increased skeletal variations in fetuses
Ema et al. (1991)	GD 7-15/olive oil gavage/ dibutyltin dichloride	maternal: 3.8 / 5.7 fetal: 1.9 / 3.8	Increased mortality and decreased body weight gain in dams; decreased body weight and increased external and skeletal malformations in fetuses
Farr et al. (2001)	GD 6-15/ olive oil gavage/ dibutyltin dichloride	maternal: 3.8 / 7.7 fetal: 3.8 / 7.7	Decreased food consumption, body weight gain, and thymus weight in dams; slight increased incidence of defects in fetuses
Seinen et al. (1977a)	GD 2-PND39/feed/ dibutyltin dichloride	pups: ND / 3.8	Reduced number of spleen cells, splenic antibody cells and body weight gain in pups

ND = not determined.

diacetate on GD 7-17 (Noda et al., 1992a, 1992b). Thymus-dependent immune function was impaired in weanling rats fed diets supplying 4.5 mg dibutyltin/kg-day as dibutyltin dichloride for 4-6 weeks (Seinen et al., 1977b). Thymus-dependent cellular immunity (allograft rejection) was impaired in newborn rats that received dibutyltin dichloride by gavage at 1 mg/kg-day, three days/week for nine weeks beginning two days after birth (Seinen et al., 1977b); averaged over a week, the doses were 0.33 mg dibutyltin/kg-day. Seinen et al. (1977b) mentioned anecdotally (possibly as a result of a range-finding study) that severe atrophy of lymphoid organs was the only pathological alteration observed in rats following postnatal intubation with 5 mg/kg dibutyltin dichloride, leading to growth stunting and increased mortality. No immunotoxicity or any other systemic effect was observed in mice that received ≤24.5 mg dibutyltin/kg-day as dibutyltin dichloride via the diet for four weeks (Seinen et al., 1977a, 1977b). No thymus effects were noted in the two-year dietary studies in rats and mice in which the animals were six weeks old at the start of exposure to dibutyltin diacetate (NCI, 1979).

The most appropriate basis for the provisional RfD for dibutyltin compounds appears to be a LOAEL for immunotoxicity (reduced thymus-dependent cellular immunity, as exemplified by delayed allograft rejection) and reduced body weight in neonatal rats exposed to 1 mg/kg-day dibutyltin dichloride by gavage 3 days/week (daily average of 0.33 mg dibutyltin/kg-day) for nine weeks beginning on postnatal day 2 (Seinen et al., 1977b). A NOAEL for dibutyltin has not been identified. The study did not provide sufficient information to support a benchmark dose analysis. The LOAEL is divided by an uncertainty factor of 1000 (10 for extrapolation from a LOAEL, 10 for interspecies extrapolation, $10^{0.5}$ for intraspecies variability, and $10^{0.5}$ for database deficiencies) to yield **provisional subchronic and chronic RfD values of 3E-4 (3 x 10^{-4}) mg dibutyltin/kg-day**, as follows:

subchronic p-RfD	=	LOAEL / UF
	=	0.33 mg dibutyltin/kg-day / 1000
	=	3E-4 mg dibutyltin/kg-day
p-RfD	=	LOAEL / UF
	=	0.33 mg dibutyltin/kg-day / 1000
	=	3E-4 mg dibutyltin/kg-day

A factor of 3 for intraspecies variability was chosen because the neonatal rats were treated during the postnatal period critical for thymus maturation (Seinen et al., 1977b), and therefore represent a sensitive population or stage of development. The primary database deficiency is the lack of a 2-generation reproduction study; the lack of a developmental toxicity study in a second species is less critical because immunotoxicity is well-established as the most sensitive effect for butyltins in general, usually occurring at exposures more than an order of magnitude below developmental toxicity levels. A subchronic-to-chronic uncertainty factor for the provisional chronic RfD appears to be unjustified considering that no thymus histopathology was noted in

rats treated with dibutyltin diacetate for two years from the age of six weeks at doses as high as 12.3 mg/kg-day (NCI, 1979). Although the short study duration of 9 weeks would generally be of concern when extrapolating to chronic exposure, it is not an issue in this case because dosing in this study included the postnatal period critical for thymus maturation. The provisional RfD for dibutyltin should be protective against developmental toxicity.

Confidence in the principal study, Seinen et al. (1977b), is medium. It examined the effect of dibutyltin on immune function during a sensitive period of thymus maturation, but the group sizes were small and limited to males only, and a NOAEL was not identified. The results of the critical experiment were supported by other functional tests in the same paper in which older (weanling) rats of one sex were exposed via the diet at slightly higher doses. Confidence in the database is low. The NCI (1979) study examined the chronic toxicity of dibutyltin diacetate in rats and mice exposed via the diet, but was designed to assess carcinogenicity and did not include evaluations of hematology, clinical chemistry, or urinalysis parameters (although the thymus was examined for histopathology). Immunotoxicity has not been examined in studies of long-term duration. In addition, there are no data on the developmental toxicity of dibutyltin compounds in a second species and no data on the potential of dibutyltin compounds to induce reproductive effects. Reflecting the low confidence in the database, there is low confidence in the provisional subchronic and chronic RfD values.

