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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: October 22, 1998

MEMORANDUM

SUBJECT: *TRICLOSAN*: - Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: K. Clark Swentzel, Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Timothy F. McMahon, Ph.D., Senior Scientist
Risk Assessment and Science Support Branch
Antimicrobials Division (7510W)

PC Code: 054901

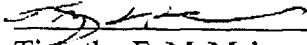
On March 10, 1998, the Health Effects Division's Hazard Identification Assessment Review committee evaluated the toxicology data base of triclosan and selected doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments, re-assessed the Reference Dose (RfD) established for chronic dietary risk assessment, and addressed the sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

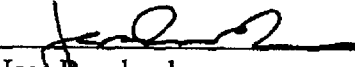
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Committee Members in Attendance

Members present were Karl Baetcke, Clark Swentzel (Chairman), John Redden, Bill Burnam, Karen Hamernik, and Jess Rowland (Executive Secretary). Members in absentia: Sue Makris, and Melba Morrow. Data were presented by Tim McMahon.

Other HED members present: Sanju Diwan

Data Presentation 
and Timothy F. McMahon, Ph. D
Report Preparation: Senior Scientist

Report Concurrence: 
Jess Rowland
Executive Secretary

I. INTRODUCTION

On March 10, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicology data base of Triclosan and selected doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments, re-assessed the Reference Dose (RfD) established for chronic dietary risk assessment, and addressed the sensitivity of infants and children from exposure to Triclosan as required by the Food Quality Protection Act (FQPA) of 1996. The application of the 10x factor for protection of infants and children from exposure to Triclosan, as required by FQPA, will be determined by the FQPA Safety Factor Committee (FQPA SFC). It is noted that a previous FQPA assessment was performed for Triclosan in 1997, with a recommendation for no additional safety factor. The HIARC's conclusions are presented below.

II. HAZARD IDENTIFICATION

A. Acute Reference Dose (Acute RfD)

Study Selected: Chronic Toxicity - Baboon

MRID. No. 257773

Executive Summaries: In a chronic toxicity study, groups of 7 baboons/sex/dose received Irgasan DP300 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 remaining 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.

Dose and Endpoint for Risk Assessment: NOAEL = 30 mg/kg/day, based on diarrhea observed 4-6 hours after dosing at the LOAEL of 100 mg/kg/day. .

Comments about Study and Endpoint: A study on the effects of triclosan on the mouse liver was available (MRID # 44389702), in which induction of several liver enzyme activities was observed at a dose of 18.4 mg/kg/day in the diet. This study was also taken under consideration for the acute dietary endpoint. The committee recognized, based on data presented by Dr. McMahon, that the pattern of enzyme induction observed with Triclosan was similar to that observed after phenobarbital administration, but did not consider this to be a treatment-related effect.

This risk assessment is required.

B. Chronic Dietary [Reference Dose (RfD)]

The RfD established in 1993 was re-assessed by this Committee pursuant to the FQPA and is discussed below:

Study Selected: Chronic Toxicity - Baboon §83-1

MRID No. 257773

Executive Summary: In a chronic toxicity study, groups of 7 baboons/sex/dose received Irgasan DP300 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 remaining 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.

Dose/Endpoint for establishing the RfD: NOAEL= 30 mg/kg/day based on diarrhea observed at 100 mg/kg/day, and hematologic alterations at 300 mg/kg/day.

Comments about Study and Endpoint: The HIARC concurred with the RfD established. The committee also noted supporting evidence for selection of the RfD from the two-year rat chronic toxicity / carcinogenicity study (MRID # 42027906) in which hepatocellular hypertrophy was observed at a dose of 52 mg/kg/day, consistent with several other studies on triclosan showing the liver to be a target organ of toxicity.

Uncertainty Factor (UF): 100 (10x for inter-species extrapolation and 10x for intra-species variation) .

$$\text{RfD} = \frac{30 \text{ mg/kg/day (NOAEL)}}{100 \text{ (UF)}} = 0.30 \text{ mg/kg/day}$$

This risk assessment is required.

