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

OCT 28 1994

OCT 29 1994

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Irgasan: Review of 3 Toxicity Studies

TO: Kathy Davis
Product Manager
Reregistration Branch
Special Review and Reregistration Division (7508W)

FROM: Deborah L. McCall
Chemical Manager
SRS / CCB / HED (7509C)

THROUGH: James Rowe, Ph.D., Section Head
TOXII / HED (7509C)

and

Marcia Van Gemert, Ph.D., Chief
TOXII / HED (7509C)

PCCODE: 054901
Caswell: 186A
Barcode: D197674

ACTION REQUESTED: Review three toxicity studies for the chemical Irgasan. The registrant (Ciba-Geigy Corporation) has submitted 3 studies in support of re-registration. Conclusions from the reviews are presented below (and the DERs are attached):

1) 90-day Study in Mice (§82-1); MRID No. 430226-05

Irgacare DP 300 was administered to CD-1 mice at dose levels of 0, 25, 75, 200, 350, 750, or 900 mg/kg/day for 13 weeks. 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 in the liver). Clinical pathology included significantly decreased erythrocytes, hemoglobin, and hematocrit at ≥ 25 mg/kg/day in males and ≥ 75 mg/kg/day in females. Enzyme changes, indicative of liver injury, included increased alkaline phosphatase and alanine aminotransferase. Based on the changes in clinical chemistry and hematology parameters as well as the lesions in the liver the Systemic LOEL = 25 mg/kg/day and a Systemic NOEL was not determined.

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This study is classified Core Minimum and satisfies the Guideline requirements (82-1), 90-day oral toxicity in mice.

2) Developmental Toxicity in Rats (§83-3a); MRID No. 430226-06

Irgacare MP was administered to Spraque-Dawley derived CD rats at dose levels of 0, 15, 50, or 150 mg/kg/day. Neither maternal or developmental toxicity was observed in this study. Therefore, the Maternal and Developmental NOEL ≥ 150 mg/kg/day; the Maternal and Developmental LOEL were not determined. This study does not meet the data requirements for a developmental toxicity study in rats (§83-3a) and is classified as CORE SUPPLEMENTARY.

2) Developmental Toxicity in Rabbits (§83-3b); MRID No. 430226-07

Irgacare MP was administered to NZW rabbits at dose levels of 0, 15, 50, or 150 mg/kg/day by oral gavage from gestation day 6 through 18, inclusive. Maternal toxicity was evidenced by significantly decreased body weight in the 150 mg/kg/day dose group on gestation days (GD) 14-16 when compared with the controls. Additionally, the 150 mg/kg/day dose group had a significant decrease in body weight change over the entire dosing period (GD's 6-19). A statistically significant decrease in mean food consumption was also noted in the 150 mg/kg/day dose group during GD's 6, 7, 8, 12, 13, 14, and 15. No statistically significant changes were noted in the cesarean section observations. A slight downward trend was noted in the total number of fetuses and fetuses per dam, (i.e., as the dose increased the total number of fetuses decreased slightly). Also, there was a slight increase in the total number of early resorptions and in the number of litters across the dose groups, as the dose increased the number of early resorptions in the mid and high dose group increased. However, the litter incidence was within the range of historical control data. No differences were noted in the number of corpora lutea, implantation sites, or preimplanation loss between the treated groups and the controls. None of the rabbits had premature deliveries or aborted pregnancies. Maternal Toxicity NOEL = 50 mg/kg/day, and the Maternal Toxicity LOEL = 150 mg/kg/day based on reduced body weight and food consumption.

No evidence of developmental toxicity (external, visceral, variations, or malformations) was indicated at dose levels up to 150 mg/kg/day. Developmental Toxicity NOEL = 150 mg/kg/day.

This study is classified Core Guideline and satisfies the Guideline requirements (83-3), Developmental Toxicity (Teratology) Study in rabbits.

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FINAL

DATA EVALUATION REPORT

IRGACARE

Study Type: Developmental Toxicity (Rat)

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer Pia Lindström Date 7/18/94
Pia Lindström, D.P.H.

Independent Reviewer William S. McLellan Date 7/18/94
William McLellan, Ph.D.

Q/A Reviewer Carol Maczka Date 7/18/94
Carol Maczka, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-64
Clement Number: 252
Project Officer: Caroline Gordon

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IRGACARE

Developmental Study (83-3)

EPA Reviewer: Deborah McCall
Special Review Section
Chemical Coordination Branch/HED (7509C)

Signature: D. McCall
Date: 8-2-94

EPA Section Head: James Rowe, Ph.D.
Review Section III
Toxicology Branch II/HED (7509C)

Signature: James N. Rowe
Date: 10/25/94

DATA EVALUATION RECORD

STUDY TYPE: Developmental Study - Rat (83-3)

P.C. CODE: 054901

MRID NUMBER: 430226-06

TEST MATERIAL: Irgacare MP

SYNONYMS: Irgasan®DP 300; Triclosan

STUDY NUMBER: 91-3665

SPONSOR: Colgate-Palmolive Company, Piscataway, NJ

TESTING FACILITY: Bio/dynamics, Inc., East Millstone, NJ

TITLE OF REPORT: A Segment II Teratology Study in Rats with Irgacare MP

AUTHOR: R.E. Schroeder

REPORT ISSUED: April 16, 1992

EXECUTIVE SUMMARY: In a developmental toxicity study, 24 or 25 Sprague-Dawley derived CD rats per group received Irgacare MP by gavage on gestation days (GDs) 6-15, inclusive, at dose levels of 0, 15, 50, or 150 mg/kg/day. Carboxymethylcellulose (0.1%) in a 20% glycerin in water suspension served as the control substance and vehicle for the test article.

Neither maternal nor developmental toxicity was observed in this study. Therefore, the Maternal Toxicity NOEL ≥150 mg/kg/day; the Maternal Toxicity LOEL was not determined. The Develcpmental Toxicity NOEL ≥150 mg/kg/day; the Developmental Toxicity LOEL was not determined.

Classification: Core Supplementary Data. This study does not satisfy the minimum requirements for a developmental study (83-3) in rats. No maternal toxicity was observed at the highest dose level and the study is therefore, not acceptable for regulatory purposes.

Special Review Criteria (40 CFR 154.7): None

MATERIALS and METHODS

A. MATERIALS

Test material:	Irgacare MP (C-P Sample No.: 38328)
Description:	Cloudy white suspension
Lot number:	13851206
Purity:	99.8%
Stability:	Not reported
CAS number:	Not reported
Vehicle:	0.1% Carboxymethylcellulose in a 20% glycerin in water suspension

Test Animals

Species:	Rat
Strain:	Sprague-Dawley derived (CD®)
Age:	Approximately 13 weeks at initiation of mating
Weight:	226-331 g on GD 0
Source:	Charles River Laboratories, Inc., Portage, MI
Housing:	1 animal/cage (except during mating)
Envr. conditions:	Temperature--66°-78°F Humidity--22%-75% Air changes--Not reported Photoperiod--12/12 hours
Acclimation:	43 days

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the potential of Irgacare MP to cause developmental toxicity in rats when administered daily by gavage on GDs 6-15, inclusive.

Mating

Females were mated in a ratio of 1 to 1 with resident proven males of the same strain. Males were proven breeders from the Bio/dynamics in-house breeding colony. GD 0 was designated as the day on which sperm were detected in a vaginal smear or a copulatory plug was observed.

Animal Assignment

Animal assignment and dose selection is presented in Table 1. Assignment was accomplished using a random procedure based on body weight.

TABLE 1. Animal Assignment

Group	Dose Level (mg/kg/day)	Number of Animals Assigned
Control	0	24
Low Dose	15	24
Mid Dose	50	25[a]
High Dose	150	25[b]

[a]Dam No. 3556 was replaced with No. 3575 due to severe weight loss on GD 8.
[b]Dam No. 4561 died on GD 7 (dosing error) and was replaced with No. 4575.