This p-RfD is for mg dibutyltin/kg-day. If soil or water concentrations at the site of concern are expressed in units of dibutyltin compound (such as dibutyltin dichloride), a molecular weight conversion can be made as follows:

$$RfD_{\text{dibutyltin compound}}\ (\text{mg/kg-day}) = RfD_{\text{dibutyltin}}\ (\text{mg/kg-day}) \times [MW_{\text{dibutyltin compound}} / MW_{\text{dibutyltin}}]$$

where: MW = molecular weight.

Tributyltin Compounds

Table 5 summarizes the results of oral toxicity studies of tributyltin acetate and tributyltin chloride (expressed as mg tributyltin per kg body weight per day).

No data are available for the oral toxicity of tributyltin compounds in humans. No chronic-duration exposure data are available for tributyltin compounds other than tributyltin oxide. Based on the available data (excluding tributyltin oxide), the most sensitive LOAEL was for hemorrhagic, partially atrophic lymph nodes in rats that received 0.4 mg/kg-day tributyltin chloride (equivalent to 0.36 mg tributyltin/kg-day) via the diet for 4 weeks (Bressa et al., 1991); a NOAEL was not identified. Relative and absolute thymus weights were reduced at higher doses in this study. Immune system toxicity following dietary exposure to tributyltin chloride was also

Table 5. Oral Toxicity of Tributyltin Compounds in Rats

Reference	Exposure Conditions: days/vehicle/doses	NOAEL / LOAEL (mg tributyltin/kg-day)	Effect
Systemic toxicity			
Snoeij et al. (1985)	2 weeks/diet/tributyltin chloride (males only)	ND / 1.8	Reduced relative spleen weight
Bressa et al. (1991)	4 weeks/diet/tributyltin chloride	ND / 0.315	Hemorrhage and partial atrophy of lymph nodes
Barnes and Stoner (1958)	3 months/diet/tributyltin acetate	4 / 8.1	Bile duct injury and reduced body weight gain
Reproductive/Developmental Toxicity			
Ogata et al. (2001)	Two generations/diet/ tributyltin chloride	1.8 / 8.9	Reduced total and live pups/litter and pup body weights, increased anogenital distance, abnormal estrous cycle, delayed vaginal opening
Omura et al. (2001)	Two generations/diet/ tributyltin chloride	0.36 / 1.8	Reduced relative prostate weight and testicular spermatid counts in F_2 males
Harazono et al. (1996)	GD 0-7/olive oil gavage/ tributyltin chloride	maternal: 7.2 / 10.9 fetal: 14.6 / ND	Reduced feed consumption and inhibited implantation in dams; no fetal effects
Ema et al. (1995a)	GD 7-8/olive oil gavage/ tributyltin chloride	maternal: ND / 35.8 fetal: ND / 35.8	Reduced body weight gain and increased postimplantation loss
Ema et al. (1995b)	GD 7-9, 10-12, or 13-15/ olive oil gavage/tributyltin chloride	maternal: ND / 22.4 fetal: ND / 22.4	Reduced body weight gain in dams; increased fetal deaths / litter losses and external malformations
Ema et al. (1996)	GD 13-15/olive oil gavage/ tributyltin chloride	maternal: ND / 44.7 fetal: ND / 44.7	Reduced body weight gain in dams; increased external malformations in pups

ND = not determined

reported in studies on weanling male rats by Snoeij et al. (1985). In the two-week study, relative spleen weights were significantly reduced at ≥1.8 mg tributyltin/kg-day, and relative thymus weights were reduced and thymus glands were depleted of lymphocytes at higher doses. In the four-week study, adverse effects on the thymus and body weight gain were observed at 13 mg/kg-day (11.6 mg tributyltin/kg-day), the only dose tested (Snoeij et al., 1985).

After immunotoxicity, the next most sensitive target of tributyltin was the male reproductive system, as reported in the two-generation reproductive study in rats dietarily exposed to tributyltin chloride (Omura et al., 2001). F_2 males appeared to be more sensitive to tributyltin chloride than F_1 males, although they were treated for 28 fewer days. In this study, the NOAEL was 0.36 mg tributyltin/kg-day; the relative weight of the ventral prostate and testicular spermatid counts were reduced in F_2 males at ≥1.8 mg tributyltin/kg-day. At higher doses, testicular and epididymal weights were reduced in F_1 and F_2 males, testicular spermatid counts were reduced in F_1 males, and epididymal sperm counts were reduced in F_2 males. In the F_0 treated dams, as reported in the companion study by Ogata et al. (2001), maternal toxicity (reduced body weight gain) and reproductive toxicity (reductions in litter size, pup survival and pup body weights at birth and weight gains during lactation and weaning) were observed at 8.9 mg tributyltin/kg-day. Female offspring were less sensitive to tributyltin chloride, as shown by the NOAEL of 1.8 mg tributyltin/kg-day (Ogata et al., 2001). At 8.9 mg tributyltin/kg-day, F_1 and F_2 females exhibited an increased anogenital distance (indicative of interference in sexual development), delayed vaginal opening (possibly related to reductions in body weight gain), and an increase in the frequency of abnormal estrous cycles.

