UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

DATE: May 14, 2008

SUBJECT: Revised **5-Chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan):** Toxicology Chapter for the Reregistration Eligibility Decision (RED) Document. Case No 2340.
PC Code: **054901. DP Barcode: 373536**

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Attached is the Toxicology Disciplinary chapter for Triclosan for the purpose of issuing a Reregistration Eligibility (RED) Decision.

TABLE OF CONTENTS

3

1.0 HAZARD CHARACTERIZATION

Triclosan (2,4,4' –trichloro-2'-hydroxydiphenyl ether) is a chlorinated aromatic compound that has functional groups representative of both phenols and ethers. It is used as a synthetic broad-spectrum antimicrobial agent in the form of a white to off-white powder. It is practically insoluble in water but is soluble in most organic solvents. Uses of triclosan include common everyday products, such as soaps, deodorants, toothpastes, laundry detergents, fabric softeners, facial tissues, and adult diapers. Triclosan is also impregnated in products such as kitchen utensils, toys, bedding, socks, and trash bags.

Acute toxicity studies in experimental animals with technical grade triclosan show that by the oral and dermal routes triclosan is of low acute toxicity (Toxicity Category IV; MRIDs 43206501 and 100178). By the inhalation route of exposure triclosan was assigned Toxicity Category II for acute exposures and is thus of higher acute toxicity by inhalation exposure than by oral or dermal exposures (MRID 42306902 and 43310501). Triclosan produces moderate irritation to the eyes (MRID 43429) and skin (MRID 42306903) with a Toxicity Category III assigned for these acute exposures. Triclosan was not a dermal sensitizer in guinea pigs using the Buehler method (MRID 43206502).

Liver toxicity in mice and rats was noted after 90 day repeated oral exposure to triclosan, characterized by fatty metamorphosis and cytomegaly, hypertrophic hepatocytes, vacuolization, inflammation, and pigmentation of Kupffer cells (MRID 43022605, 99.7% a.i.; MRID 133545, % a.i. not stated). A LOAEL of 25 mg/kg/day was obtained for the mouse study, and a NOAEL of 1000 ppm was obtained for the 90-day rat study. Similar effects on the liver including liver cell necrosis and an increase in the liver-body weight ratio were observed in a 28-day oral toxicity study in mice (MRID 44389707, > 99% a.i.). Hematological effects including significant decreases in hemoglobin and increases in thrombocytes, clinical chemistry alterations, and elevated serum enzyme activities were also evident in this study. The 28-day oral mouse systemic NOAEL was considered to be 6.48 mg/kg/day in males, and 8.25 mg/kg/day in females. In a 90-day oral toxicity study in dogs (MRID 96102, % a.i. not stated), histopathologic examination of tissues from dogs that were sacrificed or died showed evidence of hepatotoxicity resulting in obstructive jaundice with the NOAEL determined to be 12.5 mg/kg/day.

In a 90-day dermal toxicity study in rats (MRID 43328001, 99.7% a.i.), dose-related dermal irritation was present at all dose levels (10, 40, and 80 mg/kg/day) but was also reversible after a recovery period of 20 days. In addition, an increase in the incidence of occult blood in the urine was found in males and females at the 80 mg/kg/day dose level. Under the conditions of this study, the systemic LOAEL was 80 mg/kg/day; the systemic NOAEL was 40 mg/kg/day.

In two 14-day repeated dose dermal toxicity range-finding studies in mice and rats (MRID 44389708, 99.3% a.i.; MRID 44389710, 99.3% a.i.), signs of dermal toxicity were observed at application sites. A LOAEL of 6.0 mg/animal/day was obtained in the rat study. The NOAEL was 3.0 mg/animal/day. In the mouse study, systemic responses were noted including a dose-dependent increase in plasma levels of the test substance and treatment-related increases in absolute and relative liver to body and brain weights, correlated with centrilobular hepatocellular hypertrophy. Body weight gain and food consumption were affected as well. The LOAEL for this study was 1.5 mg/animal/day, based on treatment-related dermal irritation and on increased

liver weights in this treatment group. The NOAEL was 0.6 mg/animal/day.

In a 21-day inhalation toxicity study (MRID 0087996), 9 rats per dose were exposed to concentrations of 0, 50, 227/115, or 1300/301 mg/m^3 for 2 hours per day, 5 days per week. The 227 and 1300 mg/ m^3 doses were reduced to 115 and 301 after the first day, due to severe clinical signs. Twelve high-dose animals (5 males and 7 females) died during the course of the study. Toxicity was observed at all dose levels and included dyspnea, nasal discharge, muscle spasms, pallor, and diarrhea, decreased body weight, decreased body weight gain, decreased food consumption, statistically-significant increased total leukocyte count, statistically-significant increased percentage of neutrophils and decreased lymphocytes, statistically-significant increased serum glutamic-pyruvic transaminase (GPT) activity, statistically-significant increased alkaline phosphatase (AP), statistically-significant decreased serum proteins (males), and increased incidence of respiratory inflammation. Additional statistical analyses also showed a statistically-significant decrease in thrombocytes. Acute purulent inflammation with focal ulceration of the mucous membrane in the nasal cavity and in the trachea were also observed at the high concentration. The LOAEL is 50 mg/m^3 (3.21 mg/kg/day) for males based on changes in thrombocytes, total blood proteins, and alkaline phosphatase; the LOAEL for females is 115 mg/m^3 (9.91 mg/kg/day). A NOAEL could not be established for males; the NOAEL for females is 50 mg/m^3 (4.51 mg/kg/day).

In developmental toxicity studies in rats and rabbits (MRID 43817502/43817503, 99.8% a.i., MRID 43820401/43022607, 99.8% a.i.), maternal toxicity consisted of transient diarrhea, retarded body weight gain during the period of treatment, reduced food consumption, and increased water consumption. The maternal LOAEL was 300 mg/kg/day in the rat study; maternal NOAEL was 100 mg/kg/day. In the rabbit study, the maternal toxicity LOAEL was 150 mg/kg/day, and the maternal NOAEL was 50 mg/kg/day. No evidence of pre- or postnatal developmental toxicity was identified at any dose level in either study. Developmental LOAELs were therefore not identified. The developmental NOAEL in the rat study was ≥ 300 mg/kg/day, and the developmental toxicity NOAEL in the rabbit study was ≥150 mg/kg/day.

In a two-generation reproduction study in rats (MRID 40623701, ≥ 99% a.i.), reproductive and systemic effects were noted at the high dose only (150 mg/kg/day). Body weights were significantly lower in the high-dose F_1 pups on Days 14 through 21 of lactation and throughout the growth phase. The viability index was decreased in the high-dose group in both generations, and the weaning index was slightly lower in the high-dose group compared to control in the F_2 generation. The NOAEL for both reproductive and systemic effects was 1000 ppm (50 mg/kg); the LOAEL was 3000 ppm (150 mg/kg).

In a chronic toxicity study in baboons (MRID 251773, % a.i. not stated), vomiting, failure to eat, and diarrhea were noted in test animals orally administered triclosan, which occurred 4-6 hours after dosing or during the night. At necropsy, an effect on the lining of the stomach was observed at the high dose. The systemic NOAEL was determined to be 30 mg/kg/day, and the systemic LOAEL was 100 mg/kg/day, based on clinical signs of toxicity.

In a chronic toxicity/oncogenicity feeding study in rats (MRID 42027906, 99% a.i.), rats administered triclosan in the diet had decreases in erythrocyte count, hemoglobin concentration,

and hematocrit. Serum alanine and aspartate aminotransferase activities were increased in males at 168.0 mg/kg/day, and blood urea nitrogen was increased in females at 217.4 mg/kg/day. Hepatocellular hypertrophy was observed in males at all dose levels. The predominant residue of triclosan observed in blood and kidney was the sulfate conjugate of triclosan, while unconjugated triclosan was predominant in the liver. No carcinogenic potential was demonstrated for triclosan in this study. The systemic NOAEL was determined to be 52.4 mg/kg/day, based on the increase in non-neoplastic liver pathology observed in male rats at the 168.0 mg/kg/day dose.

No carcinogenic potential was demonstrated for triclosan in a chronic toxicity/carcinogenicity study in hamsters (MRID 44874001/44751101, 99.5% a.i.) as well. Beginning at 80 weeks into the study, high-dose males had an increase in mortality which correlated with deterioration in their clinical condition. Plasma urea nitrogen and urine volume were significantly increased with corresponding decreases in specific gravity and protein concentration. Microscopically, a significantly increased incidence of nephropathy was observed and was considered the main factor contributing to death in animals that died before study termination. In males tested at the high dose, a significantly increased incidence of absent spermatozoa and abnormal spermatogenic cells was observed. Increased incidence of partial depletion of one or more generations of germ cells within the testis was also observed. Also noted were lesions in the stomach, focal atypical hyperplasia of the fundic region, and distended gastric glands with or without debris. The LOAEL was 250 mg/kg/day for male and female hamsters based on decreased body weight gains, increased mortality (males), nephropathy, and histopathologic findings in the stomach and testes. The corresponding NOAEL was 75 mg/kg/day.

Triclosan was positive for carcinogenicity in the liver of mice in an oral carcinogenicity bioassay [See, 1996]. A dose-related increase in the activity of alanine aminotransferse and alkaline phosphatase, and significant decreases in albumin, total protein, and serum cholesterol suggest that triclosan can interfere with liver function. Treatment-related hematological effects included increased reticulocyte count and platelet count. Increases in mean liver weight, in the severity of hepatocellular hypertrophy, and in the incidence of nodules, discoloration, hepatocellular vacuolation/vesiculation, hepatic inflammation, necrosis, and microgranulomas was observed. After 18 months of exposure, there was a statistically significant increase in the incidence of hepatocellular adenoma and carcinoma. A systemic NOAEL of 10 mg/kg/day was established from the data in this study, based on increased incidence of liver neoplasms in male and female mice at 30 mg/kg/day.

In two independently performed microbial preincubation assays (MRID 43533301, ≥99% a.i.) and a microbial mutagenicity assay (MRID 44389705, 100.5% a.i.), there was no indication of a mutagenic response in any strain at any dose compared to the vehicle controls. Likewise, in a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 44389704, > 99% a.i.), triclosan was negative for inducing forward mutations both with and without metabolic activation. Negative results for mutagenicity were also obtained in a chromosome aberration assay [MRID 47276601, >99% a.i.], an in vivo bone marrow cytogenetic assay (MRID 43740802, 99%-100%), and an *in vitro* DNA synthesis assay [MRID 47276602, 100.5% a.i.)]. However, in an <u>in vitro</u> cytogenetic assay (MRID 43740801, 99-100% a.i.), there was a dose-related increase in the yield of cells with abnormal chromosome morphology. In the presence of S9 activation, nonsignificant but concentration dependent increases in cells bearing exchange

figures were also seen.

In a metabolism study in hamsters (MRID 45307501/45307502, 99% a.i.), urine was the major route of elimination for triclosan radioactivity. Peak plasma and blood concentrations of triclosan-derived radioactivity occurred at one hour post-dose. Area Under the Curve (AUC) measurements indicated that saturation may have been achieved at the high dose, as AUC was not proportional to dose. The major urinary metabolite detected after oral administration was the glucuronide conjugate of triclosan (U7). The major fecal metabolite was parent triclosan. The plasma, kidney, and liver eliminated triclosan equivalent rapidly. Tissue metabolite analysis showed that the glucuronide and sulfate conjugates of triclosan were the major metabolites detected. Four non-parent conjugates (M5, M6, M8, and M9) were also identified in tissues. All conjugates were acid labile and resulted in the parent compound, M2, or M3. After 1-2 hours (C_{max}), males dosed singly or repeatedly, parent glucuronide and sulfate were found in the plasma without detection of the free parent compound. The kidneys at C_{max} had parent glucuronide and free parent with little sulfate conjugate; the liver at C_{max} had free parent and sulfate conjugate with little glucuronide levels.

In an absorption, distribution, metabolism, and elimination study in mice (MRID 45307503, ~99% a.i.), triclosan was eliminated primarily through the feces, via biliary excretion. Bioretention studies indicate that values from C_{max} to $1/8C_{max}$ in the liver were higher than those in plasma following repeated administration at both dose levels, indicating that the liver is the target organ. Primary excreted compounds in the urine following single oral exposures included the unmetabolized parent compound and two parent conjugates; fecal excretion was primarily that of the free parent compound. Four conjugated metabolites (M5, M6, M8, and M9) were detected in kidney, plasma, and liver extracts in minor amounts (< 5.3%) as well. Parent and parent conjugates were rapidly eliminated and/or metabolized with half-lives ranging from 1-13 hours in the plasma, liver, and kidney. C_{max} values occurred at 4-11 hours. Mice with enlarged livers exhibited parent and parent sulfate half-lives of 13 and 14 hours, respectively. Non-parent conjugates M5, M6, M8, and M9 showed similar half-lives as parent and parent compound (<4-13 hours); however, C_{max} values were greater, occurring at 12-32 hours.

A value of 50% dermal absorption for triclosan was selected on the basis of a dermal absorption study conducted in rabbits (MRID 34335). Since that time, additional dermal absorption data on triclosan have been submitted and reviewed. *In vitro* dermal absorption studies using human skin preparations and various formulations containing triclosan (MRIDs 47261408 through 47261411) showed dermal absorption values for triclosan ranging from 11-20% in these formulations. A paper published in 2000 by Moss et al. (Food and Chemical Toxicology, Volume 38, pages 361-370) examined dermal absorption of triclosan both in vivo and in vitro using rats as well as an in vitro human skin study. These data supported the conclusion of dermal absorption of 21-23% in the rat studies, and showed in vitro dermal absorption through human skin in vitro of 6.3%. Taken together, the available data on dermal absorption suggest a lower value, around 20% for rat skin and possibly lower for human skin. Additional verification is needed.

In a liver biochemical induction study in mice (MRID 44389702, % a.i. not stated), administration of triclosan to the mouse resulted in significant hepatic effects. The biochemical

alterations observed appear to support the conclusion of a barbituate-type induction with peroxisome proliferation effects. Induction of certain liver enzyme activities as measured in this study appear to occur at the lowest dose tested in male mice, including significant increases in microsomal protein, lauric acid hydroxylation, and EROD and PROD activities. Increases in lauric acid hydroxylation, and an increase in EROD and PROD activities were also noted in a non-guideline feeding study in rats (MRID 44389703, % a.i. not stated). However, animals allowed to recover for 28 days following the 14-day administration of test chemical in this study showed no significant induction or inhibition of enzyme activities. Absolute and relative liver weights were increased at the end of the study. Cytochrome P-450 content was approximately doubled in the high dose group, while activity of glutathione-S-transferase was increased by 65%. In a third liver biochemical induction study (MRID 44389706, 99.5% a.i.), a systemic NOAEL of 700 ppm was established in hamsters, with a systemic LOAEL of 5000 ppm, based on induction of total cytochrome P-450, EROD, and PROD, and induction of Mab clo4 immunoreactive protein (CYP4A peroxisome proliferator inducible P-450) in males. The data suggest that triclosan acts as a peroxisome proliferator.

Two cell proliferation studies were conducted in mice (MRID 44389701, % a.i. not stated; Eldridge, 1995, % a.i. not stated). In the first study, hepatocellular hypertrophy was the most consistent and prominent observation. At the higher dose levels, necrosis of hepatocytes was observed. Hepatocytes were also swollen and bile stasis was evident. According to the report, cell proliferation was significantly increased over control in male mouse liver at 200 mg/kg/day and higher, and the increase was sustained from 45 to 90 days. A reviewer of this study agreed with the sustained increase, but noted that it appears that cell proliferation (as judged by labeling index and fold increase over control) is also increased significantly at the 75 mg/kg/day dose level for male mice. This result is consistent with the apparent differences in sensitivity to the hepatic effects of triclosan between male and female mice. According to the report, the distribution of hepatocellular labeling was panlobular in both sexes. The 25 mg/kg/day dose level was identified as the NOAEL for male mice, while the 75 mg/kg/day dose level was considered the NOAEL for female mice by the authors. The results of this study support a mode of action consistent with cellular regeneration as a result of hepatocellular cytotoxicity. This conclusion was also reached in the second cell proliferation study based on an increase in the labeling index at 200 mg/kg/day, in conjunction with other data which show toxicity to the liver of rats and mice. In the report, it was noted that the mode by which a chemical induces cell proliferation is an important consideration. In the case of triclosan, the evidence suggests a hepatotoxic effect followed by regenerative cell turnover, in contrast to agents which act as direct mitogens. For chemicals producing increased cell turnover through cytolethality, a threshold can be inferred below which these effects would not occur.

2.0 TOXICOLOGY DATA

The Toxicology data available for triclosan are shown in Table 1.

Test		Technical	
		Required	Satisfied
870.1100	Acute Oral Toxicity MRID 43206901	Y	Y
870.1200	Acute Dermal Toxicity MRID 100178	Y	Y
870.1300	Acute Inhalation Toxicity MRID 42306902/43310501	Y	Y
870.2400	Primary Eye Irritation MRID 94045	Y	Y
870.2500	Primary Dermal Irritation MRID 42306903	Y	Y
870.2600	Dermal Sensitization MRID 43206502	Y	Y
870.3100	Oral Subchronic (Rodent) MRID 133545, 43022605, 44389707	Y	Y
870.3150	Oral Subchronic (Non-Rodent) MRID 96102	Y	Y
870.3250	90-Day Dermal MRID 43328001	Y	Y
870.3465	21-Day Inhalation MRID 0087996	Y	N
870.3700	Developmental Toxicity (rodent) MRID 43817502 and 43817503	Y	Y
870.3700	Developmental Toxicity(non-rodent) MRID 43022607, 43820401, 43787101	Y	Y
870.3700	Developmental Toxicity (mouse) MRID 43787102 and 43817501	Y	Y
870.3800	2-Gen. Reproduction MRID 40623701	Y	N
870.4100a	Chronic Toxicity (Hamster) MRID 44874001; 44751101	Y Y	Y Y
870.4100b	Chronic Toxicity (baboon) MRID 133230	Y	Y
870.4300	Chronic/Oncogenicity (rat) MRID 42027906;161332	Y	Y
870.4200	Carcinogenicity (mouse-FDA review)…….....		
870.5100	Mutagenicity—Gene Mutation - MRID 43533301, 44389705	Y	Y
870.5300	Mutagenicity—Gene Mutation - MRID 44389704		
870.5375	Mutagenicity—Structural Chromosomal Aberrations in vitro MRID 43740801, 47276601	Y	Y
870.5385	Murtagenicity- Structural Chromosome Aberrations in vivo MRID 43740802	Y	Y
870.5500	Mutagenicity—UDS in vitro – MRID 47276602	Y	Y
870.7485	General Metabolism MRID 45307501/45307502, 45307503	Y	Y
870.7600	Dermal Penetration (MRID 34335)	Y	Y

Y - Yes; **N** - no

3.0 DATA GAPS

Although the 21-day inhalation toxicity study for triclosan has been used for regulatory purposes, a new 21-day inhalation toxicity study toxicity is being requested in order to properly assess inhalation risk. There was not enough data in the current study to convert to a Human Equivalent Concentration in accordance with the Agency's current method to assess inhalation risk.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of database for Acute Toxicity: The acute toxicity database for triclosan is considered adequate, although percent active ingredient information for the primary dermal irritation study should be supplied.