Dose Selection Rationale

Doses were selected based on the results of a range-finding study (Bio/dynamics Project No. 91-3654). The test substance was administered via gastric intubation to mated CD female rats (five per group) on GDs 6-15 at dose levels of 0, 5, 10, 25, 50, or 75 mg/kg/day. The vehicle was the same as that used in the main study. All females were sacrificed on GD 20 and examined. No mortality occurred in any group. Pregnancy rate was 100% in the control and treatment groups. Mean body weight gains for the 5-, 10-, 25-, and 50-mg/kg/day dose groups were comparable to the control group during the treatment period. Mean body weight gain in the 75-mg/kg/day dose group was lower than control on GDs 6-16 (30%) and 10-16 (40%); however, this reduction was attributed to one single animal. Mean food consumption data for the treatment groups were comparable to the control data. The 75-mg/kg/day dose group had a reduction in mean food consumption during the same intervals as the body weight loss was noted during treatment; this was also attributed to one animal. Therefore, there were no treatment-related effects noted on body weight change or food consumption for doses up to and including 75 mg/kg/day. No adverse effects were noted in the clinical observations, number of corpora lutea, uterine implantation data, litter weight data, or fetal data up to and including 75 mg/kg/day. Based on these results, the maternal and developmental NOELs were ≥75 mg/kg/day.

Dosing

All doses were in a volume of 5 mL/kg of body weight. Dosing suspensions were prepared by the sponsor. One set of dosing solutions was used throughout the study. During the dosing period, test suspensions were resuspended daily and volumes were adjusted based on the most recently recorded body weight data. Analyses for concentration and homogeneity were conducted once during the study. The analyses of the dosing solutions indicated that the solutions were within acceptable ranges.

C. OBSERVATIONS

Maternal Observations and Evaluations

The animals were checked twice daily for mortality and clinical signs of toxicity. In addition, females were given detailed physical exams approximately 1 hour after dosing on GDs 0, 6-15, and 20. On GD 20, all animals were sacrificed by exsanguination following anesthesia with CO_2 and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathology examination
- Liver weights
- Uterine weights
- Number of corpora lutea
- Number and position of implantation sites
- Number and position of resorptions (early and late) and of live and dead fetuses

Uteri from apparently nonpregnant females were stained with ammonium sulfide to detect early embryonic loss.

Fetal Evaluations

Examination of the fetuses included the following:

- Individual fetal weight and sex
- External examination of all fetuses
- Visceral examination (Staples) of one half of the fetuses
- Skeletal examination of one half of the fetuses

Statistical Analysis

The following statistical methods were employed:

- Interval data (maternal body weight and weight gain; food consumption; liver weight; numbers of corpora lutea, implantations, and live and dead fetuses; preimplantation loss; resorption/implant ratio; sex ratio; and fetal weight)--Bartlett's test for equal variance, ANOVA, Dunnett's test for multiple comparisons, and standard regression techniques for trend analysis (parametric data) or Kruskall Wallis test, Dunn's summed rank test, and Jonckheere's trend test (nonparametric data)
- Incidence data (number of litters with resorption sites, mortality rate, pregnancy rate, and developmental anomalies)--Chi-square test, Fisher's exact test with Bonferroni correction, and Armitage's trend test

Compliance

Signed and dated Good Laboratory Practice and Quality Assurance statements were provided and signed on November 22, 1993.

RESULTS

A. Maternal Toxicity

Mortality

No compound-related mortality was observed at any dose level. One female at 50 mg/kg/day was removed from the study because of severe weight loss during the predosing period. One female at 150 mg/kg/day was found dead on GD 7 because of a dosing injury. These animals were replaced with other animals (see Table 1).

Clinical Observations

No compound-related clinical signs were observed at any dose level.

Body Weight

Body weights were recorded on GDs 0, 6, 8, 10, 12, 14, 16, 18, and 20. A summary of body weight gain data is presented in Table 2. No compound-related effects in body weight or weight gain were observed at any dose level.

TABLE 2. Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 0-6)	Dosing Period (GDs 6-16)	Corrected Body Weight Gain (GDs 6-20)
0	36 ± 8	54 ± 8	41 ± 10
15	36 ± 9	52 ± 10	36 ± 11
50	36 ± 7	53 ± 11	39 ± 11
150	34 ± 9	52 ± 9	37 ± 10

[a]Data extracted from Study No. 91-3665, Tables D-2 and E-2

Food Consumption

A summary of food consumption data is presented in Table 3. Mean food consumption decreased significantly to 92% of control on GDs 6-11 in the 150-mg/kg/day dose group. Since this decrease did not affect the body weight or weight gain in the dams, it was not considered to be a relevant effect.

TABLE 3. Food Consumption (g/animal/day)[a]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 0-6)	Dosing Period (GDs 6-11)	Dosing Period (GDs 11-16)	Post-Dosing Period (GDs 16-20)
0	85 ± 6	76 ± 5	80 ± 8	81 ± 5
15	83 ± 11	73 ± 6	76 ± 4	79 ± 4
50	86 ± 7	72 ± 8	81 ± 7	83 ± 6
150	83 ± 6	70 ± 7*	78 ± 6	82 ± 5

[a]Data extracted from Study No. 91-3665, Table F-2
*Significantly different from control, p≤0.05

Gross Pathology Observations

No compound-related gross findings were observed at any dose level.

Liver Weights

No compound-related effects were observed at any dose level.

Cesarean Section Observations

Cesarean section findings are summarized in Table 4. No compound-related effects were observed at any dose level.

B. Developmental Toxicity

Summaries of major fetal external, visceral, and skeletal malformations are presented in Tables 5a, b, and c, respectively. No compound-related malformations or variations (data not shown) were observed at any dose level. At 150 mg/kg/day, incidences of delayed or incomplete ossification in some bones (supraoccipital, malar, maxilla, cervical transverse process, metacarpals, and pubis) were slightly increased above concurrent and historical controls. However, in the absence of statistical significance, a clear dose response, and any other effects, these increases were not considered to be toxicologically relevant.

DISCUSSION

A. Maternal Toxicity

No maternal toxicity was observed.

B. Developmental Toxicity

No developmental toxicity (including death/resorptions, altered growth, and anomalies) was observed.

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TABLE 4. Cesarean Section Observations[a]

Parameter	Dose Groups (mg/kg/day) 0	15	50	150
Number of animals assigned	24	24	25[b]	25
Number of animals pregnant	21	22	22	22
Pregnancy rate (%)	88	92	92	88
Maternal wastage				
Number died	0	0	0	1
Number died/pregnant	2	0	0	0
Number non pregnant	3	2	2	2
Number aborted	0	0	0	0
Number premature delivery	0	0	0	0
Total corpora lutea	359	406	392	389
Corpora lutea/dam	17.1 ± 3.0[c]	18.5 ± 1.7	17.8 ± 2.1	17.7 ± 2.0
Total implantations	336	380	366	373
Implantations/dam	16.0 ± 3.3	17.3 ± 3.5	16.6 ± 2.1	17.0 ± 1.8
Total live fetuses	303	353	346	349
Live fetuses/dam	14.4 ± 3.6	16.0 ± 3.3	15.7 ± 2.2	15.9 ± 1.8
Total resorptions	33	27	20	24
Early	33	27	20	23
Late	0	0	0	1
Resorptions/dam	1.6 ± 1.1	1.2 ± 1.2	0.9 ± 0.9	1.1 ± 1.2
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Mean fetal weight (g)	3.5 ± 0.3	3.4 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
Preimplantation loss (%)[d]	7	7	7	4
Postimplantation loss (%)[d]	11	7	6	6
Sex ratio (% male)[d]	49	54	49	53

[a]Data extracted from Study No. 91-3665, Tables G-1 to G-5
[b]Dam No. 3556 was replaced with No. 3575 on GD 8. Data from dam No. 3556 was excluded.
[c]Mean ± S.D.
[d]Calculated by reviewers, not statistically analyzed

TABLE 5a. External Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	15	50	150
Number of pups examined	303	353	346	349
Number of litters examined	21	22	22	22
Filamentous tail	0	0	1	0
Edematous fetus	0	0	0	1
Protrusion of viscera through abdominal wall	0	0	0	1
Shortened forelimbs	0	0	0	1
Absent forepaw digits	0	0	0	1
Shortened hind limbs	0	0	0	1
Absent hind paw digits	0	0	0	1
Kinked tail	0	0	0	1
Number of fetuses with any external malformations	0	0	1	1