Adverse effects on the bile duct were noted at 8.2 mg tributyltin/kg-day in rats exposed to tributyltin acetate in the diet for six months (Barnes and Stoner, 1958). However, as discussed above in the derivation for dibutyltin compounds, it is not certain that this effect is relevant to humans. Developmental toxicity studies on tributyltin chloride observed impaired implantation, post-implantation losses, and external malformations, but only at maternally toxic doses of 22.4 mg tributyltin/kg-day or more (Ema et al., 1995a, 1995b, 1996; Harazono et al, 1996).

The most sensitive endpoint in the literature was identified in the Bressa et al. (1991) 4-week rat dietary exposure study. The LOAEL of 0.332 mg/kg-day for hemorrhagic, partially atrophic lymph nodes in rats exposed to the commercial grade formulation of TBTO (equivalent to 0.315 mg tributyltin/kg-day) is selected as the basis for the subchronic RfD. A NOAEL was not identified. Data from this study were not suitable for benchmark dose analysis. The LOAEL is divided by an uncertainty factor of 1000 (10 for extrapolation from a LOAEL, 10 for interspecies extrapolation, 10 for human variability) to yield a **provisional subchronic RfD of 3E-4 (3×10^{-4}) mg tributyltin/kg-day**, as follows:

subchronic p-RfD	=	LOAEL / UF
	=	0.315 mg tributyltin/kg-day / 1000
	=	3E-4 mg tributyltin/kg-day

An uncertainty factor for database weakness was not used because the database includes a multigeneration reproduction study and developmental toxicity studies showing effects only at high doses that also produced overt toxic effects in the dams. An uncertainty factor for extrapolation from a less-than-subchronic duration was not used as chronic toxicity data for TBTO indicate that the LOAEL does not decrease with increased duration of exposure (Toxicological Review for TBTO on IRIS, U.S. EPA, 2006). The provisional subchronic RfD is expected to be protective against developmental toxicity.

Confidence in the critical study (Bressa et al., 1991) is medium; it thoroughly examined sensitive endpoints relative to immunotoxicity, but was limited by relatively small group sizes, short study duration, lack of testing in females, and failure to identify a NOAEL. Confidence in the subchronic database is medium. Supporting systemic toxicity data are limited, but adequate testing for developmental and reproductive effects showed effects only at higher doses. Confidence in the provisional subchronic RfD for tributyltin compounds other than tributyltin oxide is medium.

The 4-week study by Bressa et al. (1991) is of insufficient duration to serve as the basis for a chronic RfD. In the absence of suitable data on other tributyltin compounds, application of the verified RfD for tributyltin oxide [bis(tri-n-butyltin)oxide] (U.S. EPA, 1997b, 2005) to other tributyltin compounds was considered. A comparison of Log Kow values indicates that absorption and distribution of tributyltin oxide would be similar to that of the acetate and chloride (Table 4). Furthermore, the comparative study by Bressa et al. (1991) reported identical LOAEL values of 0.4 mg/kg-day for hemorrhagic lymph nodes in rats exposed to tributyltin oxide or tributyltin chloride for 4 weeks. Similar to the other tributyltin compounds, the extensive database for tributyltin oxide (U.S. EPA, 2005) confirms that developmental effects of treatment occur at doses higher than those effective for immunotoxicity.

The verified chronic oral RfD on IRIS (U.S. EPA, 2006) of 3×10^{-4} mg/kg-day for tributyltin oxide is adjusted for molecular weight (see Table 1) to serve as the basis for the **chronic oral RfD of 3E-4 mg tributyltin/kg-day** for tributyltin compounds.

This provisional RfD is for mg tributyltin/kg-day. If soil or water concentrations at the site of concern are expressed in units of tributyltin compound (such as tributyltin dichloride), a molecular weight conversion can be made as follows:

$RfD_{tributyltin\ compound}$ (mg/kg-day) = $RfD_{tributyltin}$ (mg/kg-day) x [$MW_{tributyltin\ compound}$ /$MW_{tributyltin}$]

where: MW= molecular weight.

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7-6-2006

Provisional Peer Reviewed Toxicity Values for

Mono-, Di- and Tri- Butyltin Compounds

(Various CASRN)

Derivation of Subchronic and Chronic Inhalation RfCs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level

MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

7-6-2006

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR MONO-, DI- AND TRI- BUTYLTIN COMPOUNDS (Various CASRN) Derivation of a Subchronic and Chronic Inhalation RfC

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

RfCs for monobutyltin, dibutyltin or tributyltin compounds are not listed on IRIS (U.S. EPA, 2006) or the HEAST (U.S. EPA, 1997a). The CARA list (U.S. EPA, 1991, 1994a) does not include any documents for butyltins. The ATSDR (2003) updated draft Toxicological Profile for tin (which includes organotin compounds) does not establish inhalation MRLs for any butyltin compound because of a lack of suitable data. ACGIH (2001, 2003), NIOSH (2003) and OSHA (2003) have established occupational exposure limits (8-hour TWA) of 0.1 mg/m^3, as Sn, for organic tin compounds, including tributyltins, to protect against irritation of the eyes, skin and respiratory tract, as well as potential adverse effects on immune function and the central nervous system. A NIOSH (1976) Criteria Document for organotin compounds, Poison Information Monographs on dibutyltin dichloride (WHO, 1993) and tributyltin compounds (WHO, 1994), an Environmental Health Criteria document on tributyltin compounds (WHO, 1990) and a review of