Acute toxicity studies in experimental animals with technical grade triclosan show that by the oral and dermal routes, triclosan is of low acute toxicity (Toxicity Category IV; MRIDs 43206501 and 100178). By the inhalation route of exposure, triclosan was assigned Toxicity Category II for acute exposures and is thus of higher acute toxicity by inhalation exposure than by oral or dermal exposures (MRID 42306902 and 43310501). Triclosan produces moderate irritation to the eyes (MRID 43429) and skin (MRID 42306903) with a Toxicity Category III assigned for both. Triclosan was not a dermal sensitizer in guinea pigs using the Buehler method (MRID 43206502).

The acute toxicity data for triclosan is summarized in Table 1.

Table 1. Acute Toxicity Profile for Triclosan

Guideline Number	Study Type/ Test substance (% a.i.)	MRID Number/ Citation	Results	Toxicity Category
870.1100 (§81-1)	Acute Oral- Rat triclosan (99.7% a.i.)	43206501	LD_{50}: >5000 mg/kg	IV
870.1200 (§81-2)	Acute Dermal- Rabbit triclosan (97% a.i.)	94044, 92084037	LD_{50}: >9300 mg/kg	IV
870.1300 (§81-3)	Acute Inhalation- Rat triclosan (100.5% a.i.)	42306902, 43310501	LC_{50}: >0.15 mg/L	II
870.2400 (§81-4)	Primary Eye Irritation- Rabbit Triclosan (97% a.i.)	94045	moderately irritating	III

Table 1. Acute Toxicity Profile for Triclosan				
Guideline Number	**Study Type/ Test substance (% a.i.)**	**MRID Number/ Citation**	**Results**	**Toxicity Category**
870.2500 (§81-5)	Primary Dermal Irritation- Rabbit triclosan (% a.i.not provided)	42306903	PII: 3.5 at 72 hours	III
870.2600 (§81-6)	Dermal Sensitization- Guinea Pig triclosan (99.7% a.i.)	43206502	Not a Sensitizer	NA

4.2 Subchronic Toxicity

Adequacy of database for Subchronic Toxicity: Acceptable subchronic toxicity data for triclosan include two 90-day oral toxicity studies in rodents (MRID 133545 and MRID 43022605), a 28-day oral toxicity study in the mouse (MRID 44389707), a 90-day oral toxicity study in dogs (MRID 96102), a 90-day dermal toxicity study in rats (MRID 43328001), in addition to non-guideline 14-day repeat dose studies in mice (MRID 44389708) and rats (MRID 44389710). An acceptable non-guideline 21-day inhalation toxicity study (MRID 0087996) is also available for triclosan.

870.3100 90-day Oral Toxicity Study (Rat)

In a 90-day feeding study in rats (MRID 133545), groups of Sprague-Dawley rats (25/sex/dose) received Irgasan (triclosan) at dietary concentrations 0, 1000, 3000, and 6000 ppm. The 6000 ppm animals showed signs of liver damage as characterized by "fatty metamorphosis and cytomegaly." Similar to a lesser degree of liver effects were also seen in the 3000 ppm groups. **The low dose, 50 mg/kg/day, was a NOAEL**.

This study is classified as **Acceptable/Guideline**. It satisfies the minimum guideline requirements for a subchronic feeding study in rats.

870.3100 90-day Oral Toxicity Study (Mouse)

In a subchronic feeding study (MRID 43022605), CD-1 mice were fed triclosan (99.7% a.i.) daily at dietary levels of 0, 25, 75, 200, 350, 750, or 900 mg/kg/day for 13 weeks (main groups, 15 mice per group) or 0, 25, 350, or 900 mg/kg/day for 7 weeks (satellite groups, 20 mice in the control group and 10 mice per treatment group). Satellite groups were run concurrently with the main groups and were mainly used to provide clinical pathology data. Animals from the satellite groups were sacrificed after- 7 weeks of exposure.

Systemic toxicity was observed at all dose levels in a dose-related manner as evidenced by clinical pathology, organ weight changes, and increased incidence or severity of histopathological lesions (especially of the liver). Clinical pathology included significantly decreased erythrocytes, hemoglobin, and hematocrit at ≥ 25 mg/kg/day in males (68%—92% of controls) and at ≥ 75 mg/kg/day in females (73%-91%). Enzyme changes, indicative of liver injury, included increased alkaline phosphatase (at ≥ 25 mg/kg/day; 1.5-4.4 fold increases in both sexes), alanine aminotransferase (at ≥ 200 mg/kg/day; 1.3-6.2 fold increases in both sexes), and aspartate aminotransferase (at ≥ 200 mg/kg/day; 1.5-2.4 fold increase in males). Absolute and relative liver/gallbladder weights increased 1.3-3.0 fold at ≥ 75 mg/kg/day in both sexes. Increased incidence or severity of histopathological lesions in the liver included hypertrophic hepatocytes, vacuolization, inflammation, necrosis, pigmented Kupffer cells and/or macrophages, mineralizition, and chronic bile duct inflammation. These lesions were evident in males at ≥ 25 mg/kg/day and in females at ≥ 200 mg/kg/day. The severity of extramedullary hematopoiesis in the spleen increased in males (≥ 200 mg/kg/day) and in females (≥ 750 mg/kg/day).

Additional findings at higher dose levels included organ weight changes (kidney, adrenal gland, Uterus, ovary, and salivary gland); clinical signs (hunched posture, thin appearance, and hypoactivity, pale appearance, and cold to touch); changes in body weight gain (a decrease to 60% and 83% in males and females, respectively, for weeks 1—6 in the satellite groups and to 83% and 67% in males and females, respectively, for weeks 1-13 in the main groups); and increased incidence or severity of cystic stomach hyperplasia, subacute kidney inflammation, uterine hypoplasia, hypertrophic adrenal cortex (males); uterine hypoplasia; chronic inflammation of the kidney (females); tubule *regeneration* of the kidney, mammary gland dilatation and epithelial hypoplasia (females), chronic heart inflammation (females); pigmented macrophages in the mandibular lymph node (males); hypercellularity of the marrow of the femur (males); and lymphoid hyperplasia in the cecum (females).

Based on changes in clinical chemistry and hematology parameters as well as lesions in the liver at the lowest dose level, the systemic toxicity LOAEL was 25 mg/kg/day; a NOAEL could not be determined.

This study is classified as acceptable/guideline and satisfies the minimum guideline requirements for a subchronic feeding study (OPPTS 870.3100) in mice.

870.3100 28-day Oral Toxicity Study (Mouse)

In a 28-day oral toxicity study (MRID 44389707), a total of 40 mice (MAGf [SPF], 5/sex/dose) received technical triclosan admixed into pelleted feed at dose levels of 0, 50, and 1000 ppm (6.48 and 135.59 mg/kg/day in males, 8.25 and 168.78 mg/kg/day in females) for 4 weeks. Five males and 5 females were given the high dose and allowed to "recover" (feeding of non-treated feed) for 2 weeks. There were no reported effects on mortality, body weight, or food consumption. Hematological effects were observed in the high dose (135.59 mg/kg/day in

males; 168.78 mg/kg/day in females), and included significant decreases in erythrocytes, hemoglobin, and hematocrit in males, and a significant decrease in hemoglobin in females. Both sexes showed significant increases in thrombocytes at this dose, the effects of which were not fully reversible after two weeks recovery. Clinical chemistry alterations (significant increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase; significant decrease in globulin fraction) were observed at the high dose in male and female mice. Elevated serum enzyme activities were evident after the two week recovery period. Absolute weight of the liver and liver/body weight ratio were significantly increased at the high dose in male and female mice. Histopathological examination of the liver showed an increased incidence of liver cell necrosis (as single cells or small cell groups), hemosiderosis of Kupffer cells in the vicinity, cytoplasmic vacuoles in hepatocytes, and liver cell hypertrophy. The presence of necrosis was still evident (2/5 males and 3/5 females) after the recovery period.

Based on the biochemical and morphological effects of triclosan treatment on the liver of male and female mice, a systemic LOAEL of 135.59 mg/kg/day for males and 168.78 mg/kg/day for females is assigned. The systemic NOAEL is considered to be 6.48 mg/kg/day in males, and 8.25 mg/kg/day in females.

This study is classified as **Acceptable/Guideline** and provides relevant toxicologic data on the effects of triclosan treatment to the liver of male and female mice. This study was not conducted to fulfill a specific guideline requirement, but provides useful data for the risk assessment of triclosan.

870.3150 Oral Subchronic (Non-rodent)

In a subchronic oral toxicity study (MRID 96102) conducted in male and female Beagle dogs, triclosan was administered orally via a gelatin capsule at doses of 0, 12.5, 25, 50, or 100 mg/kg/day seven days per week for 13 weeks. An additional test group of 2 male and 2 female dogs were given the test material at 50 mg/kg/day for 13 weeks and allowed a four week recovery period prior to termination. Body weight gain in females at 12.5 mg/kg/day was significantly lower in relation to untreated controls but body weight decrements were not observed at higher doses in either sex. One male in the 100 mg/kg/day dose group died after day 23 of dosing, and one male in the 100 mg/kg group and one female in the 50 mg/kg group were sacrificed *in extremis* on day 26 and day 57, respectively. Each of above three animals displayed weight loss, anorexia, lethargy, and symptoms of jaundice three to five days prior to death. Upon autopsy of the animals that died or were sacrificed during the study, histopathological examination of tissues revealed that the jaundice was a result of hepatotoxicity. Upon examination of all the study animals at necropsy, it was noted that there were treatment-related morphological changes in the livers of most animals at the 25, 50, and 100 mg/kg/day dose groups. The changes included focal acidophilic to granular degeneration of the cytoplasm of hepatocytes. The mean overall body weight for females receiving 12.5 mg/kg/day was significantly lower than the weights for the control group while weights for all other treatment groups showed no significant deviation from the control group. Serum alkaline phosphatase activity was elevated in dogs receiving 50 and 100 mg/kg/day. One group of animals receiving 100 mg/kg/day and then allowed a 28-day recovery period showed serum alkaline phosphatase that returned to normal after the recovery period. No other blood chemistry studies revealed any other abnormalities. **On the basis of this study the**

NOAEL was determined to be 12.5 mg/kg/day in Beagle dogs.

This study is classified as **Acceptable/Guideline**.

870. 3250 90-day Dermal Toxicity Study (Rat)

In a 90-day dermal toxicity study (MRID 43328001), groups of rats (10/sex/group) received triclosan in propylene glycol by dermal application at dose levels of 10, 40, and 80 mg/kg for 6 hrs/day for 90 days, followed by a 28 day recovery period. Dermal irritation at the application site was found in all dose groups. At the 10 mg/kg/day dose, animals were observed with erythema and edema beginning on day 21 of the study. At the 40 and 80 mg/kg/day dose levels, animals were observed with dermal reactions beginning on day 4 of the study and a greater number of animals were observed with dermal scores of +3 and +4 for erythema and edema. In the satellite group given test material at 80 mg/kg/day and allowed a 28 day recovery period beyond the 90-day dosing period, dermal irritation scores had subsided by the end of the 28-day recovery period. Systemically, an increase in the incidence of occult blood in the urine of 80 mg/kg males and females was found. No other systemic toxicity was observed from the data in this study. **Under the conditions of this study, the LOAEL for systemic toxicity was 80 mg/kg; the NOAEL was 40 mg/kg.**

This study is classified as **acceptable/guideline** and satisfies the 870.3250 guideline for a 90-day dermal toxicity study in rats.

Non-Guideline 14-Day Repeated Dose Dermal Toxicity (Mouse)

In a repeated dose dermal toxicity study (MRID 44389708), triclosan (99.3% a.i.) was applied daily in acetone to the clipped skin of ten CD-1 mice/sex/dose at dose levels of 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/day for 14 days.

Signs of dermal toxicity in both sexes at 3.0 and 6.0 mg/animal/day included erythema, edema, alopecia, fissuring, eschar, thickening and discoloration. At 1.5 mg/animal/day erythema, fissuring, eschar, thickening, and discoloration were observed in males and erythema and fissuring in females. Dermal irritation observed in mice in the 0.3, and 0.6 mg/animal/day treatment groups was comparable to that observed in the controls. Non-neoplastic skin lesions were observed at application sites and included superficial ulceration and suppurative inflammation, slight or minimal acanthosis and/or hyperkeratosis, and mild, diffuse, generally subacute/chronic inflammation of the dermis. The lesions were dose-related and occurred primarily in the 1.5, 3.0, and 6.0 mg/animal/day treatment groups. Systemic responses were observed as dose-dependent increases in plasma levels of the test substance. There were treatment-related increases in absolute and liver to body and brain weights at 1.5, 3.0, and 6.0 mg/animal/day which correlated with centrilobular hepatocellular hypertrophy at 3.0 and 6.0 mg/animal/day. There were no significant differences between the terminal body weights in the treated and control groups. For males, the overall body weight gain was significantly decreased ($p \leq 0.05$) at 6.0 mg/animal for females (↓32%) and significantly increased for males at 3.0 mg/animal/day (↑64%). Food consumption was significantly increased ($p \leq 0.05$) for the 3.0 and 6.0 mg/animal/day groups during Week 1 (females only), Week 2 (both sexes), and overall for

females only. **The LOAEL for this study is 1.5 mg/animal/day, based on treatment-related dermal irritation at the treatment site and on increased liver weights in this treatment group. The NOAEL is 0.6 mg/animal/day. Based on the results of this study, the highest recommended level for a 90-day dermal study was judged to be 1.2 mg/animal/day with inclusion of at least one level below 0.3 mg/animal/day.**

This dermal toxicity study is classified as **Acceptable/Non-Guideline** and was intended only as a range-finding study for a 90-day dermal study.

Non-Guideline 14-Day Repeated Dose Dermal Toxicity (Rat)

In a repeated dose dermal toxicity study (MRID 44389710), triclosan (99.3% a.i.) was applied daily in acetone to the clipped skin of ten Crl:CD®BR rats/sex/dose at dose levels of 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/day for 14 days.

Treatment-related dermal irritation was observed in both sexes of the 6.0 mg/animal/day group consisting of erythema, scaling, and eschar. Dose-related, non-neoplastic histopathological changes to skin were observed at application sites in the 6.0 mg/animal/day treatment group which consisted of treatment-related acanthosis of eschar in 4/10 males and 3/10 females and hyperkeratosis in 10/10 females. No unscheduled deaths occurred and no clinical signs of toxicity were observed in any treatment group. There were no treatment-related changes in organ weights. There were no significant differences between the terminal body weights or in food consumption in the treated and control groups nor were there any overall differences in body weight changes in treated males and females. **The LOAEL for this study is 6.0 mg/animal/day, based on treatment-related dermal irritation at the treatment site. The NOAEL is 3.0 mg/animal/day.**

This dermal toxicity study is classified as **Acceptable/Non-Guideline** and was intended only as a range-finding study for a 90-day dermal study.

21-Day Inhalation Toxicity Study in Rats

In a subchronic inhalation toxicity study (MRID 0087996), triclosan (purity not reported) was administered to 9 rats/dose/sex at dose levels as described in the table below:

Group	No. of Rats		Mean Concentration (mg/m^3) Air	
	Males	Females	1^{st} Day	2^{nd}-15^{th} Day
1	9*	9*	0	0
2	9	9	50	50
3	9*	9*	227	115
4	9	9	1300	301

*8 animals (2 males/2 females) from each group were kept for a 17-day recovery period, following the 21-day exposure.

Group	Mean bodyweight (kg)		Equivalent Dose (mg/kg/day)[#]	
	Males	Females	Males	Females
50 mg/m^3 (4.22 ppm)	.271	.193	3.21	4.51
115 mg/m^3 (9.71 ppm)	.251	.202	7.97	9.91
301 mg/m^3 (25.4 ppm)	.217	.170	24.14	30.81

* = (mg/m3 x 24.45)/mw = ppm
# = ((0.0087 m^3/hr *mg/m^3*hr/day)/bw), where 0.0087 m^3/hr is a default inhalation rate for young rats.

A 10% ethanol suspension of triclosan was administered "nose only" as an aerosol (5 days per week , 2 hrs per day) for 21 days. Dose levels of 0, 50, 115, or 301 mg/m^3 are equivalent to 0, 0.36, 0.77, 1.77 mg/kg/day for males, respectively, and 0, 0.36, 0.64 and 1.08 mg/kg/day for females, respectively). Treatment groups 3 and 4 initially received concentrations of 227 and 1300 mg/m^3, respectively. These concentrations were reduced after the first day of treatment because they were not tolerated well by the animals.

Twelve high-dose animals (5 males and 7 females) died during the course of the study. Toxicity was observed at all dose levels. Treatment-related effects at 1300/301 mg/m^3 included clinical signs of toxicity (dyspnea, nasal discharge, muscle spasms, pallor, and diarrhea), decreased body weight, decreased body weight gain, decreased food consumption, statistically-significant increased total leukocyte count, statistically-significant increased percentage of neutrophils and decreased lymphocytes, statistically-significant increased serum glutamic-pyruvic transaminase (GPT) activity, statistically-significant increased alkaline phosphatase (AP), statistically-significant decreased serum proteins (males), and increased incidence of respiratory inflammation. Additional statistical analyses also showed a statistically-significant decrease in thrombocytes. Macroscopic findings for the high-dose animals that died prior to scheduled sacrifice included severe acute congestion and numerous hemorrhages in all organs. Acute purulent inflammation with focal ulceration of the mucous membrane in the nasal cavity and in the trachea were also observed. Treatment-related effects at 227/115 mg/m^3 included slightly decreased body weight and body weight gain, slightly decreased food consumption, increased leukocytes, statistically-significant decreased thrombocytes, statistically-significant increased alkaline phosphatase, statistically-significant decreased serum proteins (males), and slight incidences of respiratory irritation in one male and two females. At the low dose, a statistically-significant decrease in thrombocytes and total serum proteins and a statistically-significant increase in alkaline phosphatase were observed in the males. Consequently, the LOAEL is 3.21 mg/kg/day for males based on changes in thrombocytes, total blood proteins, and alkaline

phosphatase; the LOAEL for females is 9.91 mg/kg/day. A NOAEL could not be established for males; the NOAEL for females is 4.51 mg/kg/day.

This 21-day rat inhalation study is classified **acceptable-non-guideline.** The LOAEL value established in male rats in this study may be used as a tentative LOAEL for regulatory purposes. However, there were several deficiencies in this study as noted in the full review.

4.3 Prenatal Developmental Toxicity

Adequacy of database for Prenatal Developmental Toxicity: Acceptable developmental toxicity studies (MRID 43817502/43817503 and 43820401/43022607; 43787101) in rats and rabbits are available for triclosan.

870.3700 Prenatal Developmental Toxicity (Rat)

In an oral prenatal developmental toxicity study in rats (MRID 43817502 and 43817503), triclosan (99.8%) was administered by gavage to pregnant female Colworth Wistar rats (30 rats/treated group and 60 rats in the control group) on days 6-15 of gestation at dose levels of 30, 100, or 300 mg/kg/day, with the day of mating designated as gestation day 0. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 21 of gestation, 25 rats per treated group and 50 control rats were sacrificed and necropsied; uterine weights were recorded. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation. Five rats per treated group and ten control rats were allowed to deliver their litters. Litter weight, pup mortality, and developmental milestones (presence of vibrissae, pinna unfolding, incisor eruption, eyelid opening, and completion of fur growth) were recorded. The pups were killed and necropsied on lactation day 21, and all pups were processed for skeletal examination.