[a]Data extracted from Study No. 91-3665, Table L-1
[b]Some observations may be grouped together

TABLE 5b. Visceral Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	15	50	150
Number of pups examined	156	180	178[c]	181[c]
Number of litters examined	21	22	22	22
Microphthalmia	1	0	0	0
Folded retina	0	0	0	1
Adhesion between liver and small intestine	0	0	0	1
Number of fetuses with any visceral malformations	1	0	0	2 (2)[d]

[a]Data extracted from Study No. 91-3665, Table M-1
[b]Some observations may be grouped together
[c]Fetus #13 from dam No. 3551 and fetus #6 from dam No. 4566 were processed for both skeletal and visceral evaluations; therefore the total number of fetuses evaluated for Groups III and IV will not equal the total numbers of fetuses evaluated for external effects.
[d]Number of litters

TABLE 5c. Skeletal Examination[a]

Observations[b]	Dose Groups (mg/kg/day) 0	15	50	150
Number of pups examined	147	172[c]	169[d]	169[d]
Number of litters examined	21	22	22	22
Absence of cervical transverse process	0		0	0
Presence of misc. cervical ossification	0		0	0
Absence of lumbar, sacral, and caudal vertebrae	0	0	1	0
Presence of five lumbar vertebrae	0	0	1	0
Wavy ribs	0	0	0	1
Curved scapula	0	0	0	1
Abnormal orientation of the ilia	0	0	1	0
Number of fetuses with any skeletal malformations	0	1	2 (2)[e]	2 (2)

[a]Data extracted from Study No. 91-3665, K-2
[b]Some observations may be grouped together
[c]Fetus #16 from dam No. 2552 was lost during skeletal staining procedure.
[d]Fetus #13 from dam No. 3551 and fetus #6 from dam No. 4566 were processed for both skeletal and visceral evaluations; therefore the total number of fetuses evaluated for Groups III and IV will not equal the total numbers of fetuses evaluated for external effects.
[e]Number of litters

C. Study Deficiencies

No toxicity had been noted at 75 mg/kg/day in the range-finding study. Therefore, the highest dose level in the present study was set at 150 mg/kg/day. In spite of this, maternal toxicity was not evident in the main study and the overall conclusion is that dose levels chosen for this study were too low.

D. CORE Classification: Core Supplementary Data

Maternal NOEL ≥ 150 mg/kg/day
Maternal LOEL = not determined

Developmental Toxicity NOEL ≥ 150 mg/kg/day
Developmental Toxicity LOEL = not determined

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Primary Review by: Deborah L. McCall 7-21-94
Chemical Coordination Branch, HED (7509C)
Secondary Review by: James Rowe, Ph.D., Section Head James N. Rowe 10/24/94
Review Section III, Toxicology Branch II, HED (7509C)

DATA EVALUATION RECORD

Study Type: Developmental Toxicity study in rabbits (§83-3)

EPA Identification No.s: EPA MRID (Accession) No.: 430226-07
PC Code: 054901
Caswell No.: 186A
Barcode: D197674

Test Material: Irgacare MP, or Irgasan DP 300 or Triclosan

Sponsor: Colgate-Palmolive Co., 909 River Rd, Piscataway, NJ 08855-1343 (shared data with Ciba-Geigy)

Study Number(s): 91-3666

Testing Facility: Bio/dynamic, Inc.,
P.O. Box 2360
Mettlers Road
East Millstone, NJ 08875-2360
(908) 873-2550

Title of Report: A Segment II Teratology Study in Rabbits with Irgacare MP

Author(s): R. E. Schroeder, M.S. DABT

Study Completed: April 16, 1992

EXECUTIVE SUMMARY: In a developmental toxicity study, at least 18 rabbits per dose group of the New Zealand White strain from Hazleton Research Products Inc., Denver PA received either 0, 15, 50, or 150 mg/kg/d of Irgacare MP by oral gavage from gestation day 6 through 18, inclusive. The rabbits were mated naturally.

Maternal toxicity was evidenced by significantly decreased body weight in the 150 mg/kg/day dose group on gestation days (GD) 14-16 and slightly lower body weights on GD's 8 through 12 when compared with the controls. Body weight gain was also significant decreased during GD's 6-8 and 12-14. Additionally, the 150 mg/kg/day dose group had a significant decrease in body weight change over the entire dosing period (GD's 6-19). A statistically significant decrease in mean food consumption was also noted in the 150 mg/kg/day dose group during GD's 6, 7, 8, 12, 13, 14, and 15. During the dosing period, the food consumption differences between the 150 mg/kg/day dose group and the control group ranged from -7% to 41%.

No statistically significant changes were noted in the cesarean section observations. A slight downward trend was noted in the total number of fetuses and fetuses per dam, (i.e., as the dose increased the total number of fetuses decreased slightly). Also, there was a slight increase in the total number of early resorptions and in the number of litters across the dose groups, as the dose increased the number of early resorptions in the mid and high dose group increased (control - 4/2 litters, 50 mg/kg/d - 7/6 litters, and 150 mg/kg/d - 12/7 litters). A true dose-response

relationship was not present since the low dose did not have any early resorptions.* No differences were noted in the number of corpora lutea, implantation sites, or preimplanation loss between the treated groups and the controls. None of the rabbits had premature deliveries or aborted pregnancies. **Maternal Toxicity NOEL = 50 mg/kg/day, and the Maternal Toxicity LOEL = 150 mg/kg/day based on reduced body weight and food consumption.** (* See also historical control data)

No evidence of developmental toxicity (external, visceral, or malformations) was indicated at dose levels up to 150 mg/kg/day. **Developmental Toxicity NOEL = 150 mg/kg/day.**

This study is classified Core Guideline and satisfies the Guideline requirements (83-3), Developmental Toxicity (Teratology) Study in rabbits.

1. MATERIALS AND METHODS

A. Test Compound: Purity: 99.8%
Description: White powder
Sample/Lot No.: 19851206C-P 38328
Stability: within acceptable ranges
Density: 1.051 g/mL

1. Vehicle(s): 1% (w/w) carboxymethylcellulose in a 20% aqueous glycerin solution.

B. Test Animal(s): Species: Rabbit, nulliparous female
Strain: New Zealand White
Source: Hazleton Research Products, Denver PA
Age: approximately 5 months
Weight: ♀'s - 3.4 kg; ♂'s were proven breeders 1.5 years old from the Bio/dynamic in-house breeding colony.

C. Study Design: This study was designed to assess the developmental toxicity potential of Irgacare MP when administered by the oral route to rabbits on gestation days 6 through 18, inclusive.

1. Mating: Nulliparous female rabbits were mated naturally. Each female selected for mating was placed into a male's cage. When coitus was observed the female was removed to her own cage. After 1-2 hours, the female was placed with a different second male and returned to her cage after mating. The day on which evidence of mating was observed with both males was considered gestation day '0'. Mating was conducted over a period of nine days.

2.Group Arrangement: The mated females were randomized to experimental and control groups by random numbers into 4 dosage groups of at least 18 rabbits each. The animals were identified by metal self-piercing ear tags and placed in individual cages.

Test Group	Actual[a] Concentration (mg/mL)	Dose Level (mg/kg/d)	No. of Rabbits Assigned
Control	0	0	18
Low	3.95	15	19[b]
Mid	13.1	50	18
High	39.2	150	19[c]

[a] = Actual concentration = concentration (mg/g) X density of suspensions (g/mL).
[b] = Dam #2501 was removed from study on gestation day 9 due to weight loss and clinical signs and replaced with #2519.
[c] = Dam #4501 died on gestation day 6 due to dosing accident and was replaced with #4519.

3. Range-finding Study: No justification was provided in the study report on dose selection. Also there was no mention of a range-finding study.

4. Dosing: The oral dosages of 0, 15, 50 or 150 mg/kg/d were administered at a dose volume of 4 mL/kg/d once daily to the mated rabbits on gestation days (GD) 6 through 18, inclusive.