the biological activity of organotin compounds (Snoeij et al., 1987) were consulted for relevant information. In addition, the NTP (2003a) background document for testing of methyltin and butyltin compounds, the management status documents for monobutyltin trichloride (NTP, 2003b) and dibutyltin diacetate (NTP, 2003c), and the health and safety report for dibutyltin acetate (NTP, 2003d) were also consulted. IARC (2003) has not reviewed butyltin compounds. In May,1992, literature searches were conducted in the following databases: TOXLINE (1965-1992), CANCERLINE (1963-1992), CHEM ID, HSDB, RTECS and TSCATS. In March 1995, update computer literature searches were conducted in: TOXLINE, MEDLINE, CANCERLINE, TSCATS and RTECS. More recently, update literature searches were conducted on monobutyltin, dibutyltin and tributyltin compounds for the period from 1994 to August 2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. An additional literature search was conducted through September 2004 which produced no new data.

Tributyltin oxide is the only butyltin compound for which a toxicity assessment is available on IRIS (U.S. EPA, 2006), but this did not include an RfC. Documents specific to tributyltin oxide, a Toxicological Review (U.S. EPA, 1997b) and a Concise International Chemical Assessment Document (WHO, 1999), were also consulted for this provisional value assessment.

REVIEW OF THE PERTINENT LITERATURE

Human Studies

No relevant data were located regarding the toxicity of monobutyltins, dibutyltins or tributyltins to humans following subchronic or chronic inhalation exposure.

Irritation of the upper respiratory tract and eye was reported following acute occupational exposure to tributyltin oxide (ACGIH, 2001). Portal-of-entry effects following inhalation exposure in humans would also be expected for other butyltin compounds for which acute eye irritation or skin irritation has been reported, such as tributyltin chloride and dibutyltin dichloride (ACGIH, 2001; WHO, 1993).

Animal Studies

The only relevant inhalation study in animals that was located was an unpublished 4-week inhalation toxicity study of monobutyltin trichloride in rats (Biodynamics, 1988). Groups of Sprague-Dawley rats (15/sex/concentration) were whole-body exposed to vapor/aerosol atmospheres of 95% pure monobutyltin trichloride (MW = 282.1) at mean measured

concentrations of 3.4, 18, or 24 mg/m^3 (1.3, 7.2, or 9.5 mg Sn/m^3) for 6 hours/day, 5 days/week for 4 weeks. In terms of monobutyltin (MW = 175.74), the exposure levels correspond to 2.1, 11.2 and 15.0 mg/kg-day, respectively. Controls were chamber-exposed to room air. The test atmospheres were generated from the liquid test material using a bubbler with in-line trap. The measured concentrations were much lower than the corresponding nominal values calculated based on amount of material added to the system, and the difference increased dramatically with concentration, apparently due to problems with formation of the aerosol and chemical reaction of the test material within the chamber. Measurement of the aerosols within the exposure chamber showed that a high percentage of test material in the chamber was in the form of aerosol in the low- and mid- exposure groups; the percentage was considerably lower in the high-exposure group. The mass median aerodynamic diameter MMAD (and geometric standard deviation GSD) of the aerosols in the exposure chamber were 0.91 µm (1.6), 0.98 µm (4.1), 1.7 µm (2.3), and 1.5 µm (2.0) in the control, low-, mid-, and high-exposure groups, respectively, indicating that particles in the chamber were respirable. Rats were evaluated twice daily for mortality and clinical signs, and received a weekly detailed physical examination for abnormal signs. Body weights were recorded before testing and weekly thereafter. Ophthalmoscopic examinations were conducted before testing and at termination. After 4 weeks of treatment, 15 rats/sex/group were sacrificed; of these, 10 were evaluated for hematology, clinical chemistry, gross pathology, organ weights (adrenals, brain, kidneys, liver ovaries and testes) and histopathology (more than 30 tissues examined in the control and high-concentration groups; only tissues with gross lesions examined in the low- and mid-dose groups). Overnight urine samples were collected for urinalysis from the other 5 rats, which were subsequently used for analysis of tissue tin content.

Mortality was observed in 3 males and 1 female at the highest concentration; deaths occurred after 13-15 days of exposure (Biodynamics, 1988). Deaths were considered to be treatment-related by the researchers, although cause of death could not be identified from gross necropsy. Clinical signs in high-concentration rats during the 4-week exposure period included mucoid nasal discharge, rales, lacrimation, salivation, rough coat, ano-genital staining, discoloration of the fur, and (in males only) abdominal distension. Statistically significant reductions in mean terminal body weights were seen in both males (-7%) and females (-5%) in the highest exposure group, compared to the controls. Hematological evaluation revealed no effects in treated males and only slight changes in females (small statistical increases in hemoglobin and hematocrit in the high-exposure group and erythrocyte counts in all exposed groups). There were no exposure-related ophthalmoscopic, clinical biochemistry or urinalysis findings, or effects on organ weights. The primary gross lesion was discoloration of the lungs, observed in most treated males (77-90% of each group) and females (80-100% of each group), but not in controls. Histopathological examination revealed amorphous material (hypothesized by the researchers to be the test material and/or its hydrolysis product monobutyltin dihydroxy chloride) in the alveolar sacs of treated rats (7/10, 9/10, and 9/9 males and 9/10, 8/10, and 10/10 females in the low-to-high concentration groups, respectively). This was accompanied in many cases by alveolar edema (6/10, 7/10 and 3/9 males and 3/10, 6/10 and 3/10 females in the low-to-