C. Occupational/Residential Exposure

1. Dermal Absorption

An older rabbit dermal absorption study 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). This estimate was based on 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.

Dermal Absorption Factor: 50%

2. Short-Term Dermal - (1-7 days)

Study Selected: Chronic Toxicity - Baboon

MRID No. 257773

Executive Summary: See Acute Dietary.

Dose and Endpoint for Risk Assessment: NOAEL = 30 mg/kg/day based on diarrhea observed at 4-6 hours post-dosing at the LOAEL of 100 mg/kg/day. .

Comments about Study and Endpoint: Effects were observed 4-6 hours after dosing on day 1 which is appropriate to use for this exposure period (i.e., 1-7 days). Since an oral NOAEL was identified, a dermal absorption factor of 50% should be used for this risk assessment.

This risk assessment is required..

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: Chronic Toxicity- Baboon

MRID No. 257773

Executive Summary: see Chronic Dietary

Dose and Endpoint for Risk Assessment: NOAEL = 30 mg/kg/day based on clinical signs of toxicity (vomiting, failure to eat, and diarrhea) observed at 100 mg/kg/day (LOAEL).

Comments about Study and Endpoint: None

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: Chronic Toxicity - Baboon

MRID No. 257773

Executive Summary: see Chronic Dietary

Dose and Endpoint for Risk Assessment: NOAEL = 30 mg/kg/day based on clinical signs (vomiting, failure to eat, and diarrhea) at 100 mg/kg/day (LOAEL).

Comments about Study and Endpoint: This dose was used for establishing the RfD. Since an oral NOAEL was selected, a dermal absorption factor of 50% should be used for dermal risk assessment.

This risk assessment is required.

5. Inhalation Exposure (Any-Time period)

MRID No.

Executive Summary: In a 21-day inhalation toxicity study, rats were exposed to 0.05, 0.115, and 0.301 mg/L Irgasan DP300 for 2 hours per day for 21 days. The NOAEL was determined to be 0.05 mg/L, and the LOAEL was determined to be 0.115 mg/L, based on increased total leucocyte count and increased serum alkaline phosphatase.

Dose and Endpoint for Risk Assessment: NOAEL=0.05 mg/L based on increased total leucocyte count and increased serum alkaline phosphatase at 0.115 mg/L.

Comments about Study and Endpoint: Since this is the only study available, this NOEL should be used for the Short, Intermediate and Long-Term risk assessments.

This risk assessment is required.

D. Margin of Exposure for Occupational/Residential Exposures:

A MOE of 100 should be adequate for Short, Intermediate, and Long-term **dermal and inhalation exposures for workers (occupational exposures)**. A MOE for residential exposure will be determined during risk characterization by the FQPA Safety Factor Committee.

E. Recommendation for Aggregate Exposure Risk Assessments

For acute aggregate exposure risk assessment, combine the high end exposure values from food + water and calculate % RfD.

For Short, Intermediate, and Long-term aggregate exposure risk assessments, the short-intermediate and long-term exposures should be converted to oral equivalent doses (using 50% dermal absorption rate), and these should be added to the oral exposures (from food and water), and compared to the oral NOAEL to calculate aggregate risk MOE. The NOAEL from the acute RfD should be used for short-term aggregate risk assessment and the NOAEL from the chronic RfD should be used for intermediate and long-term aggregate risk assessment.

Inhalation exposure cannot be combined since the endpoint identified is different from that identified from oral and dermal exposures.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study- Rats

§83-5

MRID No: 42027906

Executive Summary: In a chronic toxicity/oncogenicity feeding study conducted in male and female Sprague-Dawley rats, FAT 80'023 (triclosan) 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). No treatment-related effects on mortality, clinical toxicity, ophthalmology, 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 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 168.0 mg/kg/day, and the incidence of hepatic necrosis was increased 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 liver. Residual levels of triclosan were proportional to the dose administered. 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.

Discussion of Tumor Data: There was no evidence of carcinogenicity.

Adequacy of the Dose Levels Tested: The dose levels were adequately tested.