5. Observations: The animals were checked for mortality, signs of abortion, and abnormal conditions daily. Dams were sacrificed on day 30 of gestation by intravenous injection of sodium pentobarbital. Examinations at sacrifice consisted of: macroscopic pathology, number of corpora lutea, number and location of fetuses (live/dead) in each horn, and number of intrauterine resorptions. The liver was weighed for all females sacrificed on GD 30. When no uterine implants were apparent, the uterus was stained with ammonium sulfide for determination of pregnancy.

The fetuses were examined by: gross inspection, sex-determination, tagging, and individual fetal body weights. All fetuses were sacrificed by intra-peritoneal injection of sodium pentobarbital. All fetuses were evaluated viscerally by a method similar to the Staples technique. The fetuses were eviscerated and skinned and then stained with Alizarin Red S. The brain was evaluated by a transverse cut using a razor blade and then another cut just posterior to the frontal-parietal suture and then through the cerebral hemispheres under a dissecting microscope. Historical control data were provided to allow comparison with concurrent controls.

D. Statistical analysis: A copy of the statistical methods used for the data analysis is attached (Appendix A). Observations for dead fetuses and late resorptions were excluded from statistical analysis.

E. Compliance: A Quality Assurance Statement and a Statement of Compliance with FIFRA Good Laboratory Practice Standards were signed and dated November 22, 1993.

2. RESULTS

A. Analyses of Suspensions: Methods of analyses were provided in the study report. Four sets of dosing solutions were prepared for the study and the top and bottom samples were analyzed for percent concentration. The top and bottom concentrations for the samples ranged from 72-113% and the mean concentrations were within acceptable ranges.

B. Maternal Toxicity

1. Mortality: Four animals were reported to have died during the study and three of the four were attributed to dosing errors. One control (#1514), two in the 15 mg/kg/day dose group (#'s 2501 2511) and one in the 150 mg/kg/day dose group (#4501). Female

nos. 2501 and 4501 were replaced on study since they died early during the study (see Table 1 below). The pathology report did not attribute the other two deaths to the compound.

Table 1: Mortality

Female No.	Group (mg/kg/day)	GD of death	Cause of death
1514	control	13	intubation error
2501[a]	15	9	intubation error
2519	15	17	unknown
2511	15	19	unknown
4501[b]	150	6	intubation error

[a] replaced with female #2519 on GD 9.
[b] replaced with female #4519 on GD 6.

2. Clinical Observations: Cage-side observations were: staining of the skin/fur of the ano-genital area, red exudate from lip, moist rales, lacerations, alopecia and scabs. None of these findings were considered to be treatment-related clinical effects. They were considered to be average findings for this species and study type.

3. Body Weight: The animals were weighed on days 0, 6, 8, 10, 12, 14, 16, 19, 24, and 30 of gestation. The investigators supplied the following data: group mean and individual animal data. Corrected maternal body weight data was presented in the report.

The maternal body weights remained relatively stable throughout the study for the control, 15 and 50 mg/kg/day dose groups. However the 150 mg/kg/day dose group had significant decreases in mean body weights during gestation days 14 and 16 and slightly lower weights on GD's 8 through 12 when compared to the control group.

The mean body weight changes for the 15 and 50 mg/kg/day dose groups were comparable with the control group throughout the study. But there were significant differences noted in the 150 mg/kg/day dose group. During GD's 6-8 and 12-14 the dams mean weight changes were significantly decreased when compared with the controls during these periods (see Table 2). Additionally the 150 mg/kg/day dose group had a significant decrease in body weight change over the entire dosing period (GD's 6-19). These losses in body weight were considered to be treatment-related. After the dosing period (GD's 19-30), the 150 mg/kg/day dose group rebounded in body weight gain and gained more weight than the control group.

Corrected GD 30 body weights (corrected for gravid uterine weights) and mean body weight change data were comparable between the control and treated groups.

Table 2: Selected Body Weight Changes (g)

Gestation Day	Dose Groups (mg/kg/day)			
	0	15	50	150
0-6	196	186	173	183
6-8	2	-26	5	-68**
10-12	33	35	39	22
12-14	72	53	42	-30**
14-16	40	45	54	15
16-19	52	28	60	101
6-19	219	171	215	75**
19-24	104	107	104	173
19-30	220	209	237	295

* = Significantly different from the controls $p<0.05$.
** = Significantly different from the controls $p<0.01$.
(Data were extracted from Appendix D-1, page 58.)

4. Food Consumption: A pelleted, certified standard diet (Purina Certified Rabbit Chow # 5325) and tap water (automatic watering system) was provided ad libitum. Food consumption was recorded on GD 1, 3, 5-19 [daily], 24 and 29.

The mean food consumption values for the 15 and 50 mg/kg/day dose groups were comparable with the control group throughout the study. A statistically significant decrease in mean food consumption was noted in the 150 mg/kg/day dose group during GD's 6, 7, 8, 12, 13, 14, and 15 (see Table 3). During the dosing period, the food consumption differences between the 150 mg/kg/day dose group and the control group ranged from -7% to -41%. These changes in the 150 mg/kg/day food consumption were comparable with the body weight losses and they were considered to be treatment-related. After the dosing period, the food consumption values for the 150 mg/kg/day dose group were significantly higher than the controls.

Table 3: Selected Mean Food Consumption Values (g/kg/d)

Gestation Day	Dose Groups (mg/kg/day)			
	0	15	50	150
5-6	70	71	65	67
7-8	63	56	61	46**
8-9	65	60	61	48**
11-12	57	58	54	53
13-14	52	56	51	36*
14-15	58	58	56	34**
18-19	64	61	64	58

* = Significantly different from the controls $p<0.05$.
** = Significantly different from the controls $p<0.01$.
(Data were extracted from Appendix F-2, pages 70-71.)

C. Gross Pathological Observations: No significant gross pathological differences were seen in any of the four rabbits that died on study. Some of the rabbits had red/brown lungs and reddened tissues. But the pathologist did not consider any of the findings to be treatment-related either in the animals that died on test or the ones sacrificed on GD 30.

D. Organ Weight: Liver weights were obtained for all groups. No differences were noted in the mean liver weights, absolute and relative to the corrected body weights in any treatment group when compared to the controls.

E. Cesarean Section Observations: No statistically significant changes were noted in the cesarean section observations (see Table 4). A slight downward trend was noted in the total number of fetuses and fetuses per dam, (i.e., as the dose increased the total number of fetuses decreased slightly). Also, there was a slight increase in the total number of early resorptions and in the number of litters across the dose groups, as the dose increased the number of early resorptions in the mid and high dose group increased (control - 4/2 litters, 50 mg/kg/d - 7/6 litters, and 150 mg/kg/d - 12/7 litters). A true dose-response relationship was not present since the low dose did not have any early resorptions. The study included historical controls which gave a percent resorption range of 16-80% with an average around 32%. The 43% resorptions noted in the 150 mg/kg/d dose group were within the historical control ranges, but at the high end of the range.

No differences were noted in the number of corpora lutea, implantation sites, or preimplanation loss between the treated groups and the controls. None of the rabbits had premature deliveries or aborted pregnancies.