At 300 mg/kg/day, maternal toxicity consisted of transient diarrhea, retarded body weight gain during the period of treatment, and reduced food consumption and increased water consumption from the onset of treatment, throughout the gestation period. **Based on these findings: Maternal LOAEL = 300 mg/kg/day; Maternal NOAEL = 100 mg/kg/day.**

No evidence of pre- or postnatal developmental toxicity was identified at any dose level under the conditions of this study. **Developmental NOAEL $\geq$ 300 mg/kg/day); Developmental LOAEL > 300 mg/kg/day.**

This study is classified as **Acceptable/Guideline** and satisfies the 870.3700 guideline for a developmental toxicity study in rats.

870.3700 Prenatal Developmental Toxicity (Rabbit)

In a developmental toxicity study in rabbits (MRIDs 43820401, 43022607, and 43787101), triclosan (Lot No. 19851206, purity 100%) was administered by gavage to groups of 18 pregnant New Zealand white rabbits at doses of 0, 15, 50, or 150 mg/kg/day on gestation days (GD) 6-18, inclusive. The dosing solutions were administered as suspensions in 1% carboxymethylcellulose in an aqueous glycerin solution. All surviving does were sacrificed on GD 30 and subjected to gross necropsy. All fetuses were examined for external and visceral malformations/variations and processed for skeletal examination.

Maternal mortalities were due to gavage error, with one exception that was also determined not to be treatment-related. Signs of maternal toxicity in the 150 mg/kg/day group included decreased body weight and food consumption during the treatment period, and decreased body weight gain for the overall treatment period. Mean body weights for the 150 mg/kg/day does were reduced 3.3% (GD 6) to 7.9% (GD 16) compared to controls, and significantly ($p \leq 0.05$) reduced at GD 14 and 16. For GD 6-8 and 12-14. the high-dose animals showed a significant ($p \leq 0.01$) mean body weight loss. Over the entire GD 6-19 interval, the mean weight gain for the high dose group was significantly lower ($p \leq 0.01$, -65.8%) than controls. Mean food consumption (reported as g/kg day) during GD 6-19 in the high-dose group decreased compared to controls (-7% at GD 11 to -41.1% at GD 14), with significant differences ($p \leq 0.01$) on GD 6-8 and GD 12-15.

The maternal toxicity LOAEL is 150 mg/kg/day based on significant mean body weight loss and decreased body weight gain, and the maternal toxicity NOAEL is 50 mg/kg/day.

No does aborted during the study. There were no statistically significant differences in the mean number of resorptions/doe or the resorption/implant ratio between the control and treated groups. Fetal body weights of both sexes were comparable between the control and treatment groups.

The total number of fetuses/litters examined in the 0, 15, 50, and 150 mg/kg/day groups were 143/16, 126/15, 129/15, and 124/16, respectively. No treatment-related external, visceral, or skeletal malformations/variations were observed in fetuses from any treated group.

Therefore, the developmental toxicity LOAEL was not identified, and the developmental toxicity NOAEL is > 150 mg/kg/day.

This study is classified **acceptable/ guideline** and satisfies the 870.3700 guideline for a developmental toxicity study in rabbits.

870.3700 Developmental toxicity in mice

In a developmental toxicity study in mice (MRID 43817501), triclosan (99.0% a.i.) was administered to 25 Charles River CD-1 (ICR)BR female mice/dose via the diet at target dose levels of 0, 10, 25, 75, or 350 mg/kg/day (calculated achieved doses of 0, 11.2, 26.1, 81.8, and

372.1 mg/kg/day) from days 6-15 of gestation. Maternal toxicity was observed at 75 mg/kg/day by increases in absolute and relative liver weight and tan areas in the liver of one dam. At 350 mg/kg/day, maternal toxicity was evident as increases in absolute and relative liver weight and observations of tan areas of the liver in 14/22 dams. The maternal LOAEL is 75 mg/kg/day (81.6 mg/kg/day) based on increased liver weights. The Maternal NOAEL is 25 mg/kg/day (11.2 mg/kg/day).

Developmental effects were noted at the 75 and 350 mg/kg/day target dose levels as increased incidence of variations (characterized as reversible irregular ossification of the skull at 75 and 350 mg/kg/day, and phalanges at 350 mg/kg/day). Decreased fetal weight was also observed *14 and 18% decrease at 75 and 350 mg/kg/day target dose levels, respectively). The developmental LOAEL is 75 mg/kg/day (81.6 mg/kg/day) based on irregular ossification of the skull. The developmental NOAEL is 25 mg/kg/day (11.2 mg/kg/day).

This study is classified **acceptable/guideline** and satisfies the requirement for a developmental toxicity study in mice. .

4.4 Reproductive Toxicity

Adequacy of database for Reproductive Toxicity: One study is available (MRID 40623701) on the reproductive toxicity of triclosan.

870.3800 Reproduction

In a 2-generation reproduction study (MRID 40623701) triclosan was administered in the diet to groups of either 25 or 30 (F_o and F_l, respectively) male and female Crl:CD(BR) rats at dose levels of 0, 300, 1000, and 3000 ppm (0, 15, 50, and 150 mg/kg/day) for 10 weeks prior to mating and through post-natal day 21 for both generations. In the F_o generation, there were no significant decreases in parental body weight during pre-mating, but a significant increase in mean body weight was observed in 50 mg/kg/day males. Body weight in F_o females during lactation was significantly decreased on post-natal day 7, with a significant negative trend in mean body weight gain for the high dose group of for days 0-7. Increased incidence of liver discoloration in 50 and 150 mg/kg/day parental F_o males was observed. No effects on reproductive performance were observed in the F_o generation. Pups of the F_o generation (F_l pups) showed decreased mean body weight on post-natal days 14 and 21 at the 150 mg/kg/day dose. Increased pup mortality was observed on postnatal days 0-3 in high dose pups. Decreased viability index was also observed at the 150 mg/kg/day dose in F_l pups, as was an increased incidence of dilated renal pelvis. In F_l parental animals, significantly lower group mean body weights were observed during pre-mating at the 150 mg/kg/day dose. Gestational group mean body weight in F_l females was significantly decreased by 12% during the period of gestation, with a significant negative trend for gestational days 1, 7, 14, and 20. There were no differences in number of pregnant animals, mean gestation duration and mean precoital interval in F_l females. In pups of the F_l parental generation (F_2 pups), an increase in number of pups found dead or missing was increased at the 150 mg/kg/day dose. Weaning index was decreased at the high dose in F_2 pups, and increased total liter deaths was increased. The Parental Systemic NOAEL is l000 ppm (50 mg/kg/day), and the Parental Systemic LOAEL is 3000 ppm (150 mg/kg/day), based on reduced mean body weights. The

Reproductive/Developmental NOAEL is l000 ppm (50 mg/kg/day); the Reproductive/ Developmental LOAEL is 3000 ppm (150 mg/kg/day), based on reduced viability of pups and reduced body weights.

This reproductive study in the rat is classified **unacceptable/upgradable.** Information must be provided by the registrant on the meaning of unadjusted and adjusted pup body weights and a clarification of the random card draw procedure used to select parents of the F_2 generation.

4.5 Chronic Toxicity

Adequacy of database for Chronic Toxicity: Chronic toxicity data for triclosan were submitted and consist of a one-year oral toxicity study in baboons (MRID 133230), a chronic toxicity/carcinogenicity study in hamsters (MRID 44874001; 44751101), and a chronic toxicity/carcinogenicity feeding study in rats (MRID 42027906). The baboon and hamster study were considered acceptable.

870.4100b Chronic Toxicity (Baboon)

In a chronic toxicity study (MRID 133230), groups of 7 baboons/sex/dose received triclosan orally at doses of 30, 100, and 300 mg/kg/day by capsule for 52 weeks. Two males and 2 females from each dose group were sacrificed at six months, 3 males and 3 females from each dose group at 52 weeks, and the remainder of the animals after a six week recovery period following cessation of treatment. At the 100 and 300 mg/kg/day dose levels, test animals were observed with signs of vomiting, failure to eat, and diarrhea, which occurred 4-6 hours after dosing or during the night. At necropsy, an effect on the lining of the stomach was observed at the high dose. **The systemic NOAEL was determined to be 30 mg/kg/day, and the systemic LOAEL was determined to be 100 mg/kg/day, based on clinical signs of toxicity.**

This study is classified as **Acceptable/Guideline.** It satisfies the minimum guideline requirements for a non-rodent chronic toxicity study.

870.4300 Chronic Toxicity/Carcinogenicity (Rat)

In a chronic toxicity/oncogenicity feeding study (MRID 42027906) conducted in male and female Sprague-Dawley rats, [FAT 80'023 (triclosan, 99.0 % a.i.)] was administered in the diet at doses of 0, 300, 1000, or 3000 ppm (0, 15.3, 52.4, and 168.0 mg/kg/day in males ; 0, 20.0, 66.9, and 217.4 mg/kg/day in females) for 104 weeks. An additional group of 20 male and 20 female rats received triclosan in the diet at 6000 ppm (415.0 mg/kg/day [males] and 519.3 mg/kg/day [females]) for 52 weeks. No treatment related effects on mortality, clinical toxicity, opthamalogy, urinalysis, gross pathology, or neoplastic pathology were observed at any dose level tested. Erythrocyte count, hemoglobin concentration, and hematocrit were decreased in males at the 15.3, 52.4, and 168.0 mg/kg/day dose levels, and erythrocyte count was decreased in females at 66.9 and 217.4 mg/kg/day. Serum alanine and aspartate aminotrasferase activities were increases in males at 168.0 mg/kg/day, and blood urea nitrogen was increased in females at

217.4 mg/kg/day. Hepatocellular hypertrophy was observed in males at all dose levels. Increased incidence of liver necrosis was observed in males at the 300, 1000, and 3000 ppm dose levels (5/85, 4/85, and 4/85 respectively compared to 1/95 in concurrent controls). The predominant residue of triclosan observed in blood and kidney was the sulfate conjugate of triclosan, while unconjugated triclosan was predominant in the liver. Residual levels of triclosan were proportional to the dose administered. No carcinogenic potential was demonstrated for triclosan in this study. **The systemic LOAEL was determined to be 3000 ppm (168.0 mg/kg/day) based on significant decreases in body weight in male and female rats and non-neoplastic changes of the liver (cytoplasmic inclusions and hepatocellular hypertrophy) in males at 3000 ppm (168.0 mg/kg/day). The systemic NOAEL was determined to be 1000 ppm (52.4 mg/kg/day).**

This study is classified as **Core minimum**.

4.6 Carcinogenicity

Adequacy of database for Carcinogenicity: Carcinogenicity data for triclosan include a chronic/carcinogenicity study in hamsters (MRID 44874001, 44751101), a chronic toxicity/carcinogenicity feeding study in rats (MRID 42027906), and an oral carcinogenicity study in mice, a summary of which was obtained from the Food and Drug Administration's review. This FDA review was obtained and verified to be publicly citable.

870.4100a Chronic Toxicity (Rat)

In a chronic toxicity/oncogenicity feeding study (MRID 42027906) conducted in male and female Sprague-Dawley rats, [FAT 80'023 (triclosan, 99.0 % a.i.)] was administered in the diet at doses of 0, 300, 1000, or 3000 ppm (0, 15.3, 52.4, and 168.0 mg/kg/day in males ; 0, 20.0, 66.9, and 217.4 mg/kg/day in females) for 104 weeks. An additional group of 20 male and 20 female rats received triclosan in the diet at 6000 ppm (415.0 mg/kg/day [males] and 519.3 mg/kg/day [females]) for 52 weeks. No treatment related effects on mortality, clinical toxicity, opthamalogy, urinalysis, gross pathology, or neoplastic pathology were observed at any dose level tested. Erythrocyte count, hemoglobin concentration, and hematocrit were decreased in males at the 15.3, 52.4, and 168.0 mg/kg/day dose levels, and erythrocyte count was decreased in females at 66.9 and 217.4 mg/kg/day. Serum alanine and aspartate aminotrasferase activities were increases in males at 168.0 mg/kg/day, and blood urea nitrogen was increased in females at 217.4 mg/kg/day. Hepatocellular hypertrophy was observed in males at all dose levels. Increased incidence of liver necrosis was observed in males at the 300, 1000, and 3000 ppm dose levels (5/85, 4/85, and 4/85 respectively compared to 1/95 in concurrent controls). The predominant residue of triclosan observed in blood and kidney was the sulfate conjugate of triclosan, while unconjugated triclosan was predominant in the liver. Residual levels of triclosan were proportional to the dose administered. No carcinogenic potential was demonstrated for triclosan in this study. **The systemic LOAEL was determined to be 3000 ppm (168.0 mg/kg/day) based on significant decreases in body weight in male and female rats and non-neoplastic changes of the liver (cytoplasmic inclusions and hepatocellular hypertrophy) in males at 3000 ppm (168.0 mg/kg/day). The systemic NOAEL was determined to be 1000 ppm (52.4 mg/kg/day).**

This study is classified as **core minimum.**

870.4200 Carcinogenicity (Mouse)

In an 18-month carcinogenicity bioassay in mice, 5 groups of male and female CD-1 mice (70 mice/sex/dose) received triclosan in the diet at dose levels of 0, 10, 30, 100, or 200 mg/kg/day. Fifty mice/sex/dose received dietary triclosan for 18 months, while the remaining 20 mice/sex/dose received dietary triclosan for only 6 months, after which time these mice were sacrificed. Blood samples were obtained from 10 mice/sex/dose from both the 6 month and 18 month dose groups at sacrifice, for determination of triclosan plasma levels. Time of blood sampling relative to the last dose of triclosan was not stated. Parameters monitored during this study included mortality, clinical observations, body weight, food consumption, ophthalmology, clinical chemistry, urinalysis, hematology, gross and microscopic pathology, and organ weights. Reduced survival was observed in female mice receiving 200 mg/kg/day for 18 months (34/50 vs. 45/50 in control). There were no significant signs of clinical toxicity at any dose level, and no significant effects of treatment on group mean body weight, food consumption, ophthalmology, or urinalysis. A dose-related increase in activity of alanine aminotransferse and alkaline phosphatase was observed in male and female mice at 100 mg/kg/day triclosan and above in both the 6 month and 18 month dose groups. Significant decreases in both albumin and total protein were observed in males at 6 months and in females at 18 months at doses of 100 mg/kg/day and above. Serum cholesterol was markedly reduced at all dose levels including the 10 mg/kg/day dose. Treatment-related hematological effects included increased reticulocyte count and platelet count in males and females at the 200 mg/kg/day dose. Mean liver weight (absolute and relative) was increased in both male and female mice at 30 mg/kg/day and above at both 6 and 18 months. An increased incidence of nodules and discoloration of the liver was observed in both male and female mice at 100 mg/kg/day and above. A dose-related increase in severity of hepatocellular hypertrophy was observed in both male and female mice at 30 mg/kg/day and above. Dose-related increases in incidence or severity of hepatocellular vacuolation/vesiculation and hepatic inflammation, necrosis, and microgranulomas was also observed.

After 18 months of exposure, a statistically significant increase in the incidence of hepatocellular adenoma and/or carcinoma was observed in male and female mice at 100 mg/kg/day triclosan and above. The incidence was dose-related in both sexes. Combined incidence of adenoma and carcinoma was 12%, 20%, 34%, 64%, and 84% for males, and 0%, 2%, 6%, 12%, and 40% for females at the 0, 10, 30, 100, and 200 mg/kg/day dose levels, respectively. The incidence of adenoma / carcinoma combined exceeded historical control incidence at the 10 mg/kg/day dose level (17% for males, 1% for females), but became statistically significant at the 30 mg/kg/day dose level. **Therefore, a systemic NOAEL of 10 mg/kg/day can be established from the data in this study, based on increased incidence of liver neoplasms in male and female mice at 30 mg/kg/day.**

870.4300 Chronic Toxicity/Carcinogenicity (Hamster)

In a chronic toxicity/oncogenicity study (MRID 44874001), FAT 80'023/S (triclosan: 99.5% a.i.; Batch # 505017) was administered in the diet to groups of 70 male and 70 female Bio F1D Alexander Syrian hamsters at concentrations delivering doses of 0 (control 1), 0 (control 2), 12.5, 75, or 250 mg/kg/day. Actual achieved doses were: 0, 0, 12.6, 75.4 [75.5 F], and 251 mg/kg/day for males and females. Groups of 10 hamsters per sex per dose were killed after 52 weeks for interim evaluations; the remaining 60 hamsters per sex per dose were maintained on treated or control diets for up 90 weeks for females and 95 weeks for males.

No treatment-related clinical signs of toxicity were observed during the first 80 weeks of the study. After this time, high-dose males showed deterioration in their general clinical condition with signs such as lethargy, hunched posture, pallor, thin appearance, and unsteady gait. At termination of the females (week 91) the percent survival in the control 1, control 2, low-, mid-, and high-dose groups was 40%, 38%, 47%, 58%, and 48%, respectively. In contrast, high-dose males had an increase in mortality after week 80 which correlated with their deteriorating clinical condition. At termination of the males (week 96) the percent survival in the control 1, control 2, low-, mid-, and high-dose groups was 65%, 72%, 75%, 80%, and 35%, respectively.

Body weight gains by high-dose males and females were significantly ($p \leq 0.05$ or 0.01) less than one or both control groups throughout the study. Overall body weight gains by the high-dose animals through week 90 were 46-53% of the control levels. Final absolute body weights of the high-dose males and females were 84-85% and 89-90%, respectively, of the control groups. Body weights and body weight gains by the mid- and low-dose animals were unaffected by treatment. High-dose males and females had significantly ($p \leq 0.01$) reduced food consumption during weeks 1-3 as compared with both control groups. Food conversion ratios during the first 16 weeks of the study for animals in the control 1, control 2, low-, mid-, and high-dose groups were 30.5, 29.0, 29.2, 30.1, and 38.5 mg/kg/day, respectively, for males and 33.2, 32.2, 36.6, 36.5, and 50.1 mg/kg/day, respectively, for females. Water consumption was highly variable between individuals and between groups. However, for the high-dose groups, water consumption tended to be slightly increased throughout the study.

Plasma urea nitrogen was significantly ($p \leq 0.05$ or 0.01) increased to 119-156% of the control levels in high-dose males and females as compared to one or both control groups at interim sacrifice and at termination. Statistically significant changes were observed for other clinical chemistry parameters and for hematologic parameters and organ weights, but none were considered treatment related.

Throughout the study, high-dose males and females had a consistent increase in urine volume with corresponding decreases in specific gravity and protein concentration. Statistical significance ($p \leq 0.05$ or 0.01) was attained for these parameters at almost every time point when compared to one or both controls.

At interim sacrifice, irregular cortical scarring of the kidney was observed at gross necropsy in 4/10 high-dose males and 9/10 high-dose females compared with none in the control male groups and 3/19 in the control female groups combined. This corresponded to microscopic findings in

the kidneys of the high-dose groups of both sexes consisting of distended medullary tubules and radial areas of dilated basophilic tubules with or without eosinophilic colloid/fibrosis.