high concentration groups, respectively) and in some cases by subacute bronchopneumonia (2/10, 2/10 and 1/9 males and 0/10, 1/10 and 0/10 females, respectively). Severity of the pulmonary lesions was similar in all treated groups. None of these lesions were observed in controls. Other than the increase above controls, a dose-related pattern was not observed. Lung tin burdens in the mid- and high-level groups were similar, and considerably higher than the low-level group. Histological findings in other tissues were generally unremarkable, although there was some evidence for an effect on the nasal turbinates (purulent exudate observed in the anterior section of the nasal cavity in 5/9 males and 3/10 females, but not in controls) and the skin (epidermal acanthosis and hyperkeratosis observed in 8/8 males and 5/8 females, but not controls) in the high-exposure group (neither of these tissues were examined in the low- or mid-level groups). The authors of the study (Biodynamics, 1988) described the lesions as the expected response of lung tissue to the introduction of foreign corrosive material. The EPA agrees with this conclusion but considers the resulting effects to be adverse. The observed alveolar edema and bronchopneumonia is most likely a result of localized hypoxia to cells covered by the foreign material. This effect would also be expected in humans provided the tissue coverage was similar to that in the rats. However, the effects are likely dominated by the physical processes and less affected by interspecies or inter-individual physiological differences, consideration of which will reduce the overall uncertainty factor applied to the RfC (see RfC derivation section following). The lowest concentration of monobutyltin trichloride, 3.4 mg/m^3 (2.1 mg/m^3 as monobutyltin), is determined to be a LOAEL for pulmonary lesions in male and female rats. A NOAEL is not identified in this study.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR BUTYLTIN COMPOUNDS

Monobutyltin Compounds

No subchronic or chronic toxicity data are available for humans exposed to butyltins by inhalation. The only available animal study identified a LOAEL of 2.1 mg/m^3 for monobutyltin in rats exposed intermittently for 4 weeks, based on development of pulmonary lesions (Biodynamics, 1988). The data from this study were not amenable to benchmark concentration dose modeling, because incidence of lesions was high in the low-level group, and incidence and severity of effects were similar at higher exposure levels. Therefore, the LOAEL/NOAEL approach was used.

A provisional subchronic RfC for monobutyltin can be derived from the Biodynamics (1988) study using the methodology for aerosols presented in U.S. EPA (1994b). Although the exposure atmosphere included both vapors and aerosols of monobutyltin trichloride, the atmosphere at the LOAEL of 2.1 mg/m^3 (as monobutyltin) consisted largely of aerosols. In order to derive the provisional subchronic RfC, the LOAEL of 2.1 mg/m^3 is adjusted for continuous

exposure based on the exposure protocol (2.1 mg/m^3 x 6/24 hr x 5/7 d = 0.38 mg/m^3) to obtain a duration-adjusted LOAEL ($LOAEL_{ADJ}$) of 0.38 mg/m^3. The $LOAEL_{ADJ}$ is multiplied by the RDDR (regional deposited dose ratio) to calculate the human equivalent concentration ($LOAEL_{HEC}$). The RDDR for pulmonary effects in rats (average body weight of 353 g used, based on males at the LOAEL) for particles with MMAD = 0.98 μm and σ_g = 4.1 (the mean values at the LOAEL) is 0.335. The $LOAEL_{HEC}$ is 0.38 mg/m^3 x 0.335 = 0.13 mg/m^3. An uncertainty factor (UF) of 300 was calculated from factors of 10 for use of a LOAEL, 3 ($10^{0.5}$) to protect sensitive individuals, and 10 for deficiencies in the database, including the lack of reproductive, developmental, or supporting systemic studies. An additional 3-fold factor for interspecies toxicodynamic uncertainty was deemed to be unnecessary because of the predominantly physical nature of the effect. The human inter-individual uncertainty factor was reduced by half-an-order of magnitude, because toxicokinetic uncertainty is minimal. Dividing the $LOAEL_{HEC}$ of 0.13 mg/m^3 by the UF of 300 produces a **provisional subchronic RfC of 4E-4 (4×10^{-4}) mg/m^3** for monobutyltin trichloride. A provisional chronic RfC was not derived due to the short exposure duration of the Biodynamics (1988) study.

Confidence in the key study for the provisional subchronic RfC is medium. The study included adequate numbers of animals and dose groups, and examined a wide range of endpoints, including systemic and portal-of-entry effects. However, exposure duration was short, there were evident problems generating the test atmospheres, reporting of methods and results was unclear (particularly with regard to exposure concentrations experienced by test animals), and exposure levels were too high (neither a NOAEL nor a dose-response was identified). Confidence in the database is low because developmental, reproductive, and supporting systemic studies are unavailable, yielding a low confidence in the subchronic p-RfC.

Di- and Tri- Butyltin Compounds

Derivation of provisional subchronic or chronic RfCs is not feasible for dibutyltin or tributyltin compounds due to the absence of relevant data.