Table 4: Cesarean Section Observations[d] [e]

Dose (mg/kg)	Control	15	50	150
# Animals Assigned	18	19	18	19
# Nonpregnant	1	3[a]	3	3[b]
Pregnancy Rate %	94%	89%	83%	89%
Maternal Wastage				
# Died	1	3	0	1
# Aborted	0	0	0	0
# Premature	0	0	0	0
Total # of Litters	16	15	15	16
Total Corpora Lutea	155	138	141	153
Corpora Lutea/Dam	9.7 ± 1.6	9.2 ± 2.1	9.4 ± 1.4	9.6 ± 1.2
Total Implantations	147	127	136	139
Implantations/Dam	9.2 ± 1.6	8.5 ± 2.7	9.1 ± 1.3	8.7 ± 1.6
Total Live Fetuses	143	126	125	123
Live Fetuses/Dam	8.9	8.4	8.3	7.7
Resorptions				
Total # of Early	4	0	7	12
Total # of Late	0	1	0	3
Resorptions/Dam	0.3 ± 0.8	0.1 ± 0.3	0.5 ± 0.6	0.9 ± 1.5
Litters w/ resorptions (%)	2 (12.5%)	1 (6.7%)	6 (40%)	7 (43.8%)
Resorptions/Implants	0.027 ± 0.08	0.006 ± 0.02	0.052 ± 0.07	0.102 ± 0.17
Total Dead Fetuses	0	0	4	1
Litters involved			1	1
Fetal Body Weight				
Mean Fetal Wt (g)	49.7	49.3	47.5	50.4
Preimplantation Loss (%)	0.049 ± 0.078	0.087 ± 0.167	0.034 ± 0.060	0.093 ± 0.114
Postimplantation Loss (%)	2.7	0.8	8.1	11.5
(%) Male/Litter	4.3	4.2	3.6[c]	4.3
Total Male/Total Female ratio	0.9	1.0	0.8[c]	1.3

[a] = Dam #2501 was removed from the study and therefore was excluded from analysis; Dam #2519 was added to the study but died - was pregnant but excluded from analysis.

[b] = Dam #4501 was excluded from analysis - died on GD 6 and pregnancy could not be determined; the other two rabbits were not pregnant.

[c] = The fetuses for Dam #3509 were not sexed due to a technical error.

[d] = Appendix G, table G-1, page 80.

e = See attached historical control data.

F. Developmental Toxicity:

1. Fetal Observations: No differences were noted in the fetal body weight or in the percentage of males/litter between the treated groups and the controls. But when the ratio of total males to total females was examined the 150 mg/kg/d dose group had a higher ratio of males than the controls (0.9 vs 1.3).

2. External Observations: All fetuses were examined for external observations. No malformations were noted in the 15 mg/kg/d dose group (see Table 5). The control group contained one fetus each with a domed cranium and one with ectrodactyly and both were from different litters. In the 50 mg/kg/d dose group, one fetus had multiple facial defects. The 150 mg/kg/d dose group, also had one fetus with multiple defects (protruding tongue, shortened forelimbs and bowed hindlimbs). The incidences of fetuses with external malformations on a per fetus and per litter basis were not statistically significantly different between the treated groups and when compared with the controls. None of these malformations were considered to be treatment-related.

Table 5: External Malformations

Dose (mg/kg/d)	0	15	50	150
Litters examined Fetuses examined	16 143	15 126	15 129	16 124
MALFORMATIONS (affected fetuses/litter)				
Reduction in number of digits on forepaw	1/1	-	-	-
Domed Cranium	1/1	-	-	-
Protruding tongue	-	-	1[a]/1	1[b]/1
External nares	-	-	1[a]/1	-
Shortened snout	-	-	1[a]/1	-
Shortened forelimb	-	-	-	1/[b]/1
Abnormal flexure of hindlimb	-	-	-	1[b]/1

[a] = Same fetus from dam # 3516.
[b] = Same fetus from dam # 4502.
Data were extracted from Appendix L, pgs 191-206.

3. Visceral Examinations

Malformations: The control group had one fetus with distended lateral ventricles of the brain which was noted in the external examination with a domed cranium. In the 15 mg/kg/d dose group, one fetus had a small kidney. In the 50 mg/kg/d dose group, two fetuses had both the aortic arch and interventricular septal defects. One fetus (#3516-8)

from the 50 mg/kg/d dose group had multiple defects (distended lateral ventricle of the brain, aortic arch defect, and interventricular septal defect of the heart). Also this same fetus displayed multiple facial defects at the external examination. In the 150 mg/kg/d dose group, one fetus had a small atrium of the heart.

The incidences of fetuses with visceral malformations on a per fetus and per litter basis were not statistically significantly different between the treated groups and when compared with the controls. None of these malformations were considered to be treatment-related.

Variations: The presence of additional subclavian arteries was noted across all of the groups (see Table 6). The incidences of this variation per litter were as follows: control 5/4, 15 mg/kg/d - 6/5, 50 mg/kg/d - 4/4, and 150 mg/kg/d - 12/5. The high dose had a higher incidence of this variation but it was not statistically significantly different and it did not occur in a dose-related manner. Therefore, this reviewer believes it is not a treatment-related effect. The other variations, a dark red iris and a small gallbladder were not considered to be significant due to their low incidences.

The incidences of fetuses with visceral variations on a per fetus and per litter basis were not statistically significantly different between the treated groups and when compared with the controls. None of these malformations were considered to be treatment-related.

Table 6: Soft Tissue Malformations and Variations

Dose (mg/kg/d)	0	15	50	150
Litters examined Fetuses examined	16 143	15 126	15 129	16 124
MALFORMATIONS (affected fetuses/litter)				
Distended Ventricle of the Brain	1/1	-	1/1	-
Small Kidney	-	1/1	-	-
Defect of the Aortic Arch	-	-	2/1[a]	-
Interventricular Septal Defect	-	-	2/1[a]	-
Small Atrium of the Heart	-	-	-	1/1
VARIATIONS (affected fetuses/litter)				
Additional Subclavian Arteries	5/4	6/5	4/4	12/5
Small Gallbladder	-	-	1/1	-
Iris - dark red	-	-	-	1/1

[a] = Same fetus (#8) from Dam #3516.
(Data were extracted from Appendix M, pgs 207-225.)

3. Skeletal examination: Skeletal assessment was performed on all of the fetuses. The most prevalent malformation was the angulated arch of the hyoid (see Table 7). The other malformations occurred in low incidences with one fetus from one litter involved. The incidences of fetuses with skeletal malformations on a per fetus and per litter basis were not statistically significantly different between the treated groups and when compared with the controls. None of these malformations were considered to be treatment-related.

Other variations noted in this study are frequent variations seen in rabbits and no trends were evident (see Table 7). The incidences of fetuses with one or more skeletal variations were not significantly different between the treated groups and when compared with the controls. None of these variations were considered to be treatment-related.

Table 7: Skeletal Malformations and Variations

Dose (mg/kg/d)	0	15	50	150
Litters examined Fetuses examined	16 143	15 126	15 129	16 124
MALFORMATIONS (affected fetuses/litter)				
Hyoid Arches	8/6	3/3	9/6	6/5
Mandible - short/misshapen	-	-	-	1/1
VARIATIONS (affected fetuses/litter)				
Hyoid body - incompletely ossified	11/4	6/3	8/5	4/3
Interparietal - incompletely ossified	1/1	-	-	5/3
Cervical centrum - incompletely ossified	31/12	17/9	11/6	24/9
Sacral transverse process - incompletely ossified	22/9	21/12	19/8	18/11
Ribs - 13th short	29/13	31/12	20/10	14/8
Forelimb mid-phalange - incompletely ossified	12/6	11/5	14/9	18/9

(Data were extracted from Appendix N, pgs 226-279.)

G. Discussion/Conclusions:

1. Maternal Toxicity: Maternal toxicity was evidenced by significantly decreased body weight in the 150 mg/kg/day dose group on GD 14-16 and slightly lower weights on GD's 8 through 12 when compared with the controls. Body weight gain was also

significant decreased during GD's 6-8 and 12-14. Additionally, the 150 mg/kg/day dose group had a significant decrease in body weight change over the entire dosing period (GD's 6-19). A statistically significant decrease in mean food consumption was noted in the 150 mg/kg/day dose group during GD's 6, 7, 8, 12, 13, 14, and 15. During the dosing period, the food consumption differences between the 150 mg/kg/day dose group and the control group ranged from -7% to -41%.

No statistically significant changes were noted in the cesarean section observations. A slight downward trend was noted in the total number of fetuses and fetuses per dam, (i.e., as the dose increased the total number of fetuses decreased slightly). Also, there was a slight increase in the total number of early resorptions and in the number of litters across the dose groups, as the dose increased the number of early resorptions in the mid and high dose group increased (control - 4/2 litters, 50 mg/kg/d - 7/6 litters, and 150 mg/kg/d - 12/7 litters). A true dose-response relationship was not present since the low dose did not have any early resorptions. No differences were noted in the number of corpora lutea, implantation sites, or preimplanation loss between the treated groups and the controls. None of the rabbits had premature deliveries or aborted pregnancies. **Maternal Toxicity NOEL = 50 mg/kg/day, and the Maternal Toxicity LOEL = 150 mg/kg/day based on reduced body weight and food consumption.**

2. Developmental Toxicity: At doses up to 150 mg/kg/d Irgacare MP did not appear to have effects on developmental toxicity.

a. Deaths/Resorptions: No treatment-related effects were noted.

b. Altered Growth: No treatment-related effects were noted.

c. Developmental Anomalies: No treatment-related effects were noted.

d. Malformations: No treatment-related effects were noted.