At terminal sacrifice, no dose- or treatment-related gross findings were observed in males. However, in the control (combined), low-, mid-, and high-dose female groups, white nodules in the forestomach were observed in 3/46, 3/28, 5/35, and 5/29 animals, respectively, pale kidneys were observed in 14/46, 4/28, 3/35, and 10/29 animals, respectively, and irregular cortical scarring was observed in 24/46, 12/28, 16/35, and 20/29 animals, respectively. Microscopically, a significantly (p0.01) increased incidence of nephropathy was observed in high-dose males and females (decedents and survivors combined) as compared to both control groups and was considered the main factor contributing to death in animals that died before study termination. The severity of nephropathy, as calculated by the reviewer, in high-dose males and females was 3.2 and 2.8, respectively, compared with control values of 2.5-2.7 and 2.1-2.3, respectively. The incidence of nephropathy in the control 1, control 2, low-, mid-, and high-dose groups was 41/60, 38/60, 35/60, 36/60, and 56/60, respectively, for males and 19/60, 21/60, 26/60, 19/60, and 50/60, respectively, for females.

In males tested at the high dose of triclosan, a significantly increased incidence of absent spermatozoa, abnormal spermatogenic cells, and reduced numbers of spermatozoa was observed in males that died and those that were sacrificed at the end of the study. Increased incidence of partial depletion of one or more generations of germ cells within the testis was also observed in high dose male hamsters that died during the study or were sacrificed at study termination.

Lesions in the stomach were significantly ($p \leq 0.01$) increased in high-dose males and females at termination; focal atypical hyperplasia of the fundic region was observed in 11/60 males and distended gastric glands with or without debris were observed in 17/60 females. These lesions were observed in none of the control males and only one of the control females, respectively. In addition, high-dose males killed at termination and dying during the study had significantly (p0.01) increased incidences of abnormal spermatogenic cells and reduced numbers of spermatozoa in the epididymides and partial depletion of germ cells in the testes.

The LOAEL is 250 mg/kg/day for male and female hamsters based on decreased body weight gains, increased mortality (males), nephropathy, and histopathologic findings in the stomach and testes. The corresponding NOAEL is 75 mg/kg/day.

No evidence of potential carcinogenicity of the test material was observed at the doses given in this study. Neoplastic lesions did not occur in treated groups at incidences significantly higher than the incidences in control animals. The doses administered were adequate for testing carcinogenicity as evidenced by the systemic toxicity described above.

This chronic toxicity/carcinogenicity study in the hamster is **Acceptable/Guideline** and it satisfies the guideline requirement for a chronic toxicity/carcinogenicity oral study [OPPTS 870.4300 (§83-5)] in hamsters.

4.7 Mutagenicity

Triclosan has been tested for mutagenic activity in several assays, including bacterial reverse mutation tests (MRID 43533301 and MRID 44389705), an in vitro mammalian cell gene mutation test (MRID 44389704), two in vitro mammalian chromosome aberration tests (MRIDs 43740801 and 47276601), a mammalian bone marrow chromosomal aberration test (MRID 43740802), and an unscheduled DNA synthesis assay in mammalian cells in culture (MRID 47276602).

870.5100 Bacterial Reverse Mutation Test

In two independently performed microbial preincubation assays (MRID 43533301), *Salmonella typhimurium* strains TA1535, TA1537, TA98, or TA100 were exposed to triclosan doses of 0.015, 0.050, 0.15, 0.5, or 1.5 µg/plate either in the absence or the presence of 3, 10, or 30% S9 derived from Aroclor 1254-induced rat livers. The test material was delivered to the test system in dimethyl sulfoxide.

Triclosan was cytotoxic at 1.5 µg /plate -S9 and at doses ≥0.5 µg /plate with S9. **There was, however, no indication of a mutagenic response in any strain at any dose either without or with increasing concentrations of S9**. All strains responded in the expected manner to the nonactivated and S9-activated positive controls.

The study is classified as **Acceptable/Guideline**. It satisfies the guideline requirement for a gene mutation assay (§84-2).

870.5100 Bacterial Reverse Mutation Test

In a microbial mutagenicity assay (MRID 44389705), Salmonella typhimurium strains TA100 and TA1538 were exposed to triclosan (100.5% a.i.) in dimethylsulfoxide (DMSO) at concentrations of 0.005-5,000 µg/plate without mammalian metabolic activation (-S9) and 0.005-50 µg/plate with mammalian metabolic activation (±S9). Strains TA98, TA100, TA1535, TA1537, and TA2538 were evaluated for mutagenicity at 0.05-5.0 µg/plate (+S9) and all except TA100 at 0.00167-0.167 µg/plate (-S9). Without S9, TAl00 was evaluated for mutagenicity at 0.00167-0.167 µg/plate. The standard plate incorporation test was performed. S9 homogenates for metabolic activation were made from Aroclor induced rat livers.

Triclosan was tested to cytotoxic concentrations. The test article precipitated from solution at 5,000 µg/plate (-S9). In pre-screen cytotoxicity tests triclosan was not toxic to strain TA1538 at doses of 0.005 to 1.67 µg/plate with S9 activation and 0.005 µg/plate without S9 activation and was not toxic to strain TA100 at doses of 0.005 to 0.50 µg/plate +S9 and at 0.005 and 0.0167 µg/plate -S9. **There were no reproducible, dose-related differences in the number of revertant colonies in any tester strain at any dose level/condition compared to the vehicle controls.** The positive control substances induced marked increases in revertant colonies in their respective strains.

This study is classified as **Acceptable/Guideline** (§84-2) and satisfies the requirement for FIFRA Test Guideline for in vitro mutagenicity (bacterial reverse gene mutation) data.

870.5300 In Vitro Mammalian Cell Gene Mutation Test

In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 44389704), L5178Y TK +/- mouse lymphoma cells cultured in vitro were exposed to triclosan (>99% a.i.) in dimethylsulfoxide (DMSO) at concentrations ranging from 1 to 25 µg/mL without metabolic activation (-S9) and from 1 to 20 µg/mL with mammalian metabolic activation (+S9). Treatment levels were selected based on a preliminary cytotoxicity test conducted at 1 to 250 µg/mL with and without activation.

Triclosan was tested up to toxic concentrations. Mutation frequencies were determined for concentrations selected on the basis of relative growth. The first mutation assay was initiated at concentrations ranging from 1 to 25 and 1 to 20 µg/mL without S9 activation and in a second mutation assay at 1 to 20 and 0.5 to 15 µg/mL with metabolic activation. Redundant or highly cytotoxic concentrations were eliminated during the assays. Only dose levels that resulted in ≥10% survival were used to assess mutagenicity. For the final concentrations tested, relative growth ranged from 8 to 100% without activation and from 7 to 88% with activation.

In order for the test material to be considered a mutagen, it had to produce both a mutant frequency at one or more dose levels that was at least twice that of the vehicle control, as well as a dose or toxicity relationship; in addition, the effects had to be reproducible. By these criteria triclosan was negative for inducing forward mutations at the TK locus in mouse L5178Y cells both with and without metabolic activation. In both the nonactivated and activated conditions, the positive controls induced the appropriate responses.

This study is classified as **Acceptable/Guideline** (§84-2), and satisfies the requirements for FIFRA Test Guideline for in vitro mammalian forward gene mutation data.

870.5375 In Vitro Mammalian Chromosome Aberration Test

In a mammalian cell cytogenetics chromosome aberration assay (MRID 47276601), Chinese hamster ovary cells (CHO strain K_1-BH_4) were exposed to triclosan (>99% pure; Unilever sample number S15155 T01) and dissolved with DMSO. Concentrations of 0.1, 0.3, 0.5, and 1.0 µg/mL and 4.8, 9.5, 19.0, 30.0, and 38.0 µg/mL were tested for the cultures without and with metabolic activation from Aroclor 1254-induced rat livers for 24 and 6 hours, respectively. Cells were harvested 24 hours after treatment and analyzed for chromosomal aberrations.

Triclosan was tested up to the toxicity limit of 1.0 and 38.0 µg/mL, -S9 and +S9, respectively, based on a preliminary toxicity test using CHO cells that were treated at dose concentrations of 6.3, 12.5, 25.0, 50.0, 100.0, 200.0, and 400.0 µg/mL. No live cells were observed at ≥50 and ≥100 µg/mL in -S9 and +S9 cultures, respectively. There were no aberrant cells at 12.5 and 25.0 µg/mL, -S9, but the mitotic index was declined to ~29% at 6.3 µg/mL compared to the solvent control. For the cultures with +S9, the mitotic index was reduced by 27 and 77% for 50 and 25 µg/mL, respectively, but was comparable to the solvent control at 6.3 and 12.5 µg/mL. The EC_{50}

value for cultures with +S9 and –S9 were estimated to be 38 and 1 µg/mL. Hence, concentrations of 1 and 38 µg/mL were used as the highest dose for the cultures without and with S9, respectively, for the cytogenetic assay.

In the cytogenetic assay, toxicity was noted at 38 µg/mL, +S9, and was not analyzed for chromosomal aberrations. Precipitation, if observed, was not reported for any dose level. Cultures treated with 0.1, 0.3, 0.5, and 1 µg/mL (-S9) and 4.8, 9.5, 19, and 30 µg/mL (+S9) were evaluated for chromosomal aberrations. No statistically-significant increases in the number of aberrant cells or chromosomal aberrations were reported at any dose level compared to the concurrent solvent/negative control. The percentage mean number of aberrant cells with gaps (excluding and including type) was $P>0.05$ comparable to the solvent and untreated controls for all dose levels and conditions. The positive controls of mitomycin-C and cyclophosphamide displayed significant increases in the percentage of aberrations, hence eliciting a clear positive response. **There was no evidence of chromosome aberration induced over the background.**

This study is classified as **Acceptable/Guideline** because it satisfies the guideline requirement (OPPTS 870.5375; OECD 473) for *in vitro* cytogenetic mutagenicity data.

870.5375 In Vitro Mammalian Chromosome Aberration Test

In an in vitro cytogenetic assay (MRID 43740801), Chinese hamster lung fibroblasts were exposed to triclosan (99-100%) nonactivated doses of 1 µg/ml (7-hour cell harvest), 0.1-3 µg/ml (18-hour harvest), or 3 µg/ml (28-hour harvest) and S9-activated concentrations of 3 µg/ml (7- and 28-hour cell harvests) or 0.1-3 µg/ml (18-hour harvest). The S9 fraction was derived from Aroclor 1254 induced Wistar male rat livers and triclosan was delivered to the test system in ethanol.

No mitotic cells were recovered at any harvest time from cultures treated with ≥6 µg/ml -S9 or ≥ 10 µg/ml +S9. Findings with the positive controls confirmed the sensitivity of test system to detect clastogenesis. **However, nonactivated triclosan at 1 and 3 µg/ml (18-hour harvest) induced a dose-related increase in the yield of cells with abnormal chromosome morphology. The response was significant ($p\leq0.001$) at the higher concentration. A significant increase ($p\leq 0.001$) was also seen at 3 µg/ml (28-hour harvest). The most frequently observed type of chromosome damage was exchange figures. In the presence of S9 activation, nonsignificant but concentration dependent increases in cells bearing exchange figures were also seen at 1 and 3 µg/ml (18-hour harvest). The data are, therefore, sufficient to conclude that triclosan is active in this test system.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for an in vitro mammalian cell cytogenetic assay (§84-2).

870.5385 Mammalian Bone Marrow Chromosomal Aberration Test

In an in vivo bone marrow cytogenetic assay (MRID 43740802), groups of six male and six female Wistar rats received a single oral gavage administration of 4000 mg/kg triclosan (99-100%). The test material was delivered to the animals as suspensions prepared in 1% carboxymethyl-cellulose. Animals were sacrificed 6, 24, and 48 hours following compound administration and bone marrow cells from ten animals per group (5 males and 5 females) were harvested and examined for the incidence of structural chromosome aberrations.

No signs of overt toxicity or cytotoxic effects on the target organ were seen in any treatment groups. The positive control induced the expected high yield of cells with structural chromosome aberrations. **There was also no indication of a clastogenic effect at any sacrifice time.**

The study is classified as **Acceptable/Guideline** and satisfies the requirements for FIFRA Test Guideline §84-2 for in vivo cytogenetic mutagenicity data.

870.5550 Unscheduled DNA Synthesis in Mammalian Cells in Culture

In an *in vitro* DNA synthesis assay (MRID 47276602), rat hepatocytes were exposed to triclosan (batch/lot#: CC # 14663-09) dissolved in DMSO. Hepatocytes were isolated from the liver of two male Fischer 344 rats by the two-step *in-situ* perfusion. Concentrations of 0, 0.05, 0.1, 0.25, 1.0, 2.5, 5.0, 10, 25.0, 50.0, 100.0, or 250 µg/mL were tested for 18-20 hours. Cells were autoradiographed, and unscheduled DNA synthesis was evidenced by a net increase in black silver grain counts using an Artek 880 automated colony counter with microscope and connected to an Apple II computer for data analysis. The difference between the cytoplasmic grain count and the corrected grain count was calculated and the net nuclear grains (NNG) and the percentage of hepatocytes in repair were calculated.

Triclosan was tested up to the toxicity limit of 2.5 µg/mL based on the preliminary toxicity test using rat hepatocytes that were treated at dose concentrations of 10.0, 25.0, 50.0, 100.0, 250.0, and 500.0 mg/mL. Precipitation was observed ≥50 mg/mL, and turbidity was noted at 25 mg/mL. Hence 25 mg/mL was selected as the highest dose concentration for the UDS assay.

In the UDS assay, triplicate cultures were exposed to the test article, untreated control, solvent control or a positive control (2AAF). Toxicity was observed at ≥5 ug/mL in the form of low grain count. Hence dose concentrations of 0.25, 0.5, 1.0, and 2.5 µg/mL were evaluated for UDS assay. No significant increases in mean NNG counts were reported at any dose levels and the percent of cells in repair ranged from 0-6%, comparable to the solvent and untreated controls. The positive control yielded 88.7% of cell in repair and a mean NNG count of 21.2, hence eliciting a clear positive response. **There was no evidence of induction of unscheduled DNA synthesis in rat primary hepatocytes over the background.**

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirement (OPPTS 870.5550) for an *in vitro* UDS assay.

4.8 Neurotoxicity

There is one older 14-day neurotoxicity study for triclosan (HED document # 001968).

A 14-day neurotoxicity study was conducted in rats with triclosan at doses of 0, 100, 300, 1000, and 2000 mg/kg/day. Slight inhibition of movement, decreased muscular tone, polydypsia and polyuria were observed at 300 mg/kg/day, with more pronounced signs at 1000 mg/kg/day. No brain weight changes or histopathology were observed at any dose level tested. No peripheral nerve changes were observed at any dose level tested.

4.9 Metabolism and Pharmacokinetics

Adequacy of database for Metabolism and Pharmacokinetics: Data are available on metabolism and pharmacokinetic for triclosan that include absorption, distribution, metabolism, and elimination studies in hamsters (MRID 45307501, 45307502), mice (MRID 45307503), mouse, rat, rabbit, and dog (MRID 149464), mouse, rat, dog, and baboon (MRID 68161), rat (MRID 47261405), and dogs and baboons (MRID 79590). A dermal absorption study in rabbits (MRID 34335) is also available.

870.7485 General Metabolism (Hamster)

In a metabolism study (MRID 45307501 and MRID 45307502) triclosan [99% a.i., EN275927.26 (FAT 80'023/R)], and ^{14}C-triclosan [5-chloro-2-[2,4-dichlor-[^{14}C(U)]-phenoxy]-phenol; 98% a.i., Batch 0542] was administered to hamsters following the exposure scheme below. Four absorption and distribution studies (i.e., balance studies) were performed, each using 5 hamsters/sex/dose at target dose levels of 2.0 or 200 mg/kg. The first study administered a single oral dose via gavage at calculated doses of 2.0 mg/kg in both males (group 1) and females (group 3) or 213 and 203 mg/kg in males (group 2) and females (group 4), respectively. In the second study, animals were administered 2 or 200 mg/kg unlabeled triclosan via the diet for 13 days, then dosed with a comparable single oral dose of labeled triclosan via gavage (2.0 mg/kg in both males [group 5] and females [group 7], respectively, or 200 and 203 mg/kg in males [group 6] and females [group 8], respectively). A single intravenous dose was utilized with both males (group 9) and females (group 10) receiving 2.0 mg/kg ^{14}C-triclosan. A 13-day feeding regime of unlabeled triclosan was followed by a single intravenous dose of ^{14}C-triclosan at a level of 2.0 and 2.1 mg/kg in males (group 11) and females (group 12), respectively. Pharmacokinetic studies were performed on 12 hamsters/sex/dose at dose levels of 2.0 mg/kg in both males (group 13) and females (group 15) or 199 and 201 mg/kg in males (group 14) and females (group 16), respectively. Bioretention and metabolite distribution studies were performed on 16 hamster males/dose with doses of 2.0 or 201 mg/kg of ^{14}C-triclosan via gavage (groups 17 and 18, respectively) and on 16 hamster males/dose fed unlabeled triclosan for 13 days before receiving gavage doses of 2.0 or 202 mg/kg of ^{14}C-triclosan (groups 19 and 20, respectively).

After single or repeated oral doses of 2.0 or 200 mg/kg ^{14}C-triclosan, urine was the major route of elimination for triclosan radioactivity (60-80% of the administered radioactivity). Fecal

elimination represented from 12-35% of administered radioactivity across the oral dose groups. Compared to the low dose, administration of a single high or repeated dose resulted in a shift toward urinary elimination and a decrease in fecal elimination.

Intravenous administration at the low dose resulted in a similar pattern of elimination in male and female hamsters as those receiving the low oral dose. Administration intravenously after feeding of unlabelled triclosan for 13 days also did not appear to have any significant effect on the general pattern of elimination. Greater than 90% of the administered radioactivity was excreted in 7 days post-dose after oral or intravenous administration. Organ/tissue radioactivity levels were <1% of the dose received after 7 days, which also included the plasma.

After oral administration of a single low or high dose (2.0 or 200 mg/kg), peak plasma and blood concentrations of triclosan-derived radioactivity occurred at one hour post-dose for both the low and high oral doses. Concentration in plasma appeared higher than in whole blood at each sampling time. There did not appear to be any significant sex differences in blood or plasma kinetics at the low or high dose. However, Area Under the Curve (AUC) measurements indicated that saturation may have been achieved at the high dose, as AUC was not proportional to dose (in males, AUC increased 34x from the low to high dose; in females, AUC increased 54 times from the low to high dose).

The major urinary metabolite detected after oral administration was the glucuronide conjugate of triclosan (U7) at 32-37% of the administered radioactivity at the low single and low repeated dose level in males; 55-57% of the administered RA in high single and high repeated dose males; 9.5-43% of the administered RA in low single and low repeated dose females; and 60% in high single and high repeated dose females. The major fecal metabolite was parent triclosan in all oral dose groups (1-8). After intravenous administration, triclosan glucuronide was also detected as the major urinary metabolite. Notably, in female hamsters, intravenous administration of triclosan resulted in a significant increase in the percentage of the glucuronide metabolite found in urine compared to oral dosing (41.4% vs 9.5%). This is similar to what occurred after the single oral dose was increased from 2.0 to 200 mg/kg in female hamsters (60.2 vs. 9.5%). Distribution patterns in the orally and intravenously dosed animals were similar between the single- and repeat-dosed groups with the highest residual activity found in the kidney, liver, lung, and plasma; however, the levels were low. No organ demonstrated accumulation of triclosan with highest levels of triclosan equivalent in the plasma 7 days after dosing. The plasma, kidney, and liver eliminates triclosan equivalent rapidly with 1/8 of the concentration eliminated by hours 48-56.