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6-12-2006

Provisional Peer Reviewed Toxicity Values for

Mono-, Di- and Tri- Butyltin Compounds

(Various CASRN)

Derivation of a Carcinogenicity Assessment

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
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OSF	oral slope factor
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p-RfD	provisional oral reference dose
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ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
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RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

6-12-2006

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR MONO-, DI- AND TRI- BUTYLTIN COMPOUNDS (Various CASRN)
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Background

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Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Tributyltin oxide (CASRN 56-35-9) is the only butyltin compound for which there is a carcinogenicity assessment on IRIS (U.S. EPA, 1997a, 2006). In this assessment (consensus review date 07/02/1997), tributyltin oxide is assigned to U.S. EPA (2005) cancer weight-of-evidence category *"Inadequate Information to Assess Carcinogenic Potential."* Tributyltin oxide is not mutagenic to bacteria, but yields positive results in mammalian systems *in vitro* and *in vivo*. Since an assessment is available on IRIS, tributyltin oxide is not considered further in this issue paper. However, some documents on this compound were consulted for possible information on other butyltin compounds: the IRIS summary sheets (U.S. EPA, 2006) and Toxicological Review (U.S. EPA, 1997a), and a Concise International Chemical Assessment Document (WHO, 1999).

No carcinogenicity assessments are available for monobutyltin, dibutyltin, or tributyltin compounds (other than tributyltin oxide) on IRIS (U.S. EPA, 2006), the HEAST (U.S. EPA, 1997b), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002). The CARA database (U.S. EPA, 1991, 1994) does not list any document covering organotin compounds. A NIOSH (1976) Criteria Document for organotin compounds, a draft of the ATSDR (2003) updated draft Toxicological Profile for tin (which includes organotin compounds), Poison Information Monographs on dibutyltin dichloride (WHO, 1993) and tributyl tin compounds (WHO, 1994), an Environmental Health Criteria document on tributyl tin compounds (WHO, 1990) and a review of the biological activity of organotin compounds (Snoeij et al., 1987) were consulted for relevant information. In addition, the NTP (2003a) background document for testing of methyltin and butyl tin compounds, the management status documents for monobutyltin trichloride (NTP, 2003b) and dibutyltin diacetate (NTP, 2003c) and the health and safety report for dibutyltin acetate (NTP, 2003d) were also consulted. IARC (2003) has not reviewed organotin compounds. In August 1992, computer literature searches of TOXLIT (1965-1992), TOXLINE (1965-1992), CHEM ID, RTECS (through August, 1992), and TSCATS databases were conducted for monobutyltin oxide and dibutyltin oxide. In March 1995, update literature searches of TOXLINE, MEDLINE, EMIC, HSDB, DART, and RTECS were performed for dibutyltin and tributyltin compounds. More recently, update literature searches were conducted for the period from 1994 to August 2003 for monobutyltin, dibutyltin and tributyltin compounds in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. An additional literature search was conducted through September 2004 which produced no new data.

Monosubstituted organotins have had limited application as stabilizers in PVC films. Dialkylorganotin compounds such as dibutyltin are used in the chemical industry as heat stabilizers in the production of PVC, curing agents for silicon rubber, and catalysts in the production of polyurethane. Tributyltin compounds are used mainly for their biocidal properties as molluscicides, fungicides, insecticides and miticides. Tetrasubstituted organotin compounds are mainly used as intermediates in the preparation of other organotin compounds (Boyer, 1989; Bulten and Meinema, 1991; Magos, 1986; Nicklin and Robson, 1988; NIOSH, 1976; WHO, 1980).

REVIEW OF THE PERTINENT LITERATURE

Human Studies

No data were located on the carcinogenicity of mono-, di- or tri- butyltin compounds in humans.

Animal Studies

Monobutyltin Compounds

No data were located on the carcinogenicity of monobutyltin compounds in animals.

Dibutyltin Compounds

Carcinogenicity data for dibutyltin compounds in animals are limited to oral-exposure studies for dibutyltin acetate in rats and mice (NCI, 1979).

Fischer 344 rats (50/sex/dose group) received dibutyltin diacetate at time-weighted average dietary levels of 66.5 or 133 ppm for 78 weeks, and then received control diets for a period of 26 weeks (NCI, 1979). Twenty animals/sex served as controls. Based on approximate average body weights in the study of 0.350 kg for males and 0.225 kg for females, and using a food consumption rate calculated as described in U.S. EPA (1988), it can be estimated that males ingested 0, 5.3, or 10.6 mg/kg-day and females ingested 0, 6.2, or 12.3 mg/kg-day. Endpoints examined included body weight and food consumption throughout the study, and gross and microscopic examination of major tissues and organs at sacrifice and from animals that died early. Body weights of high-dose male rats were lower than controls throughout the experiment, but statistical analysis was not reported; body weights in females were not affected by treatment with dibutyltin diacetate. Survival was significantly reduced in high-dose male rats (apparently due to pneumonia), but remained adequate for assessment of risk due to late-developing tumors (26/50 survived to termination). Survival in treated females was not significantly lower than controls, and was adequate for assessment of late-developing tumors (32/50 survived to termination in the high-dose group and 42/50 in the low-dose group). However, tissues from 17 out of 50 high-dose females were lost, limiting the adequacy of the evaluation in this group. The only tumorigenic response of note in rats was an apparent increase in neoplasms of the uterus in low-dose females, that did not, however, achieve statistical significance. The incidence of uterine neoplasms was 1/19 (5%), 10/49 (20%), and 2/33 (6%) in the control, low-dose, and high-dose groups, respectively. Uterine neoplasms included adenocarcinomas, leiomyomas, endometrial stromal polyps, and hemangiomas. The lack of increase in the high-dose group is confounded by tissues that were lost from 17/50 high-dose females. Of these 17 animals, 5 were noted as having undetermined uterine tumors on the basis of gross observation. NCI (1979) concluded that there was no evidence for carcinogenicity in male rats, and that the study in female rats was inadequate because tissues from many high-dose rats were not analyzed.