Developmental Toxicity NOEL = 150 mg/kg/day.

H. Study Deficiencies: Page 24 of the report, 1st paragraph indicates that Group II female #2519 died on study. In the 2nd paragraph, last sentence the report indicates that female #2501 was replaced with female #2519. The 1st paragraph may be in error.

I. Core Classification: Core Guideline

This study satisfies the Guideline requirements (83-3), Developmental toxicity (Teratology) Study in rabbits.

011304

DATA EVALUATION REPORT

IRGACARE

Study Type: Subchronic Oral Toxicity in Mice

Prepared for:

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Health Effects Division
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by:

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Independent Reviewer: Carrie Rabe, Ph.D. Date: 4/19/94

QA Reviewer: William McLellan, Ph.D. Date: 4/19/94

Contract Number: 68D10075
Work Assignment Number: 3-64
Clement Number: 253
Project Officer: Caroline Gordon

Guideline Series 82-1. Subchronic Oral Toxicity in Mice 01130

EPA Reviewer: Deborah McCall
Special Review Section, CCB
Health Effects Division (7509C)

Signature: DMcCall
Date: 8.2.94

EPA Section Head: James Rowe, Ph.D.
Review Section III, Toxicology Branch II
Health Effects Division (7509C)

Signature: James N. Rowe
Date: 10/25/94

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity - Mice (82-1)

P.C. CODE: 054901

MRID NUMBER: 430226-05

TEST MATERIAL: Triclosan

SYNONYMS: Irgacare; Irgasan DP 300

STUDY NUMBER: HWA 483-287

SPONSOR: CIBA-GEIGY Inc., Greensboro, NC

TESTING FACILITY: Hazleton Washington Inc., Vienna, VA

TITLE OF REPORT: 13-Week Subchronic Oral Toxicity Study of Triclosan in CD-1 Mice

AUTHOR: J.A. Trutter

REPORT ISSUED: January 28, 1993

EXECUTIVE SUMMARY: In a subchronic feeding study, 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 increases 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, mineralization, and chronic bile duct inflammation. These lesions were evident in males (≥25 mg/kg/day) and in females (≥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 LOEL was 25 mg/kg/day; the NOEL could not be determined.

Classification: Core Minimum. This study satisfies the minimum guideline requirements for a subchronic feeding study in mice.

Special Review Criteria (40 CFR 154.7): None

A. MATERIALS

Test Material

Description:	White powder
Batch number:	5.2.0211.0
Purity:	99.7%
Stability:	Not reported; on file with the sponsor
CAS number:	3380-34-5
Vehicle:	None; test material was administered in the diet.

Test Animals

Species:	Mouse
Strain:	Crl:CD-1
Age:	At least 6 weeks at initiation
Weight:	Males--22-28 g at week -1 Females--18-22 g at week -1
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Individual
Temperature:	72°± 6°F (intended range; no deviations outside this range were reported)
Humidity:	50% ± 20% (intended range; room humidity was lower on six occasions and higher on one occasion; the magnitude of these deviations was not reported)
Air changes:	10 or more/hour
Photoperiod:	12/12 hours light/dark
Acclimation:	11 days

B. STUDY DESIGN

Animal Assignment

Animals were assigned to the test groups in Table 1 using a random allocation scheme based on body weight. Each animal was uniquely identified by an identification number with an implanted microidentification device. Mean body weights of the various treatment groups at all doses were comparable at the start of the study.

TABLE 1. Study Design

Test Group	Dose in diet (mg/kg/day)	Male	Female
	Main Groups		
I	0	15	15
II	25	15	15
III	75	15	15
IV	200	15	15
V	350	15	15
VI	750	15	15
VII	900	15	15
	Satellite Groups		
VIII	0	20	20
IX	25	10	10
X	350	10	10
XI	900	10	10

Rationale for Dose Selection

Concentrations were chosen by the sponsor based on the results of previous 28-day studies. The results of these studies were not presented.

Diet Preparation and Analysis

Diets were prepared weekly by mixing appropriate amounts of triclosan that had been ground into a fine powder with feed (Purina Certified Rodent Chow #5002). Target concentrations were varied each week to achieve constant mg/kg/day intake. No adjustment was made for % active ingredient. Analyses for homogeneity and stability were conducted on test formulations from the low and high dose groups in the main study prior to the dosing period. Analyses for concentration were conducted weekly for 14 weeks on test formulations from all dose levels.

Results

Homogeneity analysis: 100%-109% of target
Stability analysis: 98%-104% of target (10 days, room temp.)
Concentration analysis: 90%-109% of target

Average concentrations at every test level from two samples each for males and females were as follows:

TABLE 2. Achieved Dietary Concentrations*

Expected Dose (mg/kg/day)	Percent Nominal (%)	
	Males	**Females**
25	101.4	97.7
75	100.6	100.5
200	101.8	100.8
350	102.4	101.9
750	101.8	100.9
900	101.5	100.3

*Data extracted from Study No. HWA 483-287, Table 1

Animals received Purina Certified Rodent Chow #5002 and water *ad libitum* throughout the acclimation and study periods.

Statistical Analyses

Body weight, weight gain, food consumption, clinical pathology (except cell morphology), and organ weights (except liver) were analyzed by Levene's test for homogeneity of variances, ANOVA, and Dunnett's test for group comparisons. Data without homogeneous variances were transformed until homogeneity was achieved. Hematology data (red cell parameter only) and liver and kidney weights were also analyzed using Terpstra-Jonckheere's trend test, simple linear regression of untransformed or rank-transformed data, and regression ANOVA.

Compliance

Signed and dated Quality Assurance and Good Laboratory Practice statements were submitted and dated November 10, 1993.

C. METHODS AND RESULTS

Observations

Animals were observed twice daily for mortality and moribundity and once daily for clinical signs of toxicity. In addition, detailed physical examinations were performed weekly.

Results

Mortality

In the main groups, unscheduled deaths included two males (one found dead, one sacrificed moribund during weeks 2 and 3) and one female (found dead during week 8) at 900 mg/kg/day. The two males exhibited clinical signs of toxicity prior to death including hunched posture, hypoactive, pale appearance, and/or thin appearance; thus, these mortalities may have been treatment related. The cause of the female unscheduled death could not be determined. Three additional deaths occurred at 200 mg/kg/day; nothing, however, indicated that they were treatment related.

Clinical Signs

A summary of selected daily clinical signs observed in the main groups is presented in Table 3. Compound-related effects were observed at 750 and 900 mg/kg/day and included hunched posture, pale appearance, thin, hypoactivity, and cold to touch. In the satellite groups at 900 mg/kg/day, one female was hypoactive and had hunched posture and one male had hunched posture. These signs were not observed among controls.

Body Weight and Weight Gain

Animals were weighed prior to initiation of treatment, weekly throughout the study, and prior to sacrifice. Summaries of body weight and weight gain data are presented in Tables 4 A (main groups) and 4 B (satellite groups).

Results

Compound-related effects on body weight gain were observed in both sexes at 900 mg/kg/day. In the main groups among the 900 mg/kg/day males, weight gain decreased non-significantly to 80% of controls on weeks 1-6 and 83% on weeks 1-13. For the 900 mg/kg/day females, it decreased significantly to 40% of controls on weeks 1-6 and to 67% on weeks 1-13. In the satellite groups, weight gain de-creased non-significantly to 60% and 83% of controls for males and females, respectively, on weeks 1-6. Incidental (but statistically significant) decreases below or increases above controls on body weight gain were noted in the main groups among males at 350 and 750 mg/kg/day (weeks 1-6 and 1-13) and among females at 200 mg/kg/day (weeks 1-13) and at 350 mg/kg/day (weeks 1-6 and 1-13).