Tissue metabolite analysis showed that the glucuronide and sulfate conjugates of triclosan were the major metabolites detected at the low and high oral single doses. The percentage of the sulfate conjugate increased significantly in the plasma and liver from the low to high dose while the percentage of the glucuronide conjugate decreased only in liver. This pattern was also observed in the single orally dosed female hamsters. Four non-parent conjugates (M5, M6, M8, and M9) were also identified in tissues. All conjugates were acid labile and resulted in the parent compound, M2, or M3. The two dose levels suggest reduced phase 1 metabolism of triclosan in hamsters at the higher dose level of 200 mg/kg as compared to the low dose of 2.0 mg/kg ^{14}C-triclosan.

After 1-2 hours (C_{max}), males dosed single or repeatedly parent glucuronide and sulfate were found in the plasma without detection of the free parent compound. The kidneys at C_{max} had parent glucuronide and free parent with little sulfate conjugate; the liver at C_{max} had free parent and sulfate conjugate with little glucuronide levels. The major urinary metabolite was the parent glucuronide conjugate with little free parent compound detected; the major feces metabolite was the free parent compound with a little parent glucuronide conjugate; neither urine or feces had detectable levels of the parent sulfate conjugate. Comparing the low- to the high-dose groups, the liver and kidney levels of free parent compound were similar between the dose groups. The percentage of parent conjugates increased in the plasma, liver, and kidneys after 200 mg/kg/day compared to the 2 mg/kg/day. The parent sulfate was the most pronounced in the plasma with levels of 7-9% in the low-dose group and 28-59% in the high-dose group and liver with levels of 8-16% in the low-dose group and 36-38% in the high-dose group. Parent glucuronide increased after feeding for 13 days in the plasma from 24 to 56% and kidneys from 48 to 60% in the low- to high-dose groups, indicating the hamsters have an increased conjugating capacity with the higher-dose group (200 mg/kg). Triclosan sulfate conjugate concentrations in the liver were enhanced by 250-600 times when comparing the high- to the low-dose groups.

The metabolite data suggest that the sulfate conjugate produced in the liver is transported to the kidney where it undergoes hydrolysis and re-conjugation with glucuronic acid and then is eliminated in urine. However, sulfation is usually predominant at lower doses while glucuronidation normally increases with increasing dose.

This metabolism study in the hamster is classified **Acceptable/Guideline** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in hamsters.

870.7485 General Metabolism (Mouse)

In an absorption, distribution, metabolism, and elimination study (MRID 45307503), ^{14}C-triclosan (98% pure) was administered to mice in various separate experiments. These separate experiments were as follows:

- Balance and distribution studies, in which male and female mice were administered either (1) a single oral low (2 mg/kg) or high (200 mg/kg) dose (groups 1-4) of ^{14}C-triclosan or (2) a repeated (13X) food supply of non-labeled test article followed by a single oral administration of ^{14}C-triclosan at the low or high dose (groups 5-8).
- Balance and distribution studies, in which male and female mice were intravenously administered a low dose (2 mg/kg) of ^{14}C-triclosan with and without repeated (13X) food supply of non-labeled test article (groups 9-12).
- Blood/plasma level studies, in which male and female mice were administered a single oral low (2 mg/kg) or high (200 mg/kg) dose of ^{14}C-triclosan (groups 13-16).
- Bioretention studies from Cmax to 1/8Cmax, in which male mice received a single oral administration of ^{14}C-triclosan at either the low (2 mg/kg) or high (200 mg/kg) dose with or without repeated (13X) food supply of non-labeled test article (groups 17-20).

Metabolite characterization was determined by using mice excreta from balance study groups (1-12). Plasma from the bioretention study groups (17-20) and liver and kidney pools from the bioretention study groups (17-20) were analyzed for metabolite characterization.

The test material was rapidly absorbed following oral administration, and was eliminated primarily through the feces, via biliary excretion. Urinary excretion was secondary to that in the gastrointestinal tract. This excretory pattern was consistent following I.V. administration as well. Most organs/tissues showed comparable levels of radioactivity after oral and intravenous administration. Only organs with the highest blood supply (lung, liver, and spleen) showed higher levels of radioactivity (~3-15 times) following I.V. administration. Bioretention studies indicate that values from Cmax to 1/8Cmax in the liver were higher than those in plasma following repeated administration at both dose levels, indicating that the liver is the target organ. Primary excreted compounds in the urine following single oral exposures included the unmetabolized parent compound and two parent conjugates (parent sulfate and parent glucuronide); fecal excretion was primarily that of the free parent compound, as small amounts of glucuronide were detected, and no sulfate was detected. It was speculated that intestinal microflora were responsible for deconjugating the parent glucuronide prior to elimination in the feces. Interestingly, parent glucuronide was detected in the fecal matter of females, but not males, following I.V. dosing.

Because parent sulfate was detected in plasma, liver, and kidney, and the parent glucuronide was absent in the liver and kidney, but present in the plasma and urine, it is assumed that the glucuronide, once formed, is rapidly eliminated from both the liver and kidney. It was also suggested that the kidney would reconjugate any sulfate conjugate that was transported there from the liver, to the glucuronide, prior to excretion in the urine. This seems to happen predominantly in females following a low single oral dose, in females at low and high single oral doses, and in both sexes following repeated high oral doses.

Additionally, 4 conjugated metabolites (M5, M6, M8, and M9) were detected in kidney, plasma, and liver extracts in minor amounts (<5.3%). The compounds were generally present in plasma only after a single, low oral dose, increasing slightly in kidney (and potentially liver—the values were illegible in the study report) at the high single oral dose. M6 and M9 were not present in these tissues after repeated dosing at either dose level. In general, as the dose increased, the urinary excretion shifted to free parent and parent glucuronide.

Parent and parent conjugates were rapidly eliminated and/or metabolized with half-lives ranging from 1-13 hours in the plasma, liver, and kidney. Cmax values occurred at 4-11 hours. Mice with enlarged livers (i.e., group 20) exhibited parent and parent sulfate half-lives of 13 and 14 hours, respectively. Non-parent conjugates M5, M6, M8, and M9 showed similar half-lives as parent and parent compound (<4-13 hours); however, Cmax values were greater, occurring at 12-32 hours.

This absorption, distribution, metabolism and elimination study is **Acceptable/Guideline** and satisfies the guideline requirement for a metabolism study OPPTS 870.7485.

Metabolism and Pharmacokinetics - Multiple Species

CITATION: Stierlin, H. (1972). GP 41 353: Study of pharmacokinetics and metabolism in mouse, rat, rabbit, and dog. Pharma Research, Pharmacological Chemistry. CIBA-GEIGY, Ltd. Basle. Project No. GP 41 353. Report No. 33/1972. MRID 149464. December 1, 1972. Unpublished.

EXECUTIVE SUMMARY: In a metabolism study (MRID 149464), rats, mice, rabbits, and dogs were administered [^{14}C]-GP 41 353 or [^{3}H]-GP 41 353 (radiochemical purity 99%; chemical purity 99.5%; batch/lot nos. not provided) intravenously, intraduodenally, or orally (gavage) at doses of 10 mg/kg (mice), 0.4 mg/kg (rats), 5 mg/kg (rats, rabbits, dogs), or 50 mg/kg (rabbits). Radioactivity levels in the blood, tissues, and excreta were measured for time intervals up to 168 hours. Additionally, biliary elimination was also analyzed in rats given a single intraduodenal dose.

There was no indication of any toxic effects in the test animals. Overall recovery of administered radioactivity in rats ranged from 99.67% to 104.53% at 2 to 8 hours after dosing. Data were unavailable in the study report to accurately determine radioactivity inventory for the other species tested.

Absorption of the test material was reported by the study author as 70-80% as determined by comparisons of areas under-the-blood concentration curve for oral and intravenous administrations. Absorption in rats could also be estimated based upon biliary and urinary elimination data where, over a 7.5 to 10-hour period, biliary elimination accounted for 62.5% of a 5 mg/kg gavage dose and 67% of a 5 mg/kg intraduodenal dose and urinary excretion at 6 hours accounted for 76.60% of a 5 mg/kg gavage dose. These data affirm that absorption exceeded 70%. Absence of biliary secretion data for the other test species precludes assessment of absorption. Time-course concentration data revealed that peak blood levels occurred within 30 minutes in rats following a single oral or intravenous dose of 5 mg/kg and at 2-4 hours for dogs.

Tissue distribution patterns were similar among mice and rats, and exhibited only slight quantitative variability for intravenous versus oral dosing. Following a single oral dose in rats, radioactivity in tissues was low (generally <1 µg/g tissue) with the exception of blood and the organs associated with excretory function (e.g., liver, gall bladder, kidneys). Following an intravenous dose to rats and mice, tissue levels were also greatest in highly perfused organs or those associated with excretory function. Based upon data from rats, tissue burdens declined appreciably over 24 hours with no indication of accumulation/sequestration.

Excretion of GP 41 353 was examined in two strains of rats, rabbits, and dogs. Biliary elimination was also assessed in rats. Urinary excretion appeared to be a minor route of elimination in rats and dogs, accounting for 3-17% of the administered oral dose in rats over a 168-hour period, and 8.3-8.8% in dogs over a 120-hour period. Urinary elimination in dogs was somewhat greater following intravenous administration; 12.9-17.7% over 120 hours. For rabbits, urinary excretion was a significant route of elimination and accounted for 74.1% of a single 50 mg/kg oral dose and 60.4% of a single 5 mg/kg oral dose over a 72-hour period. The biliary secretion data in rats showed that most of the fecal radioactivity could be attributed to biliary elimination products rather than unabsorbed test material. Biliary elimination was also affirmed by data from the mouse showing very high concentrations of radioactivity in the gall bladder at 5 minutes to two hours following a 10 mg/kg, i.v. dose.

Analysis of bile samples from the rats indicated that the test material underwent Phase II biotransformation. Treatment of samples with β-glucuronic acid revealed that most of the biliary product was glucuronide conjugates while some was unchanged parent compound.

The results of this multi-species study indicated that at least 70% of an oral dose of GP 41 353 is absorbed from the gastrointestinal tract and that biliary secretion and subsequent fecal elimination is a major excretory route in the rat and dog. Urinary excretion appeared to be a major route of elimination in the rabbit. Tissue accumulation was minimal and primarily associated with highly perfused tissues and organs with excretory function. Metabolite data in rats revealed glucuronide conjugates and unchanged parent compound as biliary metabolites.

This metabolism study in rats is **Acceptable/Guideline** and satisfies the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)]. Although information regarding dose confirmation, homogeneity, and stability were lacking, the consistency of results across several species by several dose routes would seem to preclude potential problems pertaining to these parameters. The data are also consistent with findings from other reports in humans (MRID 68163, 68162), and other laboratory species (MRID 68161, 79590).

Metabolism and Pharmacokinetics - Rats, Dogs, and Baboons

CITATION: Stierlin, H., K. Schmid, A. Sutter (1977). GP 41 353: Comparison of pharmacokinetic and metabolic parameters of triclosan and HCP in the mouse, rat, beagle, dog and baboon. Part A. Survey of findings. Part B. Detailed account of the study. Pharma Research, Pharmacological Chemistry. CIBA-GEIGY, Ltd. Basle. Report No. B 1/1977. MRID 68161. January 27, 1977. Unpublished

EXECUTIVE SUMMARY: In a metabolism study (MRID 68161), ^{14}C-triclosan (>98% radiochemical purity, lot/batch no. not specified) was administered to male rats by gavage at doses of 5 or 30 mg/kg (single or 14-day repeated). In addition, two male beagle dogs and two male baboons received single 5 mg/kg doses in gelatine capsules and 10 male mice were given a single 10 mg/kg intravenous dose. Blood levels were monitored in mice up to 2 hours, in rats at

24 hours postdose, in the dogs up to 72 hours and in monkeys up to 168 hours postdose. Urinary and fecal excretion was monitored in monkeys and dogs for 6-10 days. Tissue distribution was assessed in mice and rats.

There were no test article-related toxic effects reported. Recovery of administered radioactivity was marginal; 86.9% and 74.1% for each of two dogs, and 83.1% and 80.5% for each of two monkeys. Administered radioactivity was widely distributed among tissues/organs in mice after an intravenous injection (10 mg/kg) and in rats following a single oral administration or the last dose of a 14-day repeated oral administration (5 or 30 mg/kg). Based on the radioactivity distribution data, there was no evidence indicating sequestration of the test material or its metabolites in either mice or rats.

Time-course analysis of blood/plasma radioactivity in rats revealed that peak concentrations were attained within three hours after a single oral dose of 5 mg/kg and that the concentrations declined approximately five-fold within 24 hours. For dogs, somewhat greater blood t_{max} values were observed but were variable (2- 8 hours) for the two dogs tested. Generally, the test article did not exhibit especially rapid partitioning into or clearance from the blood for either species. Time-course analysis of tissues from mice given a single intravenous dose (10 mg/kg) of ^{14}C-triclosan showed that the highest concentrations of radioactivity were initially associated with highly perfused organs/tissues. These levels significantly declined within 30 minutes but the decline was somewhat less rapid for organs /tissues associated with metabolism and elimination and notably increased for the gall bladder.

Both urinary and fecal elimination were identified as major routes of excretion. The relative contribution of each to overall elimination of administered radioactivity exhibited species variability. For monkeys, urinary excretion accounted for 56.69% of the administered dose and fecal elimination accounted for 25.15%. For dogs, urinary and fecal elimination accounted for 12.16% and 68.30%, respectively, of the administered dose. In both species, most of the urinary and fecal excretion occurred within 48 hours.

Definitive characterization of metabolites was not performed. Preliminary investigations using acid and enzyme hydrolysis, indicated that very little (<1%) of the blood/plasma radioactivity was associated with unchanged triclosan. In the brains of rats, however, 35-50% of the radioactivity was attributed to parent compound.

Overall, this study demonstrated that ^{14}C-triclosan is readily absorbed from the gastrointestinal tract, has a potential wide volume of distribution, and can cross the blood-brain barrier. Blood absorption and clearance is not especially rapid, but the compound does not appear to undergo sequestration in the species tested. Most of the circulating radioactivity was attributed to metabolism products (probably conjugation products based upon preliminary experiments using acid and enzymatic hydrolysis). Both the urine and the feces are significant routes of excretion with the relative importance appearing to be species dependent.

This metabolism study in multiple species, which predates GLP guidelines, is **Unacceptable/Nonguideline** and does not satisfy the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)]. The study was properly conducted and

provided data regarding excretion and plasma/blood kinetics of triclosan in monkeys, dogs, and rats, and tissue distribution data in rats and mice. Dose confirmation, homogeneity, and stability were not provided, and overall recoveries of administered radioactivity were marginal. Additionally, a quality assurance statement was not provided. The results of experiments assessing excretion and blood time-course, and tissue distribution, however, do affirm findings of companion studies (MRID 149464, 79590) in animal species and studies (MRID 68162, 68163) in human volunteers.

Metabolism and Pharmacokinetics-Rat

CITATION: van Dijk, A. (1996) Absorption, distribution and execution (ADE) after single oral and repeated oral administration. RCC Umwelchemie AG (Itingen, Switzerland). RCC Project 341998, July 19, 1996. MRID 47261405. Unpublished.

EXECUTIVE SUMMARY**:** In a metabolism study (MRID 47261405), ^{14}C-labeled triclosan (99% a.i., Batch # B-71.32A) was administered to two groups of 10 Wistar rats/dose and four groups of 36 Wistar rats/dose. The levels of radioactivity appearing in the feces, urine, plasma, liver, and kidney were studied. Groups 1 and 2 were administered a single dose of 2.3 mg/kg or 211 mg/kg of radiolabeled triclosan, respectively, by gavage and sacrificed at 96 hours. Groups 3 and 4 were administered a single dose of 2.2 mg/kg or 199 mg/kg of radiolabeled triclosan, respectively, by gavage and groups of 4 rats were sacrificed at 0.5, 1, 2, 4, 8, 24, 48, 72, or 96 hours. Groups 5 and 6 were administered thirteen doses of 2.0 mg/kg or 201 mg/kg of non-radiolabeled triclosan, respectively, through food followed by a single gavage dose of radiolabeled triclosan.

The radioactive dose was rapidly excreted and elimination was nearly complete by 72 h. Fecal excretion predominated at both dose levels and dosing regimes. At 96 hours after administration of the low dose (Group 1), 11.2% of the administered radioactivity was found in the urine, 81.2% in the feces, 2.4% in the cage wash, 0.1% in the intestinal tract, <0.05% in the organs/tissues, 0.3% in the carcass, and 0.2% in the plasma. At 96 hours after administration of the high dose (Group 2), 12.2% of the administered radioactivity was found in the urine, 82.2% in the feces, 3.5% in the cage wash, 0.1% in the intestinal tract, <0.05% in the organs/tissues, 0.2% in the carcass, and <0.05% in the plasma. Further single oral dose studies (Groups 3 and 4) showed that at 48 hours after administration most of triclosan or its equivalent were excreted with, 10.7% (low dose) and 11.8% (high dose) of the administered dose excreted in the urine and 79.5% (low dose) and 80.2% (high dose) excreted in the feces.

At both dose levels in single and repeated oral administration, the maximum level of radioactivity in the plasma was reached 1 to 4 hours post administration. Half lives for triclosan and its equivalents in the plasma ranged from 10.0-12.6 hours, single (12.6 or 10 hours, low- or high-dose, respectively) and repeated (11.7 or 10.7 hours, low- or high-dose, respectively) dose groups were comparable. At both dose levels in single and repeated oral administration, the maximum level of radioactivity in the liver was always reached at 1 to 4 hours post

administration. Half lives for triclosan and its equivalents in the liver for all groups ranged from 9.4-11.0 hours (single dose: 11.0 or 9.8 hours, low- or high-dose, respectively; repeated dose: 9.4 or 10.0 hours, low- or high-dose, respectively). At both dose levels in single and repeated oral administration, the maximum level of radioactivity in the kidneys was always reached at 1 to 4 hours post administration. Half lives for reactivity metabolism in the kidney for all groups ranged from 10.8-14.1 hours (single dose: 13.0 or 12.0 hours, low- or high-dose, respectively; repeated dose: 14.1 or 10.8 hours, low- or high-dose, respectively).

The majority of the metabolites found in the urine and liver were in the form of conjugated and/or non-parent compound. Analysis of the metabolites found in the urine revealed that 3.7% (single low dose) and 1.7% (single high dose) of the radioactivity administered was in the form of free parent compound while 9.3% (single low dose) and 10.3% (single high dose) was in the form of conjugated parent and/or non-parent compound. Analysis of the metabolites found in the liver revealed that 91.1% (single low dose) and 75.6% (single high dose) of the metabolites found in the livers were in the form of free parent compound while 5.2% (single low dose) and 21.7% (single high dose) were in the form of conjugated parent or non/parent compound. Repeated dose groups showed similar breakdowns.

Repeated doses of triclosan did not cause significant changes to the pharmacokinetics of triclosan and/or its metabolites in the rat, as compared to single dose administration. In addition, the results indicate that triclosan exhibits saturation of absorption and a higher conjugating/metabolizing capacity at approximately 200 mg/kg, as compared to approximately 2 mg/kg.