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2. Carcinogenicity Study- Mice

§83-2b

A second carcinogenicity study for Triclosan is currently unavailable, but is being performed as a dermal carcinogenicity study in rats.

3. Classification of Carcinogenic Potential: The committee was unable to assign a carcinogenicity classification to triclosan, due to the lack of a study in a second species.

IV. MUTAGENICITY

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** (§84-2), and satisfies the requirements for FIFRA Test Guideline for in vitro mammalian forward gene mutation data.

In a microbial mutagenicity assay (MRID 44389705), *Salmonella typhimurium* strains TA100 and TA1538 were exposed to tolylfluanid (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, TA100 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.

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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 (§84-2)** and satisfies the requirement for FIFRA Test Guideline for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

In an *in vivo* bone marrow chromosome aberration assay (MRID 44389711), 6 male and 6 female Chinese hamsters were given 3 oral doses of triclosan (purity not reported) per week for 12 weeks in 0.7% aqueous carboxymethylcellulose (CMC) at levels of 75, 150, 300, or 600 mg/kg body weight. Bone marrow was sampled 6 hours after the last treatment.

Lethality occurred in 8/12 animals in the 600 mg/kg test group and in 1/12 animals in the 75 mg/kg group. Clinical signs of toxicity or body weight depression were not reported to have been evaluated at any treatment level. There was no significant increase in the incidence of chromosome damage at any treatment level compared to vehicle controls. Only one chromatid break was observed (per 400 metaphase spreads) each at 300 and 600 mg/kg. A positive control was not included in the assay.

This study is classified as **unacceptable/not upgradable (§84-2)** and does not satisfy the guideline requirements for *in vivo* cytogenetic mutagenicity studies because: (i) a positive control was not included in the study, (ii) the form and purity of the active ingredient tested were not reported, (iii) the test system was not fully identified (strain, source, and age of test animals), and (iv) the animals were sampled only at 6 hours after the last dosing.

V. FOPA CONSIDERATIONS

1. Neurotoxicity Data

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, polydipsia 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. The committee concluded on the basis of these data and the lack of any evidence for neurotoxicity in subchronic and chronic toxicity studies with triclosan that triclosan was negative for neurotoxicity in the submitted studies.

2. Developmental Toxicity

In a developmental toxicity study in rabbits, Irgacare (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 to the high dose (150 mg/kg/day) does 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.

3. Reproductive Toxicity

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 lower 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.

4. 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.

5. 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.**

6. Determination of the FQPA Safety Factor:

The application of the FQPA safety factor to ensure the protection of infants and children from exposure to triclosan, as required by FQPA, will be determined by the FQPA Safety Factor Committee. However, the HIARC, based solely on the hazard assessment, recommends to the FQPA Safety Factor Committee that the additional 10x 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.

VI. DATA GAPS

A carcinogenicity study in a second species was identified as a data gap, but is currently being conducted by the registrant.

VII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected and Margins of Exposures for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOEL = 30	diarrhea 4-6 hours after dosing	Chronic Toxicity - Baboon
	UF = 100	Acute RfD = 0.30 mg/kg/day	
Chronic Dietary	NOEL = 30	diarrhea 4-6 hours after dosing; hematological changes at high dose	Chronic Toxicity - Baboon
	UF = 100	RfD = 0.30 mg/kg/day	
Short-Term (Dermal) ^a	NOEL = 30	Diarrhea observed 4-6 hours after dosing.	Chronic Toxicity - Baboon
Intermediate-Term (Dermal) ^a	NOEL = 30	clinical signs of toxicity (vomiting, failure to eat, and diarrhea)	Chronic Toxicity - Baboon
Long-Term (Dermal) ^a	NOEL = 30	clinical signs of toxicity (vomiting, failure to eat, and diarrhea)	Chronic Toxicity - Baboon
Inhalation (Any Time Period)	NOEL = 0.05 mg/L	Increased total leukocyte count and increased serum alkaline phosphatase	21-Day Inhalation-Rat

^a = The use of a 50% dermal absorption rate is required since an oral NOAEL was selected for these risk assessments.

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