TABLE 3. Main Groups - Selected Daily Clinical Signs[a]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
Males							
Hunched posture	0/15	0/15	0/15	0/15	0/15	1/15	2/15
Pale-entire body	0/15	0/15	0/15	0/15	0/15	0/15	3/15
Thin	0/15	0/15	0/15	0/15	0/15	1/15	2/15
Hypoactivity	0/15	0/15	0/15	0/15	0/15	1/15	2/15
Females							
Hunched posture	0/15	0/15	0/15	0/15	0/15	1/15	1/15
Cold to touch	0/15	0/15	0/15	0/15	0/15	0/15	1/15
Pale-entire body	0/15	0/15	0/15	0/15	0/15	0/15	1/15
Thin	0/15	0/15	0/15	0/15	0/15	0/15	1/15
Hypoactivity	0/15	0/15	0/15	0/15	0/15	1/15	4/15

[a]Data extracted from Study No. HWA 483-287, Table 3A.

TABLE 4 A. Main Groups - Mean Body Weight and Weight Gain (g ± s.d.)*

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
				Males			
Body weight							
Week -1	25 ± 1.2	24 ± 1.8	25 ± 1.4	25 ± 1.0	25 ± 1.2	25 ± 1.2	25 ± 1.4
Week 3	28 ± 1.6	29 ± 1.9	29 ± 1.9	30 ± 1.8	29 ± 1.7	26 ± 2.2	26 ± 2.5
Week 7	31 ± 1.4	30 ± 1.6	31 ± 2.3	33 ± 1.9	33 ± 2.5	30 ± 3.0	30 ± 2.0
Week 10	31 ± 1.9	33 ± 2.1	33 ± 2.7	34 ± 2.1	34 ± 2.0	30 ± 2.7	31 ± 2.5
Week 13	32 ± 1.8	33 ± 1.7	33 ± 2.8	34 ± 2.2	34 ± 1.9	31 ± 2.4	31 ± 2.5
Weight Gain							
Weeks 1- 6	5 ± 1.8	5 ± 1.2	5 ± 1.7	6 ± 1.6	7 ± 1.8*	3 ± 2.8	4 ± 1.9
Weeks 1-13	6 ± 2.0	7 ± 1.2	7 ± 2.1	6 ± 2.8	8 ± 1.6	4 ± 2.0*	5 ± 2.8
				Females			
Body weight							
Week -1	20 ± 0.9	20 ± 1.4	20 ± 0.9	20 ± 1.1	20 ± 1.2	20 ± 1.0	20 ± 1.0
Week 3	24 ± 1.1	25 ± 1.6	24 ± 1.1	25 ± 1.5	25 ± 1.9	23 ± 1.6	22 ± 1.6
Week 7	27 ± 1.9	27 ± 2.0	27 ± 1.1	28 ± 1.6	28 ± 1.9	26 ± 2.0	25 ± 2.0*
Week 10	27 ± 2.1	28 ± 2.1	27 ± 1.5	28 ± 1.3	29 ± 2.0	27 ± 2.0	25 ± 1.9
Week 13	28 ± 1.6	28 ± 2.1	28 ± 1.2	29 ± 2.1	30 ± 2.3	27 ± 2.5	25 ± 2.2
Weight Gain							
Weeks 1- 6	5 ± 1.4	4 ± 1.6	5 ± 1.2	6 ± 1.3	7 ± 1.5*	5 ± 1.9	2 ± 1.4*
Weeks 1-13	6 ± 1.9	6 ± 1.5	7 ± 1.7	8 ± 1.4*	9 ± 1.8*	6 ± 2.0	4 ± 1.8*

*Data extracted from Study No. MAA 483-287, Tables 4 A and 5 A
*Significantly different from control, p≤0.05

TABLE 4 B. Satellite Groups - Mean Body Weight and Weight Gain (g ± s.d.)[1]

Observation	Dose Level (mg/kg/day) 0	25	350	900
		Males		
Body weight				
Week -1	25 ± 1.5	25 ± 1.7	25 ± 1.8	25 ± 1.6
Week 3	29 ± 1.8	29 ± 1.8	29 ± 2.8	25 ± 2.1
Week 7	31 ± 1.8	31 ± 2.3	32 ± 1.8	29 ± 2.2
Weight Gain				
Weeks 1-6	5 ± 0.9	5 ± 1.5	6 ± 1.0	3 ± 1.5*
		Females		
Body weight				
Week -1	21 ± 1.2	20 ± 1.1	20 ± 1.3	20 ± 1.0
Week 3	23 ± 1.0	22 ± 1.2	24 ± 1.1	22 ± 1.4
Week 7	27 ± 1.4	26 ± 1.2	28 ± 1.4	26 ± 1.9
Weight Gain				
Weeks 1-6	6 ± 1.0	5 ± 1.5	6 ± 1.1	5 ± 2.2

[1]Data extracted from Study No. HWA 483-287, Tables 4 B and 5 B
*Significantly different from control, p≤0.05

No compound-related effects were observed on body weight. Incidental (but statistically significant decreases below controls) were noted in females from the main group at 900 mg/kg/day on weeks 7 and 13 (Table 4 A).

Food Consumption and Compound Intake

Food consumption was determined weekly and compound intake was subsequently calculated.

Results

Food Consumption

Weekly food consumption data revealed no treatment-related effects. Overall, food consumption could not be accurately assessed because of frequent food spillage (especially at the higher concentrations).

Compound Intake

Compound intake values were, with one exception (week 13 means: 83% to 123%), within the range of expected values at all dose levels (85% to 117%). Mean values were as follows:

	Dose Levels (mg/kg/day)					
Groups	25	75	200	350	750	900
Main Groups, Weeks 1-13						
Males	24.8	74.5	198.8	348.0	766.4	907.6
Females	25.1	75.5	199.8	350.7	745.0	897.7
Satellite Groups, Weeks 1-6						
Males	25.1	----	-----	368.2	-----	894.6
Females	28.0	----	-----	356.8	-----	820.2

Data extracted from Study No. HWA 483-287, Tables 7A.

Ophthalmoscopic Examinations

Eye examinations were conducted on animals from the main groups only prior to the dosing period and on week 13 of treatment after dilation of the pupils with a mydriatic agent (1% Mydriacyl).

Results

No compound-related effects were observed.

Clinical Pathology

Hematology and clinical chemistry analyses were performed on 10 animals per sex and group from the satellite groups on day 45 and from the main groups at termination. Prior to the dosing period, hematology and clinical chemistry analyses were also performed on 10 animals per sex from the satellite control group and on an additional 8 males and 7 females not used in the study. Blood samples for hematology were collected from the orbital sinus and samples for clinical chemistry were collected from the abdominal vena cava. Animals were fasted overnight prior to sampling at study termination. The parameters marked with an "X" below were examined.

Hematology

X Hematocrit (HCT)*	X Leukocyte differential count*
X Hemoglobin (HGB)*	Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	Mean corpusc. HGB conc. (MCHC)
X Erythrocyte count (RBC)*	Mean Corpusc. volume (MCV)
X Platelet count*	Reticulocyte count
Blood clotting measurements	X Cell morphology
(Thromboplastin time)	
(Prothrombin time)	

* Required for subchronic and chronic studies

Results

Summaries of selected hematology parameters are presented in Table 5. Compound-related decreasing trends in hematocrit, hemoglobin, and red blood cell counts among males and females were observed at 7 weeks and at termination. At 7 weeks, females at 25 mg/kg/day had significantly decreased hemoglobin. In males and females at 350 and 900 mg/kg/day (satellite group), decreases in red cell count, hemoglobin, and hematocrit were observed. At study termination, dose-related decreases in these parameters were observed in males at all dose levels and in females at ≥75 mg/kg/day. Dose-dependent increases in segmented neutrophils and white blood cells were also observed among females (but not in males) at both 7 and 14 weeks (significant for segmented neutrophils at ≥750 mg/kg/day). In addition, the severity of polychromasia (data not shown) after 14 weeks increased slightly at ≥750 mg/kg/day. At 750 mg/kg/day, two females showed marked polychromasia; at 900 mg/kg/day, one male and one female showed severe polychromasia and one male showed marked polychromasia. At <750 mg/kg/day after 14 weeks and at all doses after 7 weeks, all animals (including those in the control groups) showed slight to moderate polychromasia.