This Tier I metabolism study in rats is classified **ACCEPTABLE, GUIDELINE** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats. The study does not satisfy the requirements for a Tier II study; an intravenous dose was not administered, nor was a biliary excretion study conducted.

Metabolism and Pharmacokinetics - Dogs and Baboons

CITATION: Stierlin, H. (1976). GP 41 353: Isolation and identification of the main metabolites in the blood of the beagle and baboon and in the urine of the latter following oral administration of ^{14}C-labelled triclosan. Pharma Research, Pharmacological Chemistry. CIBA-GEIGY, Ltd. Basle. Report No. B 14/1976. MRID 79590. March 16, 1976. Unpublished.

EXECUTIVE SUMMARY: In a metabolism study (MRID 79590), two baboons and two dogs were administered a single oral dose (5 mg/kg) of [^{14}C]-GP 41 353 (radiochemical purity 99%; chemical purity 99.5%; batch/lot nos. not provided). Blood samples were taken at 3 hours postdosing from one dog and at 8 and 12 hours postdosing from one baboon. Another dog and baboon were killed at 6 and 7 hours postdosing to obtain sufficient blood samples for metabolite characterization. Urine samples were collected from the dog and baboon that were not sacrificed for blood sample acquisition.

There were no adverse effects associated with the test article. At 3 hours postdosing, total radioactivity was 6.08 µg eq./mL blood in the dog. For the baboon total radioactivity was 1.24 and 1.03 µg eq./mL blood, respectively at 8 and 12 hours postdosing. For the dog and baboon terminated for sample acquisition, total blood radioactivity was 4.86 µg eq./mL blood for the dog at 6 hours and 2.29 µg eq./mL blood for the baboon at ~7 hours postdosing. Urinary elimination accounted for 32% of the administered dose to the surviving baboon by 72 hours. Urinary excretion by the dog was minimal and accounted for "only a few percent of the administered dose".

Hydrolysis of the biological samples with arylsulfatase and β-glucuronidase resulted in the release of free triclosan indicating that the measured radioactivity was associated with sulfate and glucuronide conjugation products. For the dog, glucuronide conjugates accounted for 7% of the blood radioactivity and sulfate conjugates accounted for 88% of the radioactivity sampled at three hours. At six hours, ~86% of the radioactivity in the blood was associated with glucuronide/sulfatase conjugates. For the baboon, glucuronide conjugates represented about 25% and sulfate conjugates represented about 33% of the circulating radioactivity at 8 hours postdosing. Similar analysis with blood collected at 12 hours postdosing, revealed somewhat greater amounts of sulfate conjugates (~88%). For the baboon, analysis of 0-72 hour urine samples revealed that approximately 6% of the urinary radioactivity was due to unchanged triclosan. Up to 75% of the urinary radioactivity underwent spontaneous hydrolysis presumably due to endogenous urinary β-glucuronidase. No analyses were conducted for the dog due to the minimal urinary elimination of radioactivity.

In summary the results of this study showed that the major blood metabolites in the baboon and beagle dog were sulfate and glucuronide conjugation products. The major urinary metabolite in the baboon was a glucuronide conjugate but analysis of urinary metabolites in the dog was precluded by negligible urinary products as determined by radioactivity.

This metabolism study in rats is **Acceptable/Nonguideline** and does not satisfy the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)]. Although not designed as an 85-1 Guideline study (no tissue distribution data), it was properly conducted and provided data regarding the characterization of blood and urinary metabolites in the baboon and blood metabolites in the dog following a single oral dose. These data complement the findings from other studies in humans (MRIDs 68162, 68163) and laboratory species (MRID 149464, 68161).

870.7600 Dermal Absorption

An older in vivo rabbit dermal absorption study is available (MRID 34335). In this study, up to 48% of an applied dermal dose of 0.89 mg triclosan (3% a.i.) was absorbed. The in vivo rabbit dermal absorption data are in agreement with the estimate of dermal absorption of 50% derived from comparison of the LOAEL's from a rat 90-day dermal toxicity study (MRID 43328001) and a rat 2-generation reproduction study (MRID 401623701). This estimate was based on a reduced

mean body weight observed in the reproduction study at 150 mg/kg/day, and occult blood in urine observed at 80 mg/kg/day in the dermal study.

Additional dermal absorption data on triclosan have been submitted and reviewed. *In vitro* dermal absorption studies using human skin preparations and various formulations containing triclosan (MRIDs 47261408 through 47261411) showed dermal absorption values for triclosan ranging from 11-20% in these formulations. A paper published in 2000 by Moss et al. (Food and Chemical Toxicology, Volume 38, pages 361-370) examined dermal absorption of triclosan both in vivo and in vitro using rats as well as an in vitro human skin study. These data supported the conclusion of dermal absorption of 21-23% in the rat studies, and showed in vitro dermal absorption through human skin in vitro of 6.3%. Several human *in vivo* dermal absorption studies were cited in the 2007 CANTOX report on the Toxicological Evaluation of Triclosan that also suggest a lower value for dermal absorption than 50%, but none of these studies have been reviewed by the Agency's Human Studies Review Board for scientific and ethical conduct and are thus not cited in this risk assessment. Taken together, the available data on dermal absorption suggest a lower value, around 20% for rat skin and possibly lower for human skin. Additional verification is needed.

4.10 Special Studies

Three liver biochemical induction studies (MRID 44389702, 44389703, and 44389706) were performed with triclosan, in addition to two liver cell proliferation studies (MRID 44389701, Eldridge, 1995). Although these studies do not fulfill a guideline requirement, they provide additional data that may be used to characterize the toxicity of triclosan, specifically the potential mode(s) of action in regard to the formation of the liver tumors observed in the oral carcinogenicity study in mice.

Liver Biochemical Induction (Mouse)

In this study (MRID 44389702), triclosan technical was administered to CD-1 mice in a pelleted rodent diet for a 14-day period at doses of 0, 25, 75, 350, and 900 mg/kg/day and 0, 25, 350, or 900 mg/kg/day for males and females, respectively. An additional group of male mice received either 0 or 900 mg/kg/day for 14 days followed by a 4-week recovery period. Decreased absolute body weight and body weight gain was observed in male mice receiving 900 mg/kg/day or greater following 14 days of test article administration and weight gain was decreased by 75% vs. control and absolute group mean body weight was decreased by 16% vs. control. No effect on body weight or body weight gain was observed in female mice.

Liver weight in male mice receiving 75 mg/kg/day and above and in female mice at 350 and 900 mg/kg/day was significantly increased in comparison to the respective controls. Liver weight effects were reversible in recovery groups.

Significant increases in the activity of all biochemical parameters were observed at the top dose in male mice and significant increases were observed for microsomal protein (25% increase), lauric acid hydroxylation, and EROD (82% increase) and PROD (431% increase) activities at the

lowest dose tested in males. These effects were reversible in the recovery group.

In female mice, there were no significant increases in liver biochemical parameters at the low dose with the exception of PROD activity (268% over control). Increase in liver biochemical parameters were observed for female mice at the mid dose and above.

Total microsomal hydroxylation of testosterone was significantly increased at all dose levels tested in male mice, and were dose-dependant. Formation of the 2β-, 6β-, 15β-, and 16β-metabolites were increased 11.6 fold, 10.9 fold, 5.3 fold, and 7.6 fold, respectively, at the high dose level. Hydroxylation of testosterone at the 7 position is associated with CYP2A1 and isoenzymes of the peroxisome proliferator inducible P-450 family CYP4A in the rat.

In male mice, electron microscopy revealed marginal to moderate proliferation of endoplasmic reticulum. Rough endoplasmic reticulum membranes were distinctly reduced and disorganized at 54.7 mg/kg/day and above, leading to a mixture of rough and smooth ER membranes. Peroxisomes showed a moderate (at 54.7 mg/kg/day) to striking (1346.8 mg/kg/day) proliferation and were increased in size. At 54.7 mg/kg/day and above, lipid vacuoles were encountered in hepatocyte nuclei and, at the top dose, nearly all nuclei contained numerous lipid vacuoles of various sizes.

In female mice, the same morphological alterations were observed at the top dose (1105.6 mg/kg/day) as were observed in male mice.

From these data it is apparent that administration of triclosan to the mouse results in significant hepatic effects. The biochemical alterations observed appear to support the conclusion of a barbituate-type induction with peroxisome proliferation effects. Induction of certain liver enzyme activities as measured in this study appear to occur at the lowest dose tested in male mice, including significant increases in microsomal protein, lauric acid hydroxylation, and EROD and PROD activities.

Liver Biochemical Induction (Rat)

In a non-guideline feeding study (MRID 44389703), the effect of triclosan on selected biochemical liver parameters was examined at concentrations of 0, 300, 1500, and 6000 ppm. Reversibility of effects was assessed in a single group of five animals, who received 6000 ppm in the diet for 14 days followed by a 28 day recovery period.

There were no clinical signs of toxicity reported. Food consumption was reduced in all test groups with the exception of the 6000 ppm dose group which had a reduced food intake on day 1 and then an almost 3-fold increase over the control group. Group mean body weight was not significantly changed except in the 6000 ppm group which was slightly decreased (4-8%) over

the first 8 days of the study. At the end of the study, rats receiving 6000 ppm showed a significant increased absolute and relative liver weight.

Significant effects were observed for several biochemical parameters in the liver at the 6000 ppm dose level. Cytochrome P-450 content was approximately doubled in the high dose group, while activity of glutathione-S-transferase was increased by 65%. Other enzymes affected at the 6000 ppm dose level included an increase in lauric acid hydroxylation and an increase in PROD and EROD activity. In general, those animals allowed to recover for 28 days following the 14-day administration of test chemical showed no significant induction or inhibition of enzyme activities.

Liver Biochemical Induction (Hamster)

In a liver biochemical induction study (MRID 44389706), triclosan (purity 99.5%) was administered to 4 groups of young adult male and female Syrian Hamsters (five/sex/group) in a pelleted standard hamster diet (Nafag 924) at concentrations of 0, 700, 5000, and 15000ppm [approximately 0, 49.9, 309.8, 799.0 mg/kg/day (males) and 46, 314.3, 958.8 mg/kg/day (females)] for 14 days. Separate recovery groups of five males and five females received either 0 or 15000 ppm triclosan in the diet for 14 days followed by a 28 day recovery period.

Significant treatment-related effects were observed in male and female hamsters at the 5000 (309.8 mg/kg/day in males, 314.3 mg/kg/day in females) and 15000 ppm (799 mg/kg/day in males, 958.8 mg/kg/day in females) treatment levels. At 5000 ppm triclosan, significant induction of total hepatic microsomal cytochrome P-450 and activities of ethoxyresorufin-o-deethylase (EROD) and pentoxyresorufin-o-dependentylase (PROD) was observed, as was an increase in Mab clo4 immunoreactive protein in male hamster liver. At 15000 ppm, the above effects were also observed, and in addition, abnormal histopathology of the kidneys in females (randomly distributed spots or white patches of white pigmentation on the surface of the kidney) was observed after 14 days of treatment. Total activity towards testosterone was not affected by triclosan feeding in the diet, but specific hydroxylation reactions were affected. Of note in males, formation of androstenedione was increased in a dose-related manner, as was the formation of the 16-β metabolite. A noticeable dose-response was observed only for androstenedione formation, however.

In female hamsters, a dose-related increase in formation of both the 7-∝ and 15-∝ hydroxyl metabolites was noted as a result of triclosan treatment (formation of the 7-∝ metabolite: activities of 22.16, 35.74, 39.33, and 46.66 nmol/min/g at the 0,700, 5000, and 15000 ppm dose levels, respectively; formation of the 15-∝ metabolite: 8.61, 12.78, 18.29, and 29.87 nmol/min/g at 0, 700, 5000, and 15000 ppm triclosan, respectively). Androstenedione formation was also slightly increased with dose of triclosan, with a doubling of activity at the high dose (42.72 nmol/min/g) in relation to control activity (21.14 nmol/min/g).

Significant treatment-related increases in lauric acid hydroxylation were observed in male and female hamsters at 15000 ppm triclosan, as were significant decreases in activity of cytosolic glutathione-S- transferase and increases in bilirubin and morphine glucuronyltransferase activity.

Total immunoreactive protein towards the Mab clo4 antibody (indicative of induction of CYP4A P-450, a peroxisome proliferator inducible form) was observed in male and female hamsters at 15000 ppm. Together with the data presented on the effects of the model inducers phenobarbital, 3-methylcholanthrene, nafenopin, and pregnenolone 16 ∝ -carbonitrile, the data suggest that triclosan acts as a peroxisome proliferator, as observed in other work with rats and mice. Hamsters, however, appear less sensitive to triclosan treatment relative to rats and mice.

Based on the results of this study, a Systemic NOAEL of 700 ppm can be established, with a Systemic LOAEL of 5000 ppm, based on induction of total cytochrome P-450, EROD, and PROD in male and female hamsters, and induction of Mab clo4 immunoreactive protein (CYP4A peroxisome proliferator inducible P-450) in male hamsters).

This study is classified as **Acceptable/Non-guideline**. The study provides important information on the mechanistic basis of triclosan induced liver toxicity, and also provides information on the relative sensitivity of the hamster to the hepatic effects of triclosan.

Liver Cell Proliferation

A cell proliferation study (MRID 44389701) was conducted as supplemental to a previously reviewed subchronic feeding study in mice (MRID 43022605) to determine whether cell proliferation was induced in the liver of male and female mice after 45 and 90 days' dietary administration of triclosan (% a.i. not stated). Liver tissue from mice receiving 0, 25, 75, 200, 350, or 900 mg/kg/day was obtained--as formalin-fixed wet tissue. Slides prepared from paraffin embedded tissue were stained with hematoxylin and eosin for histopathology evaluation, or stained for proliferating cell nuclear antigen (PCNA) using immunohistochemical methods.

Positive staining for PCNA was identified by uniform dark red nuclear staining of hepatocytes in the S-phase of the cell cycle. Homogeneity of a cell proliferative response was evaluated by perusing liver sections from each individual animal, and found to be similar among the lobes examined from each animal. At least 1000 hepatocellular nuclei were counted in six fields using a 20X objective and an eyepiece containing a 10 x 10 mm grid. Labeling index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of nuclei counted, and expressing the result as a percentage.

Histopathological evaluations are presented below. Severity of lesions was graded from 0 to 4 with 4 being most severe.

GROUP	Lipofuscin	Hepatocyte Hypertrophy	Bile Retention	Necrosis	Lipid Vacuolization	Biliary Hyperplasia
01 MM	0	0	0	0	0	0
02 MM	0	0	0	0	0	0
03 MM	0.2	1	0.2	0.2	0.2	0.2
04 MM	1	1	0.8	0.6	0.4	0.8
05 MM	1	1	2	1.2	0.6	0.4

GROUP	Lipofuscin	Hepatocyte Hypertrophy	Bile Retention	Necrosis	Lipid Vacuolization	Biliary Hyperplasia
07 MM	1.4	2	1.2	1.8	0.8	1
08 MM	0	0	0	0	0	0
09 MM	0	1.6	0	0	0	0
10 MM	1.2	3.6	0.4	1.2	0.4	0.8
11 MM	2.2	3.4	1.2	3	0.8	2
01 FM	0.2	0	0	0	0	0
02 FM	0	0	0	0	0	0
03 FM	0	0.4	0	0	0	0
04 FM	0.2	0.8	1	1.2	0.4	0.6
O5 FM	1.4	1	1.4	0.6	0.4	1
07 FM	1	1.4	1	1.6	0.8	1.6
08 FM	0	0	0	0	0	0
09 FM	0	1	0	0	0	0
1O FM	1	3	0.4	1	0	0.8
11 FM	1.8	3.4	1	2.4	1.2	2.2

MM = male mice; FM = female mice. **Dose groups:** (male and female 90 day dose groups): **01**, 0 mg/kg/day; **02**; 25 mg/kg/day; **03**, 75 mg/kg/day; **04**, 200 mg/kg/day; **05**, 350 mg/kg/day; **07**, 900 mg/kg/day. **Dose groups** (male and female 45 day dose groups): **08**, 0 mg/kg/day; **09**, 25 mg/kg/day; **10**, 350 mg/kg/day; **11**, 900 mg/kg/day.

At the 45 day time point (dose groups 08 through 11), hepatocellular hypertrophy was the most consistent and prominent observation. This lesion could be observed in male and female mice at 25 mg/kg/day (dose group 09), and the severity generally increased with increasing dose. At the higher dose levels of 350 and 900 mg/kg/day, necrosis of hepatocytes was observed, usually in large areas surrounded by proliferating bile duct epithelial cells, fibroblasts, and/or Kupffer cells. Along with the "fibrosis" were macrophages with yellow-brown pigment compatible with lipofuscin or ceroid, breakdown products of cellular organelles. Bile stasis between hepatocytes, in the canaliculi of the liver lobules, was, according to the report, not a prominent feature at 45 days, but was more prominent at 90 days. As noted above, male mice were scored higher for severity of this lesion than female mice at the 350 and 900 mg/kg/day dose levels at 90 days.

At the 90 day time point, similar liver lesions were observed. Hepatocellular hypertrophy was observed at 75 mg/kg/day and above in male and female mice. With increasing dose, severity of this lesion also increased. In addition to hypertrophy, necrosis of hepatocytes was observed at 75 mg/kg/day and above in male mice and at 200 mg/kg/day and above in female mice, again with a dose- related increase in severity. The report stated that with increasing dose, necrosis became more severe, involving groups of cells, and in the most severe cases, involved all cells of individual lobules (panlobular). Hepatocytes were also swollen and many had cytoplasmic yellow-brown granules at the periphery, near bile canaliculi. This lesions was classified as bile stasis.

For many of the pathological changes in the liver, male and female mice at the top dose showed higher severity scores at 45 days than 90 days, with the possible exception of bile retention, which appeared to increase with time.

Results of cell proliferation experiments are shown below:

GROUP	Mean Labeling Index	SEM	Fold Increase Over Control	GROUP	Mean Labeling Index	SEM	Fold Increase Over Control
01 MM	0.035	0.016		**01 FM**	0.042	0.036	
02 MM	0.008	0.008	0.0	**02 FM**	0.046	0.015	1.1
03 MM	0.167	0.082	4.8	**03 FM**	0.065	0.009	1.5
04 MM	0.124	0.031	3.5	**04 FM**	0.140	0.050	3.3
05 MM	0.398	0.063	11.0	**05 FM**	0.256	0.218	6.1
07 MM	0.536	0.195	15.0	**07 FM**	0.300	0.060	7.1
08 MM	0.090	0.018		**08 FM**	0.058	0.029	
09 MM	0.112	0.089	1.2	**09 FM**	0.064	0.021	1.1
10 MM	0.292	0.096	3.2	**10 FM**	0.242	0.072	4.2
11 MM	0.726	0.218	8.0	**11 FM**	0.380	0.080	6.6

MM = male mice; FM = female mice. **Dose groups** (male and female 90 day dose groups: **01**, 0 mg/kg/day; **02**, 25 mg/kg/day; **03**, 75 mg/kg/day; **04**, 200 mg/kg/day; **05**, 350 mg/kg/day; **07**, 900 mg/kg/day. **Dose groups** (male and female 45 day dose groups: **08**, 0 mg/kg/day; **09**, 25 mg/kg/day; **10**, 350 mg/kg/day; **11**, 900 mg/kg/day.