In the mouse study, B6C3F1 mice (50/sex/dose level) were treated with dibutyltin diacetate at time-weighted average dietary levels of 76 or 152 ppm for 78 weeks, and then received control diets for an additional 14 weeks (NCI, 1979). A control group was composed of 20 mice/sex. Based on estimated average body weights of 0.037 kg for males and 0.034 kg for females in this study, and using a food consumption rate calculated as described in U.S. EPA

(1988), the doses were estimated as 0, 13.0, or 26.0 mg/kg-day for male mice and 0, 13.4, or 26.8 mg/kg-day for female mice. The endpoints examined were the same as in the rat study. In mice, body weight gain did not appear to be significantly affected by treatment with dibutyltin diacetate. Survival was significantly reduced in high-dose female mice starting at about week 45 of the study, but remained adequate for evaluation of late-developing tumors (29/50 survived to study termination). The cause of death in high-dose females was not discussed. The study found marginal evidence for a treatment-related increase in liver tumors (see Table 1). The incidence of hepatocellular adenomas or carcinomas (combined) appeared to be increased in treated males, but the differences from control were not statistically significant. Females did not have carcinomas. There was a significant trend for hepatocellular adenomas in females, but only a marginal pairwise increase in the high-dose group. NCI (1979) determined that under the conditions of the study, there was no conclusive evidence for the carcinogenicity of dibutyltin diacetate in male or female mice.

Table 1. The Incidence of Liver Tumors in Mice Studied by NCI (1979)

Tumor	Dose Group		
	Control	Low	High
Male			
Hepatocellular adenoma	2/19 (11%)	9/49 (18%)	13/49 (27%)
Hepatocellular carcinoma	0/19 (0%)	2/49 (4%)	2/49 (4%)
Hepatocellular adenoma or carcinoma	2/19 (11%)	11/49 (22%)	15/49 (31%)
Female			
Hepatocellular adenoma	1/20 (5%)[a]	4/47 (9%)	12/43 (28%)[b]

[a] significant trend (p=0.006) by Cochran-Armitage test (reported by NCI, 1979)

[b] marginally significant pairwise increase (p=0.03) by Fisher Exact test that is not significant after Bonferroni correction for multiple comparisons (reported by NCI, 1979)

Tributyltin Compounds

Aside from the oral studies on tributyltin oxide, the only information on carcinogenicity of tributyltin compounds in animals comes from a dermal exposure study by Sheldon (1975). Sheldon (1975) applied tributyltin fluoride to the shaved backs of male Swiss albino mice (50/dose group) three times per week for 6 months. Two treated groups were included: one that received 15 mg of a 10% solution of the compound in propylene glycol for 26 weeks and one that received a 30% solution for the first 3 weeks of the study, but had the concentration lowered to5% for the remaining 23 weeks due to irritant effects. A control group received the solvent alone, and a positive control group was treated with a known carcinogen identified as R-911-10 in propylene glycol. Ten animals treated with 30% and then 5% solution showed hyperplastic skin changes, attributed by the authors to the irritant effects of the 30% solution. No neoplastic changes were seen in this group. Mice treated with the 10% solution showed no neoplastic or nonneoplastic skin lesions. The incidence of skin lesions in the positive control group was 56%, 86% of which were neoplastic. Negative controls had no lesions.

Other Studies

Toxicokinetics

Data summarized by Boyer (1989) indicate that alkyltin compounds are metabolized mainly in the liver by the P-450 monooxygenase system. Tributyltin acetate is metabolized by isolated rat microsomes to form alpha-, beta-, gamma- and delta-hydroxybutyldibutyltin derivatives, as well as 1-butanol and 1-butane. Further oxidation of the gamma-hydroxy compound yields the corresponding ketone. Tetrabutyltin incubated with the liver microsome fraction produces tributyltin derivatives; similarly, dibutyltin diacetate produces monobutyltin derivatives. Several of the metabolites that are produced *in vitro* have also been detected in the liver and feces of mice exposed to tributyltin acetate or dibutyltin diacetate by gavage. In rats, an initial increase in tributyltin observed in the liver after exposure to tributyltin fluoride by gavage was followed by an increase in dibutyltin, monobutyltin and inorganic tin. Biliary excretion represents the main route of excretion of butyltin compounds.