According to the report, cell proliferation was significantly increased over control in male mouse liver at 200 mg/kg/day and higher, and the increase was sustained from 45 to 90 days. The reviewer would agree with the sustained increase, but it appears that cell proliferation (as judged by labeling index and fold increase over control) is also increased significantly at the 75 mg/kg/day dose level for male mice. This result is consistent with the apparent differences in sensitivity to the hepatic effects of triclosan between male and female mice, as cell proliferation in female mice was not affected at 75 mg/kg/day, but was increased at 200 mg/kg/day, consistent with the observed difference in liver histopathology.

According to the report, the distribution of hepatocellular labeling was panlobular in both sexes. The results of this study support a mode of action consistent with cellular regeneration as a result of hepatocellular cytotoxicity. The **25 mg/kg/day** dose level was identified as the **NOAEL** for **male** mice in this study, while the **75 mg/kg/day** dose level was considered the **NOAEL** for **female** mice by the authors. The reviewer agrees with this interpretation, as liver responses in female mice, while evident at 75 mg/kg/day, were not significant enough to support a true effect level. Males, by contrast, did show hepatic responses at the 75 mg/kg/day dose which would support an effect level.

This study is classified as **Acceptable/Non-guideline**.

Liver Cell Proliferation

A cell proliferation study (Eldridge, 1995; MRID 44389701) was conducted to examine whether cell proliferation was induced in male and female mice which had been the subject of an earlier subchronic toxicity study in mice exposed to dietary triclosan, % a.i. not stated at dose levels of 0, 25, 75, 200, 350, 750, or 900 mg/kg/day for 7 or 13 weeks (MRID # 430026-05).

Formalin-fixed tissue was obtained from the 0, 25, 75, 200,350, and 900 mg/kg/day dose groups from the 90 day time point, and tissue was also obtained from the 0, 25, 350, and 900 mg/kg/day dose at the 45 day time point for cell proliferation analysis. Tissue slides were stained for proliferating cell nuclear antigen using immunohistochemical methods.

Positive staining for PCNA was identified by uniform dark staining of hepatocytes in the S-phase of the cell cycle. Homogeneity of a cell proliferative response was evaluated by examining liver sections from each individual animal and was found to be similar among the lobes examined, although which lobes were examined was not detailed in this report. A labeling index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of nuclei counted. Student's t-test for the inequality of unpaired data sets was used to determine significant differences in labeling index between controls and treated groups.
The results of this study are shown below. It is noted that the study report itself appeared to be an abbreviated version with no detail on procedures for cell proliferation analyses.

Cell Proliferation in Male and Female Mice Administered Dietary Triclosan for 90 Days

Group (males, mg/kg/day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg/day)	Mean Labeling Index	SEM	Fold Increase Over Controls
0	0.035	0.016	-	**0**	0.042	0.036	-
25	0.008	0.008	0.0	**25**	0.046	0.015	1.1
75	0.167	0.082	4.8	**75**	0.065	0.009	1.5
200	0.124	0.031	3.5	**200**	0.140	0.050	3.3
350	0.398	0.063	11.0	**350**	0.256	0.218	6.1
900	0.536	0.195	15.0	**900**	0.300	0.060	7.1

Cell Proliferation in Male and Female Mice Administered Triclosan in the Diet for 45 Days

Group (males, mg/kg/day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg/day)	Mean Labeling Index	SEM	Fold Increase Over Controls

0	0.090	0.018	-	**0**	0.058	0.029	-
25	0.112	0.089	1.2	**25**	0.064	0.021	1.1
350	0.292	0.096	3.2	**350**	0.242	0.072	4.2
900	0.726	0.218	8.0	**900**	0.380	0.080	6.6

[a]Data obtained from Table III of the report (no page number). N = 5 except for control at 90 days, where N = 7 for males and N = 5 for females.

Although it appears that not all of the animals from the subchronic toxicity study were evaluated (in the subchronic study, groups of 10-20 mice/sex/dose were used), an increase in the labeling index was apparent at 200 mg/kg/day and above for male and female mice. The labeling index was increased at day 45 in both sexes at 350 and 900 mg/kg/day, and as noted, this continued at these dose levels at 90 days as well as the increase observed at 200 mg/kg/day at 90 days for both sexes. The observation of an increase in labeling index from this study, in conjunction with other data which show toxicity to the liver of rats and mice, indicate cytolethality of triclosan which is followed by induced cellular regeneration. However, there may be species differences in the response to triclosan hepatotoxicity. Hepatic necrosis was observed in a two-year rat chronic toxicity/carcinogenicity study at 300 ppm, 1000 ppm, and 3000 ppm, but there was no significant increase in tumor incidence. The mouse also shows evidence of hepatic necrosis, but individual animal tumor data are not available to make a comparison to the rat.

In the report, it was noted that the mode by which a chemical induces cell proliferation is an important consideration. In the case of triclosan, the evidence suggests a hepatotoxic effect followed by regenerative cell turnover, in contrast to agents which act as direct mitogens. For chemicals producing increased cell turnover through cytolethality, a threshold can be inferred below which these effects would not occur. This scenario could apply to triclosan based on the available data.

This study is classified as **Acceptable/Non-Guideline**

5.0 TOXICITY ENDPOINT SELECTION

5.1 See Section 7.1, Summary of Toxicological Doses and Endpoint Selection, Table 3.

5.2 Dermal Absorption

An older rabbit dermal absorption study (MRID 34335) was available from the one-liner database (HED document # 001958. In this study, up to 48% of an applied dermal dose of 0.89 mg triclosan was absorbed. In addition, literature data available on dermal absorption in the mouse show dermal absorption up to 70%. These data are in agreement with the estimate of dermal absorption of 50% derived from comparison of the LOAEL's from a rat 90-day dermal

toxicity study (MRID # 43328001) and a rat 2-generation reproduction study (MRID # 40623701).

In vitro dermal absorption studies using human skin preparations and various formulations containing triclosan (MRIDs 47261408 through 47261411) showed dermal absorption values for triclosan ranging from 11-20% in these formulations. A paper published in 2000 by Moss et al. (Food and Chemical Toxicology, Volume 38, pages 361-370) examined dermal absorption of triclosan both in vivo and in vitro using rats as well as an in vitro human skin study. These data supported the conclusion of dermal absorption of 21-23% in the rat studies, and showed in vitro dermal absorption through human skin in vitro of 6.3%. Several human *in vivo* dermal absorption studies were cited in the 2007 CANTOX report on the Toxicological Evaluation of Triclosan that also suggest a lower value for dermal absorption than 50%, but none of these studies have been reviewed by the Agency's Human Studies Review Board for scientific and ethical conduct and are thus not cited in this risk assessment. Taken together, the available data on dermal absorption suggest a lower value, around 20% for rat skin and possibly lower for human skin. Additional verification is needed.

5.3 Classification of Carcinogenic Potential

On March 10, 1998, the Health Effects Division's HIARC committee examined the available carcinogenicity data for triclosan and was unable to assign a classification to triclosan at that time since data for only one species (rat) were submitted for evaluation of carcinogenicity. Since this determination, a chronic toxicity/carcinogenicity study in the hamster (MRID 44874001) and a carcinogenicity study in the mouse reviewed by the Food and Drug Administration were submitted and/or obtained by the Agency. The Agency was not able to obtain the individual animal data records for the mouse carcinogenicity study but was able to obtain the FDA's review and Expert Panel reports on the significance of the mouse study results. On July 25, 2007, the Health Effects Division's Carcinogenicity Assessment Review Committee met to discuss the additional data submitted as well as the biochemical studies conducted with triclosan in support of a mode of action involving peroxisome proliferation as a causative factor in the positive tumorigenic results observed in the mouse carcinogenicity study.
The overall weight of the evidence supports activation of peroxisome proliferator-activated receptor alpha (PPARά) as the mode of action of triclosan-induced hepatocarcinogenesis in mice. Key precursor events and the tumor response in mice were concordant with respect to both time and dose. The data did not support either a mutagenic mode of action or a mode of action involving cytotoxicity followed by regenerative proliferation as alternative modes of action. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans, it is quantitatively implausible and unlikely to take place in humans based on quantitative species differences in PPARά activation and toxicokinetic differences between the mouse and human.

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC classified triclosan as "Not Likely to be Carcinogenic to Humans". This decision is based on the weight-of-evidence that supports activation of peroxisome proliferator-activated receptor alpha (PPARά) as the mode of action for triclosan-induced hepatocarcinogenesis in mice. The data did not support either mutagenesis or cytotoxicity followed by regenerative proliferation as alternative modes of action. While the proposed mode of action for liver tumors in mice is theoretically plausible in humans, hepatocarcinogenesis by this mode of action is quantitatively implausible and unlikely to take place in humans based on quantitative species differences in PPARά activation and toxicokinetics. The quantification of risk is not required.

6.0 FQPA CONSIDERATIONS

6.1 Reproductive Toxicity Study Conclusions

In a 2-generation reproduction study (MRID # 40623701), triclosan was administered to 25 rats/sex/dose at dietary levels of 300, 1000, and 3000 ppm (nominal doses of 15, 50, and 150 mg/kg/day). Significant body weight reduction was observed in adult rats at the high dose during weeks 0-12, gestation, and lactation. The Systemic NOAEL = 1000 ppm, and the Systemic LOAEL = 3000 ppm, based on reduced mean body weight. Body weights in high dose F1 pups were significantly lover on days 14 and 21 of lactation. F2 pups displayed significantly lower body weights at birth which did not persist at day 4 of lactation or greater. Viability index was decreased at the high dose in both generations of pups and the weaning index was slightly lower in high dose F2 pups vs control. The Reproductive NOAEL = 1000 ppm, and the Reproductive LOAEL = 3000 ppm, based on reduced pup weights and equivocal reduced pup viability in both generations.

6.2 Pre-and/or Postnatal Toxicity

In a developmental toxicity study in rabbits, triclosan (100% a.i.) was administered by gavage to pregnant female New Zealand White rabbits (18/group) on gestation days 6-18 at dose levels of 15, 50, or 150 mg/kg/day. Rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. On day 30 of gestation, rabbits were sacrificed and necropsied; gravid uterine weights were recorded. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external, visceral and skeletal anomalies. They were then examined by the Staple's dissection procedure. Evidence of treatment-related toxicity at the high

dose (150 mg/kg/day) consisted of reduced body weight gain and food consumption over the period of treatment. The Maternal NOAEL = 150 mg/kg/day, based on decreased body weight gain and food consumption during treatment. The Maternal NOAEL = 50 mg/kg/day. No developmental toxicity was observed under the conditions of this study. The Developmental LOAEL = not determined; the developmental NOAEL = 150 mg/kg/day.

Triclosan was administered by gavage to pregnant female Wistar rats (30 rats/group, 60/group in control) on days 6-154 gestation at dose levels of 30, 100, or 300 mg/kg/day. At 300 mg/kg/day, maternal toxicity was evident and consisted of transient diarrhea, decreased body weight gain during treatment, and reduced food consumption and increased water consumption from onset of treatment through gestation. Based on these findings, the Maternal NOAEL = 100 mg/kg/day, and the Maternal LOAEL = 300 mg/kg/day. There was no evidence of pre- or post-natal developmental toxicity at any dose level in this study. The Developmental LOAEL = not determined (> 300 mg/kg/day); the Developmental NOAEL $\geq$ 300 mg/kg/day.

A. Determination of Susceptibility

The data base is complete and there are no data gaps pertaining to developmental or reproductive toxicity. The data provided no indication of increased sensitivity of rats or rabbits to *in utero* and post-natal exposure to triclosan. Two prenatal developmental toxicity studies, one in rats and one in rabbits, failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels which resulted in evidence of parental toxicity.

B. Proposed Hazard-based Special FQPA Safety Factor(s):

The hazard-based FQPA factor should be removed because:

(I) The data provided no indication of increased susceptibility of rats or rabbits to *in utero* and/or postnatal exposure to triclosan.

(ii) No evidence of developmental anomalies, including abnormalities in the development of the fetal nervous system, were observed in the pre- and/or post-natal studies.

(iii) Although the toxicology data base is not complete (see below), there are no data gaps for evaluation of increased susceptibility to infants and children.

6.3 Recommendation for a Developmental Neurotoxicity Study

The committee considered the available data on triclosan for evaluation of neurotoxicity, including the 14-day neurotoxicity study in rats, developmental and reproductive toxicity

studies in rats and rabbits, and subchronic and chronic data in rats and mice. There was no evidence of a neurotoxic effect of triclosan in any of these studies. **Thus, the committee did not recommend a developmental neurotoxicity study for triclosan.**

7.0 SUMMARY OF TOXICOLOGICAL DOSES AND ENDPOINTS FOR TRICLOSAN FOR USE IN HUMAN RISK

7.1 Summary Table of Toxicological Dose and Endpoint Selection (Table 2)

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (gen. pop.)	NOAEL = 30 mg/kg UF = 100	FQPA SF = 1x	Chronic Toxicity study in Baboons MRID 133230
Acute Dietary (females 13+)	Endpoint not identified in the database		
Chronic Dietary (all populations)	NOAEL = 30 mg/kg UF = 100	FQPA SF = 1x	Chronic Toxicity study in Baboons MRID 133230 LOAEL = 100 mg/kg/day, based on clinical signs of toxicity
Short-Term/ Intermediate-Term Incidental Oral (1-30 days; 30 days- 6 months)	NOAEL = 30 mg/kg UF = 100	FQPA SF = 1x	Chronic Toxicity study in Baboons MRID 133230 LOAEL = 100 mg/kg/day, based on clinical signs of toxicity
Dermal (short-term)	NOAEL = 0.6 mg/animal (100 $\mu g/cm^2$)	MOE = 10	14-day dermal toxicity study in the mouse MRID 44389708 LOAEL = 1.5 mg/kg/day, based on treatment-related dermal irritation at the treatment site and on increased liver weights
Dermal (intermediate term)	NOAEL = 40 mg/kg	MOE = 100	90-day Dermal Toxicity in Rats MRID 43328001 LOAEL = 80 mg/kg/day, based on increased incidence occult blood in the urine.

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal (long-term)	NOAEL = 40 mg/kg	MOE = 100	90-day Dermal Toxicity in Rats MRID 43328001 LOAEL = 80 mg/kg/day, based on increased incidence occult blood in the urine.
Inhalation (all durations)	LOAEL = 3.21 mg/kg/ay	MOE = 1000	21-Day Inhalation Toxicity study in the rat MRID 0087996 LOAEL = 3.21 mg/kg/day [males], based on increased total leucocyte count and increased serum alkaline phosphatase
Cancer (oral)	Not likely to be carcinogenic in humans.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

NOTE: The Special FQPA Safety Factor recommended by the ADTC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

8.0 TOXICITY PROFILE TABLES

8.1 Acute Toxicity Profile Table - (See Section 4.1, Acute Toxicity, Table 1).

8.2 Subchronic, Chronic and Other Toxicity Profiles Table (Table 3)

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.3100 (§82-1) 90-day Oral - Rat Triclosan Purity: Not Reported	133545 **Acceptable/Guideline** 0, 1000, 3000, and 6000 ppm	NOAEL: 1000 ppm The test compound caused a nonspecific dose-related liver toxicity at 3000 and 6000 ppm
870.3100 (§82-1) 90-day Oral - Rat Triclosan Purity: 99.7%	43022605 **Acceptable/Guideline** 0, 25, 75, 200, 350, 750, and 900 mg/kg/day	LOAEL = 25 mg/kg/day based on the changes in clinical chemistry and hematology parameters as well as lesions in the liver. NOAEL = Not Determined
870.3100 (§82-1) 28-day Oral - Mouse Triclosan Purity: >99%	44389707 **Acceptable - Guideline** 0, 6.48, and 135.59 mg/kg/day (M), 0, 8.25, and 168.78 mg/kg/day (F)	**Oral Toxicity** LOAEL (M): 135.59 mg/kg/day LOAEL (F): 168.78 mg/kg/day (biochemical and morphological effects on the liver) NOAEL(M): 6.48 mg/kg/day NOAEL (F): 8.25 mg/kg/day
870.3150 (§82-1) Oral Subchronic (non-rodent) Triclosan Purity: Not Reported	96102 **Acceptable-Guideline** 0, 12.5, 25, 50, and 100 mg/kg/day	**Systemic Toxicity** NOAEL: 12.5 mg/kg/day LOAEL: 25 mg/kg/day based on histopathologic changes in the liver of treated dogs
870.3250 (§82-2) 90-day Dermal - Rat Triclosan Purity: 99.7%	43328001 **Acceptable/Guideline** 0, 10, 40, or 80 mg/kg/day	**Systemic Toxicity** NOAEL: 40 mg/kg/day (excluding dermal findings) LOAEL: 80 mg/kg/day
Other 14-day Repeated Dose Dermal Toxicity - Mouse Triclosan Purity: 99.3%	44389708 **Acceptable - Non-Guideline** 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/day	LOAEL: 1.5 mg/animal/day based on treatment-related dermal irritation at the treatment site and on increased liver weights in this treatment group. NOAEL: 0.6 mg/animal/day
Other 14-day Repeated Dose Dermal Toxicity - Rat Triclosan Purity: 99.3%	44389710 **Acceptable - Non-Guideline** 0, 0.3, 0.6, 1.5, 3.0, or 6.0 mg/animal/day	LOAEL: 6.0 mg/animal/day based on treatment-related dermal irritation at the treatment site NOAEL: 3.0 mg/animal/day

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.3700a (§83-3) Developmental – Rat (oral) Triclosan Purity: 99.8%	43817502, 43717503 **Acceptable - Guideline** 30, 100, or 300 mg/kg/day	**Maternal:** LOAEL: 300 mg/kg/day NOAEL: 100 mg/kg/day At 300 mg/kg/day, transient diarrhea, retarded body weight gain during the period of treatment, and reduced food consumption and increased water consumption from the onset of treatment, throughout the gestation period. **Developmental:** LOAEL: Not Determined (> 300 mg/kg/day) NOAEL: ≥300 mg/kg/day No evidence of pre- or postnatal developmental toxicity
870.3700a (§83-3) Developmental - Rabbit (oral) Triclosan Purity: 99.8%	43820401, 43022607 **Acceptable- Guideline** 15, 50, or 150 mg/kg/day	**Maternal:** LOAEL: 150 mg/kg/day NOAEL: 50 mg/kg/day **Developmental:** LOAEL: Not Determined (>150 mg/kg/day) NOAEL: ≥ 150 mg/kg/day
870.3800 (§83-3) Reproduction - Rat (oral) Triclosan Purity: ≥99%	40623701 **Supplementary** 0, 300, 1000, and 3000 ppm	**Reproductive:** NOAEL: 1000 ppm LOAEL: 3000 ppm for decreased viability **Systemic Parental:** NOAEL: 1000 ppm LOAEL: 3000 ppm for a decrease in body weights and food consumption at various points during premating, gestation, and lactation for both F0 and F1 parents **Developmental:** NOAEL: 1000 ppm LOAEL: 3000 ppm for decreased body weight and increased mortality
870.4300 (§83-3) Chronic Toxicity - Baboon (oral) Triclosan Purity: Not Reported	133230 **Acceptable/Guideline** 30, 100, and 300 mg/kg/day	NOAEL: 30 mg/kg/day LOAEL: 100 mg/kg/day based on clinical signs of toxicity.