Ueno et al. (1997) examined the hepatic metabolism of di- and tributyltin chlorides in male mice exposed by gavage. Three hours after exposure to 0.18 mM/kg tributyltin chloride, the main hepatic metabolites were dibutyltin dichloride (40%) and dibuty(3-carboxylpropyl)tin (12%); by 24 hours the content had changed to 36.4% and 25.6%, respectively. In contrast, 24 hours after treatment with dibutyltin chloride, most of the hepatic butyltin was the parent compound (94.8%). Pretreating mice with an inhibitor to cytochrome P-450 significantly reduced hepatotoxicity caused by exposure to tributyltin chloride, but did not affect the hepatic toxicity caused by exposure to dibutyltin chloride. The authors suggested that dibutyltin is the primary agent for hepatotoxicity in mice.

In an *in vitro* study, Ohhira et al. (2003) compared the metabolism of tributyltin by hepatic microsomes derived from rats, hamsters, male humans, and female humans. Under comparable reaction conditions, rat hepatic microsomes exhibited the highest dealkylation and dearylation activity compared to the other samples. The percentage of total metabolites (dibutyltin, monobutyltin, and inorganic tin) to parent tributyltin was 214, 55.1, 11.4 and 27.6, respectively, for rat, hamster, male human, and female human hepatic microsomes. The authors suggested that the hamster is more appropriate than the rat as a model for evaluating tributyltin effects in humans.

Tributyltin compounds may reduce cellular levels of glutathione, increasing susceptibility of cells to oxidative stress. Following a 15 minute incubation, tri-*n*-butyltin chloride, but not tetrabutyltin chloride, at concentrations of ≥ 3 nM, reduced the cellular content of glutathione in cultured rat thymocytes (Okada et al., 2000). At a concentration of 300 nM, the glutathione content was nearly depleted.

Genotoxicity

Mono-, di-, and tri- butyltin compounds are mutagenic in some bacterial systems. Dibutyltin diacetate was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 in the presence or absence of metabolic activation from induced rats or hamsters (Boyer, 1989). Using a modified assay for mutagenicity in *S. typhimurium* TA100, significant increases in the number of revertants were observed for mono-n-butyltin oxide, n-butyltin trichloride, di-n-butyltin dichloride, tri-n-butyltin chloride and bis-(tri-n-butyltin) oxide (Hamasaki et al., 1993). Only di-n-butyltin dichloride had positive results when the *S. typhimurium* TA98 strain was used (Hamasaki et al., 1993). Positive results were found in the SOS chromotest with *Escherichia coli* PQ37 for mono-n-butyltin oxide, n-butyltin trichloride and di-n-butyltin dichloride (Hamasaki et al., 1992); negative results were observed for tri-n-butyltin chloride. In the rec-assay for mutagenicity in *Bacillus subtilis*, positive results were found for di-n-butyltin dichloride and tri-n-butyltin chloride, while negative results were found for mono-n-butyltin oxide and n-butyltin oxide (Hamasaki et al., 1992).

Dibutyltin compounds gave mixed results for genotoxicity in mammalian cells *in vitro*. Dibutyltin dichloride increased the frequency of mutations (HGPRT assay) in cultured Chinese hamster ovary cells (Li et al., 1982). In tests without metabolic activation, tributyltin chloride did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO K1) cells (Sasaki et al., 1993). Treatment with dibutyltin chloride or tributyltin chloride did not significantly increase the frequency of aneuploidy in cultured peripheral lymphocytes taken from one human donor (non-smoking healthy female) (Jensen et al., 1991). Tributyltin chloride caused a slight elevation in the frequency of hyperdiploid cells, but the increase was not statistically significant. Neither n-butyltin trichloride nor di(n-butyltin) dichloride induced breaks in double-stranded lambda DNA (Hamasaki et al., 1995). Di-*n*-butyltin dichloride at concentrations between 100 and 1500 nM promoted morphological transformation in previously

initiated, cultured murine C3H/10T1/2 cells (Parfett et al., 2000). The compound also induced the expression of mRNA species associated with cell transformation: the proliferin gene (a growth hormone with angiogenic properties) and members of the fos and jun proto-oncogene families.

In vivo, dibutyltin diacetate was inactive in *Drosophila melanogaster* in an assay for sex-linked recessive lethal mutations in which the male flies were either fed or injected the compound before mating (Woodruff et al., 1985). Micronucleus formation in bone marrow erythrocytes of mice exposed orally to dibutyltin dichloride was observed after 48 or 72 hours of administration, but not after 24 hours, suggesting that biotransformation was needed for the effect (Life Science Research, 1991).

In summary, there is evidence for the genotoxicity of mono-, di- and tri- butyltin compounds in various *in vitro* and *in vivo* systems.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR MONO-, DI-, AND TRI- BUTYLTIN COMPOUNDS

There are no data for the carcinogenicity of mono-, di- or tributyltin compounds in humans or for the carcinogenicity of monobutyltin compounds in animals. Data for dibutyltin compounds in animals are limited to the NCI (1979) study, which reported negative or inconclusive evidence in rats and mice exposed in the diet to dibutyltin diacetate. Data for tributyltin compounds (excluding tributyltin oxide) are limited to a skin painting assay by Sheldon (1975) in which no neoplastic changes were noted in mice exposed to tributyltin fluoride. Short-term studies suggest that the butyltin compounds may produce genotoxic effects. Under the guidelines for carcinogen risk assessment (U.S. EPA, 2005), there is inadequate information to assess the carcinogenic potential of butyltin compounds. The lack of positive cancer data precludes the derivation of provisional quantitative risk estimates for these compounds.

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