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.4300 (§83-3) Chronic/Oncogenicity - Rat Triclosan Purity: 99%	42027906 **Supplementary** 2-year study: 0, 300, 1000, or 3000 ppm (0, 15.3, 52.4, and 168.0 mg/kg/day in males; 0, 20.0, 66.9, and 217.4 mg/kg/day in females) 52 week study: 6000 ppm.	**Systemic Toxicity:** NOAEL: 52.4 mg/kg/day based on the increase in non-neoplastic liver pathology observed in male rats at the 168.0 mg/kg/day dose. LOAEL: 15.3 mg/kg/day based on the histopathological incidence of hepatic necrosis
870.4200 (§83-3) Carcinogenicity- Mouse Triclosan Purity: 99%	See, Norman A. (1996) **FDA Review** 0, 10, 30, 100, or 200 mg/kg/day	NOAEL: 10 mg/kg/day in regard to tumorigenicity (and all other effects with the exception of reduced plasma cholesterol)
870.4300 (§83-3) Chronic/Oncogenicity - Hamster Triclosan Purity: 99.5%	44874001, 44751101 **Acceptable/Guideline** 0, 12.5, 75, and 250 mg/kg/day	NOAEL: 75 mg/kg/day LOAEL: 250 mg/kg/day for increased mortality (males), nephropathy, histopathologic findings in the stomach and testes and general clinical condition deterioration - lethargy, hunched posture, pallor, thin appearance, unsteady gait
870.5100 (§84-2) Bacterial Reverse Mutation Test Triclosan Purity: ≥99%	43533301 **Acceptable/Guideline** 0.015, 0.050, 0.15, 0.5, or 1.5 ug/plate	**Negative** Triclosan was cytotoxic at 1.5 ug/plate without S9 and at doses of ≥ 0.5 ug/plate with S9. No mutagenic response was seen at any dose levels with or without S9.
870.5100 (§84-2) Bacterial Reverse Mutation Test Triclosan Purity: 100.5%	44389705 **Acceptable/Guideline** TA100 and TA1538 were exposed to 0.005-5,000 ug/plate (-S9) and 0.005-50 ug/plate (±S9). Strains TA98, TA100, TA1535, TA1537, and TA2538, were evaluated for mutagenicity at 0.05 - 5.0 ug/plate (+S9) and all except TA100 at 0.00167-0.167 ug/plate (-S9). Without S9, TA100 was evaluated for mutagenicity at 0.00167-0.167 ug/plate.	**Negative** There were no reproducible, dose-related differences in the number of revertant colonies in any tester strain at any dose level/condition compared to the vehicle controls.
870.5300 (§84-2) In Vitro Mammalian Cell Gene Mutation Test Triclosan Purity: > 99%	44389704 **Acceptable/Guideline** 1 to 25 ug/ml (-S9) and from 1 to 20 ug/mL (+S9)	**Negative** Triclosan was negative for inducing forward mutations at the TK locus in mouse L5178Y cells both with and without metabolic activation.

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.5375 (§84-2) In Vitro Mammalian Chromosome Aberration Test Triclosan Purity: > 99%	47276601 **Acceptable/Guideline** 0 (DMSO), 0.1, 0.3, 0.5, and 1.0 ug/ml (+S9) and 0, 4.8, 9.5, 19, 30, and 38 ug/ml (-S9)	**Negative** There was no treatment-related increase in clastogenicity at any dose.
870.5375 (§84-2) In Vitro Mammalian Chromosome Aberration Test Triclosan Purity: 99% - 100%	43740801 **Acceptable/Guideline** Nonactivated doses of 1 ug/mL (7-hour cell harvest), 0.1-3 ug/ml (18-hour harvest), or 3 ug/ml (28-hour harvest) and S9-activated concentrations of 3 ug/ml (7- and 28-hour cell harvests) or 0.1-3 ug/ml (18-hour harvest).	**Positive** Nonactivated FAT 80'023/Q at 1 and 3 ug/mL (18-hour harvest) induced a dose-related increase in the yield of cells with abnormal chromosome morphology. The response was significant ($p \leq 0.001$) at the higher concentration. A significant increase ($p \leq 0.001$ was also seen at 3 ug/ml (28-hour harvest). The most frequently observed type of chromosome damage was exchange figures. In the presence of S9 activation, nonsignificant but concentration dependent increases in cells bearing exchange figures were also seen at 1 and 3 ug/ml (18-hour harvest). The data are, therefore, sufficient to conclude that FAT 80'023/Q is active in this test system.
870.5385 Mammalian Bone Marrow Chromosomal Aberration Test Triclosan Purity: 99% - 100%	43740802 **Acceptable/Guideline** 4000 mg/kg	**Negative** No signs of overt toxicity or cytotoxicity effects on the target organ were seen in any treatment group. There was also no indication of a clastogenic effect at any sacrifice time.
870.5550 Unscheduled DNA Synthesis in Mammalian Cells in Culture Triclosan Purity: 100.5% a.i.	47276602 **Acceptable/Guideline** 0.25, 0.5, 1.0, and 2.5 µg/mL	**Negative** There was no UDS in cultured rat hepatocytes at the concentrations tested. The positive controls functioned as expected.

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.7485 General Metabolism Triclosan Purity: 99% a.i.	45307501, 45307502 **Acceptable/Guideline** 2 or 200 mg/kg	After single or repeated oral doses of 2.0 or 200 mg/kg ^{14}C-triclosan, urine was the major route of elimination for triclosan radioactivity (60-80% of the administered radioactivity). Fecal elimination represented from 12-35% of administered radioactivity across the oral dose groups. Compared to the low dose, administration of a single high or repeated dose resulted in a shift toward urinary elimination and a decrease in fecal elimination. Peak plasma and blood concentrations of triclosan-derived radioactivity occurred at one hour post-dose for both the low and high oral doses. Concentration in plasma appeared higher than in whole blood at each sampling time. There did not appear to be any significant sex differences in blood or plasma kinetics at the low or high dose. The major urinary metabolite detected after oral administration was the glucuronide conjugate of triclosan. The major fecal metabolite was parent triclosan in all oral dose groups (1-8). After intravenous administration, triclosan glucuronide was also detected as the major urinary metabolite. Tissue metabolite analysis showed that the glucuronide and sulfate conjugates of triclosan were the major metabolites detected at the low and high oral single doses.
870.7485 General Metabolism Triclosan Purity: 99% a.i.	45307503 **Acceptable/Guideline** 2 or 200 mg/kg	The test material was rapidly absorbed following oral administration, and was eliminated primarily through the feces, via biliary excretion. Urinary excretion was secondary to that in the gastrointestinal tract. This excretory pattern was consistent following I.V. administration as well. Primary excreted compounds in the urine following single oral exposures included the unmetabolized parent compound and two parent conjugates (parent sulfate and parent glucuronide); fecal excretion was primarily that of the free parent compound, as small amounts of glucuronide were detected, and no sulfate was detected

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
870.7600 Dermal Absorption rabbit Triclosan Concentration: 3%	34335	48% of the applied dermal dose was absorbed
Liver Biochemical Induction – Mouse (oral) Triclosan Purity: Not Reported	44389702 0, 25, 75, 350, and 900 mg/kg bw for M and 0, 25, 350, and 900 mg/kg bw for F	The test article is a strong, but reversible barbiturate-type and peroxisome proliferator-type inducer of foreign compound metabolizing enzymes in male and female mice
Liver Biochemical Induction - Rat Triclosan Purity: Not Reported	44389703 0, 300, 1500, and 6000 ppm.	Food consumption was reduced in all test groups with the exception of the 6000 ppm dose group which had a reduced food intake on day 1 and then an almost 3-fold increase over the control group. Group mean body weight was not significantly changed except in the 6000 ppm group which was slightly decreased (4-8%) over the first 8 days of the study. At the end of the study, rats receiving 6000 ppm showed a significant increased absolute and relative liver weight. Significant effects were observed for several biochemical parameters in the liver at the 6000 ppm dose level. Cytochrome P-450 content was approximately doubled in the high dose group, while activity of glutathione-S-transferase was increased by 65%. Other enzymes affected at the 6000 ppm dose level included an increase in lauric acid hydroxylation and an increase in PROD and EROD activity. In general, those animals allowed to recover for 28 days following the 14-day administration of test chemical showed no significant induction or inhibition of enzyme activities.
Liver Biochemical Induction - Hamster Triclosan Purity: 99.5%	44389706 **Acceptable/Non-Guideline** 0, 700, 5,000, and 15,000 ppm [approximately 0, 49.9, 309.8, 799.0 mg/kg/day (M) and 46, 314.3, and 958.8 mg/kg/day (F)] or 0 or 15000 ppm (recovery groups)	**Systemic Toxicity** NOEL: 700 ppm LOEL: 5000 ppm based on induction of total cytochrome P-450, EROD, and PROD in male and female hamsters, and induction of Mab clo4 immunoreactive protein (CYP4A peroxisome proliferators inducible P-450) in male hamsters.
Liver Cell Proliferation - Mouse Triclosan Purity: Not Reported	44389701 **Acceptable/Guideline** 0, 25, 75, 200, 350, or 900 mg/kg/day	NOAEL(M): 25 mg/kg/day NOAEL(F): 75 mg/kg/day

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/ Classification/ Doses	Results
Liver Cell Proliferation - Mouse Triclosan Purity: Not Reported	Eldridge, 1995 44389701 **Acceptable/Guideline** 0, 25, 75, 200, 350, 750, or 900 mg/kg/day	An increase in the labeling index was apparent at 200 mg/kg/day and above for male and female mice. The labeling index was increased at day 45 in both sexes at 350 and 900 mg/kg/day, and as noted, this continued at these dose levels at 90 days as well as the increase observed at 200 mg/kg/day at 90 days for both sexes. The observation of an increase in labeling index from this study, in conjunction with other data which show toxicity to the liver of rats and mice, indicate cytolethality of triclosan which is followed by induced cellular regeneration. However, there may be species differences in the response to triclosan hepatotoxicity. In the report, it was noted that the mode by which a chemical induces cell proliferation is an important consideration. In the case of triclosan, the evidence suggests a hepatotoxic effect followed by regenerative cell turnover, in contrast to agents which act as direct mitogens. For chemicals producing increased cell turnover through cytolethality, a threshold can be inferred below which these effects would not occur. This scenario could apply to triclosan based on the available data.

9.0 REFERENCES

MRID 0087996:

MRID 34335: Stierlin, H. (1968) "Percutaneous Absorption of GP 41 353 in the Rat and the Rabbit." (Unpublished study; prepared by J.R. Geigy, AG, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:000506-M)

MRID 43429: Stenger, E.G.; Scharer, (1963) "Local Effects on Rabbit's Eye." (Unpublished study; prepared by J.R. Geigy, AG, Switzerland, submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:108210-J)

MRID 96102: Burther, B.R.; Gordon, D.E. (1973) "Report to Ciba-Geigy Corporation: 90-day Subacute Oral Toxicity Study with Irgasan DP-300 in Beagle Dogs: IBT No. C1435." (Unpublished study; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:008400-C)

MRID 100178/130187: Schoenig, G. (1967) "Report to Geigy Chemical Company: Acute Dermal Toxicity Study on CH 3565: IBT No. A5460." (Unpublished study; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:000503-D)

MRID 133545: Goldsmith, L. (1983) 90-Day Oral Toxicity Study in Rats with Fat 80'023/H: LBI Project No. 22188. Final Report. (Unpublished study received Dec. 23, 1983 under 100-502; submitted by Ciba-Geigy Corp.)

MRID 251773: Drake, J.C. & Buxtorf, A. (1976) 1 Year Oral Toxicity Study in Baboons with Compound FAT 80 023/A. Geigy Pharmaceuticals, Toxicology Department.

MRID 40623701: Morseth, S.L. (1988) "Two-Generation Reproduction Study in Rats with FAT 80'02390" Hazleton Laboratories America, Inc.

MRID 42027906: Study 1 - Yau, E.T. and Green, J.D. (1986) Fat 80'023 2 Year Oral Administration to Rats; Study 2 – Parkes, D.G. (1986) Determination of Fat 80'023 in Blood and Tissue Samples Taken During a Two-Year Chronic Oral Toxicity/Oncogenicity Study in Albino Rats (24-month Final Report). Ciba-Geigy Corporation.

MRID 42306902, 43310501: Duchosal F. and Ph. Thevenaz (1990) "4-Hour Acute Inhalation Toxicity Study with FAT 80'023/Q in Rats" Ciba-Geigy Ltd.

MRID 42306903: Sachsse, K.; Ullman, L. (1975) Skin Irritation in the Rabbits after Single Application of FAT 80'023/A. Unpublished Study Conducted by Ciba Geigy Ltd., Basel Switzerland. Project No. Siss 4719. Submitted to EPA by Chemical Division of Ciba Geigy Corp. (9/11/75).

MRID 43022605**:** Trutter, Janet A. (1993) "13-Week Subchronic Oral Toxicity Study of Triclosan in CD-1® Mice." Hazleton Washington, Inc.
MRID 43206501: Wnorowski, Gary. (1994) "Acute Toxicity Limit Test for Triclosan (Irgasan® DP 300) Product Safety Labs.

MRID 43206502: Wnorowski, G. (1994) Dermal Sensitization Test-Buehler Method for Triclosan (Irgasan® DP 300) Lot No. 5.2.0211.0. Product Safety Labs; Study No. 2635. Submitted to EPA by Ciba-Geigy Corp. Unpublished.

MRID 43328001: Trimmer, Gary W. (1994) "90-Day Subchronic Dermal Toxicity Study in the Rat with Satellite Group with Irgasan DP300 (MRD-92-399)" Exxon Biomedical Sciences, Inc.

MRID 43533301: Jones, E., Wilson, L.A. (1988) "Ames Metabolic Activation Test to Address the Potential Mutagenic Effect of Triclosan (Irgasan DP 300)" Huntingdon Research Center, Ltd. Cambridgeshire, England.

MRID 43740801: Heidemann, A. (1990) "Chromosome Aberration Assay in Chinese Hamster V79 Cells In Vitro with FAT 80'023/Q (Irgasan® DP 300); Cytotest Cell Research GMBH & Co. KG, Federal Republic of Germany; Study No. 179100. Unpublished.

MRID 43740802: Volkner, W. (1991) "Chromosome Aberration Assay in Bone Marrow Cells of the Rat with FAT 80'023/Q (Irgasan® DP300); Cytotest Cell Research GMBH & Co. KG, Federal Republic of Germany; Study No. 218305. Unpublished.

MRID 43817502, 43817503: Denning, H.J., Sliwa, S., and Willson, G.A. (1992) "Triclosan: Effects on Pregnancy and Post-Natal Development in Rats: Volume 1, Volume 2 and Appendices 1-17" Unilever Research, Colworth/ Welwyn Lab.

MRID 43820401, 43022607: Schroeder, R.E. et al. (1992) "A Segment II Teratology Study in Rabbits with Irgacare MP" Bio/dynamics Inc.

MRID 44389701: Eldrige, S. (1993) Cell Proliferation in Rodent Liver: (Triclosan): Final Report: Lab Project Number: 142. Unpublished study prepared by Pathology Associates, Inc. 14 p.

MRID 44389702: Molitor, E., Persohn, E., Thomas, H. "The Effect of FAT 80'023/Q (Irgasan DP 300) on Selected Biochemical Liver Parameters Following Subchronic Dietary Administration to Male and Female Mice. Ciba-Geigy Limited, Switzerland, Report CB 91/18, May 22, 1992.

MRID 44389703: Molitor, E.; Persohn, E. (1993) The Effects of FAT 80'023/Q (Irgasan DP 300) on Selected Biochemical and Morphological Liver Parameters Following Dietary Administration to Male Rats: Lab Project Number: CB 92/28. Unpublished study prepared by Ciba-Geigy Ltd. 70p.

MRID 44389704: Henderson, L.M., et al. (1988) An Assessment of the Mutagenic Potential of Triclosan Using the Mouse Lymphoma TK Locus Assay. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Study No. ULR 216/88644. Unpublished.
MRID 44389705: Stankowski, L.F., Jr. et al. (1993) Amended Final Report, Ames/Salmonella Plate Incorporation Assay on Test Article 39316 (CC #14663-09). Pharmakon USA, P.O. Box 609, Waverly, PA. Laboratory Study Report No. PH 301-CP-001-93. Unpublished.

MRID 44389706: Thomas, Rer. Nat. H. (1994) The Effect of FAT 80'023/Q and the Model Inducers Phenobarbitone, 3-Methylcholanthrene, Pregnenolone 16 ∞ -carbonitrile and Nafenopin on Selected Biochemical and Morphological Liver Parameters in the Syrian Hamster. Study conducted by Ciba-Geigy Limited. Study number CB 93/40. Unpublished.

MRID 44389707: Thevenaz, Dr. Phil. (1987) Final Report: FAT 80023: 28-Day Toxicity Study in Mice (Administration in Feed) with Special Reference to Histopathology. Ciba-Geigy Ltd., Basle, Switzerland. Unpublished.

MRID 44389708: Burns, J.M. (1997) 14-Day Repeated Dose Dermal Study of Triclosan in CD-1 Mice. Corning Hazleton Incorporated (CHV), 9200 Leesburg Pike, Vienna, Virginia. Laboratory Study No. CHV 2763-100. April 29, 1997. Unpublished.

MRID 44389710: Burns, J.M. (1997) 14-Day Repeated Dose Dermal Study of Triclosan in Rats. Corning Hazleton Incorporated (CHV), 9200 Leesburg Pike, Vienna, Virginia. Laboratory Study No. CHV 6718-102. Unpublished.

MRID 44874001, 44751101: Chambers, P.R. (1999) "Potential Tumorigenic and Chronic Toxicity Effects in Prolonged Dietary Administration to Hamsters." Huntingdon Life Sciences Ltd., Huntingdon, England. CBG 756/972896.

MRID 45307501, 45307502: Van Dijk, Dr. A. (1994) "^{14}C- Triclosan: Absorption, Distribution, Metabolism, and Elimination after Single/Repeated Oral and Intravenous Administration to Hamsters." RCC Umweltchemie AG. RCC Project No. 351707.

MRID 45307503: Van Dijk, Dr. A. (1995) "^{14}C- Triclosan: Absorption, Distribution, Metabolism, and Elimination after Single/Repeated Oral and Intravenous Administration to Mice." RCC Umweltchemie AG. RCC Project No. 337781.

Tox Record No. 001955, 001956 (1968)

Tox Record No. 001968 (1977)

MRID 47276601 Broker, P.C., Gray, V.M., Howell, A. (1988) "Analysis of Metaphase Chromosomes Obtained from CHO Cells Cultured *in vitro* and Treated with Triclosan." Huntingdon Research Center, Ltd. ULR 214/88731; Unilever Test #: KC880171. Unpublished.

Eldrige, S. (1995) Cell Proliferation in Rodent Liver. Study conducted by Pathology Associates, Inc. Submitted to EPA (no MRID). Unpublished.

MRID 47276602 San Sebastian, J.R., Morgan, J.M. (1993) "Rat Hepatocyte Primary Culture/DNA Repair Test on 39317" Pharmakon Research International, Inc. Pharmakon Study #: PH311-CP-001-93. Unpublished.

See, Norman A. (1996) Review and Evaluation of Pharmacology and Toxicology Data Division of Dermatologic and Dental Drug Products (HFD-540) Food and Drug Administration.