Clinical Chemistry

Electrolytes	Other
Calcium*	X Albumin*
Chloride*	X Albumin/globulin ratio
Magnesium	X Blood creatinine*
Phosphate*	X Blood urea nitrogen*
Potassium*	X Globuline
Sodium*	X Total protein*
	X Glucose*
Enzymes	X Total bilirubin
	X Triglycerides
X Alkaline phosphatase (ALP)	X Total cholesterol*
X Alanine aminotransferase (ALT)	Phospholipid
X Aspartate aminotransferase (AST)	Protein fraction
X Gamma glutamyltransferase (GGT)	Serum electrophorese
X Lactate dehydrogenase (LDH)	
Glutamic oxaloacetic transaminase	

* Required for subchronic and chronic studies

TABLE 5. Selected Hematology Parameters[a,b]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
WEEK 7 MALES							
RBC (mi/uL)	10.6 ± 0.4†	10.4 ± 0.5			9.5 ± 0.4*		8.9 ± 1.2*
HGB (g/dL)	17.4 ± 0.5†	17.2 ± 0.8			15.0 ± 0.7*		14.2 ± 1.8*
HCT (%)	50.1 ± 1.9†	50.5 ± 3.1			44.5 ± 2.1*		43.3 ± 5.4*
SEG (th/uL)	1.1 ± 0.5	1.3 ± 0.9			2.6 ± 2.8		2.3 ± 1.1
WBC (th/uL)	4.0 ± 1.7	3.4 ± 1.7			5.7 ± 3.3		4.0 ± 1.2
WEEK 7 FEMALES							
RBC (mi/uL)	10.5 ± 0.4†	10.1 ± 0.5			9.4 ± 0.4*		9.6 ± 0.4*
HGB (g/dL)	18.0 ± 0.8†	17.0 ± 0.9*			15.5 ± 0.7*		15.7 ± 0.8*
HCT (%)	51.7 ± 3.0†	49.1 ± 2.5			45.6 ± 2.1*		45.8 ± 2.8*
SEG (th/uL)	0.9 ± 0.7	1.1 ± 0.7			1.7 ± 1.6		4.3 ± 2.8*
WBC (th/uL)	2.4 ± 1.0	2.7 ± 1.3			4.3 ± 3.3		7.2 ± 3.2*
WEEK 14 MALES							
RBC (mi/uL)	10.6 ± 0.6†	9.8 ± 0.3*	9.8 ± 0.6*	9.1 ± 1.3*	9.0 ± 0.6*	8.7 ± 0.7*	7.4 ± 1.2*
HGB (g/dL)	17.2 ± 0.9†	15.8 ± 0.3*	15.9 ± 0.5*	14.5 ± 2.3*	14.6 ± 1.0*	13.6 ± 1.1*	11.7 ± 1.6*
HCT (%)	50.3 ± 3.1†	45.5 ± 1.5*	46.4 ± 1.9*	43.4 ± 6.8	43.5 ± 2.9*	41.1 ± 2.9*	35.9 ± 4.7*
SEG (th/uL)	1.0 ± 0.4	1.8 ± 1.0	1.4 ± 0.7	1.6 ± 0.6	2.4 ± 2.4	2.3 ± 1.6	2.3 ± 1.4
WBC (th/uL)	3.9 ± 0.8	5.6 ± 1.8	5.1 ± 1.5	5.3 ± 2.9	6.4 ± 2.7	5.6 ± 2.9	4.7 ± 3.0
WEEK 14 FEMALES							
RBC (mi/uL)	10.3 ± 0.5†	9.9 ± 0.5	9.4 ± 0.4*	9.0 ± 0.4*	8.7 ± 1.0*	8.4 ± 1.1*	7.7 ± 1.7*
HGB (g/dL)	17.0 ± 0.8†	16.4 ± 0.6	15.2 ± 0.9*	15.0 ± 0.8*	14.3 ± 1.6*	13.9 ± 1.9*	12.4 ± 2.7*
HCT (%)	48.4 ± 2.6†	47.7 ± 1.6	45.1 ± 2.6	45.1 ± 2.8	41.9 ± 4.6*	41.2 ± 5.2*	36.9 ± 7.1*
SEG (th/uL)	0.8 ± 0.5	1.7 ± 1.0	1.9 ± 1.9	1.8 ± 0.7	1.8 ± 1.6	3.4 ± 2.4*	6.1 ± 7.3*
WBC (th/uL)	3.1 ± 1.8	4.0 ± 1.9	4.1 ± 2.9	5.2 ± 1.6	5.0 ± 3.4	5.4 ± 2.6	9.1 ± 9.3

[a]Data Extracted From Study No. 483287, Tables 8 A and 8 B
[b]Values not determined for satellite groups at 75, 200, and 750 mg/kg/day
*Significantly different from control, p≤0.05
†Significant decreasing trend, p≤0.05

Results

Interpretation of most clinical chemistry data was severely limited by the large number of determinations that were coded "No sample received" or "Quantity not sufficient". Only ALT, alkaline phosphatase, AST, and LDH were analyzed for sufficient number of animals for data to be meaningful. Summaries of selected clinical chemistry parameters are presented in Table 6.

Treatment-related increases in interim and final ALT levels were seen among both sexes. At week 7, values were significant at ≥350 mg/kg/day for both sexes. At week 14, values were significant for males at ≥350 mg/kg/day and for females at ≥750 mg/kg/day.

Treatment-related increases in interim and final ALK levels were seen among both sexes. At week 7, values were significant for males at ≥350 mg/kg/day and for females at ≥25 mg/kg/day. At week 14, values were significant for males and females at ≥200 mg/kg/day.

Levels of AST increased at week 14 (significant for males at 350 and 900 mg/kg/day; nonsignificant for females) and at week 7 (nonsignificant for both sexes at 900 mg/kg/day. Levels of LDH increased non-significantly at week 14 (for males at ≥350 mg/kg/day and for females at ≥750 mg/kg/day) and at week 7 (for males only at ≥350 mg/kg/day). These increases may also have been treatment related.

Sacrifice and Pathology

All animals found dead as well as those surviving to the scheduled 14-week sacrifices were subjected to a complete gross examination. At necropsy of interim sacrificed animals, only the liver was examined. Animals were sacrificed by an injection of sodium pentobarbital. Tissues marked with an "X" below were preserved in 10% neutral buffeted formalin from mice in the 14-week groups. Organs marked with an "XX" were also weighed at necropsy. Only liver weights were recorded from animals at the 7-week sacrifice. A section of the left lateral lobe of the liver from animals in the satellite groups was preserved in 100% methanol. Histopathology was performed on all tissues from animals in the control and high-dose groups in the 14-week groups as well as any animals that died or were sacrificed moribund during the study. If treatment-related effects were observed in any tissue in the high-dose group, the next lowest dose group was examined and so on until the effect was no longer observed. Histopathological examinations were not conducted on tissues collected from animals in the 7-week groups.

TABLE 6. Selected Clinical Chemistry Parameters[a,b]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
WEEK 7 MALES							
AST (u/L)	209 ± 66	195 ± 60			204 ± 80		356 ± 352
ALT (u/L)	49 ± 13	49 ± 12			111 ± 43*		334 ± 447*
ALK P (u/L)	72 ± 15	71 ± 12			227 ± 224*		213 ± 111*
LDH (u/L)	937 ± 403	792 ± 207			1124 ± 643		1464 ± 1071
WEEK 7 FEMALES							
AST (u/L)	266 ± 81	256 ± 71			258 ± 92		329 ± 164
ALT (u/L)	53 ± 7	58 ± 21			93 ± 32*		214 ± 206*
ALK P (u/L)	104 ± 5	145 ± 27*			150 ± 83*		236 ± 141*
LDH (u/L)	1208 ± 306	1157 ± 427			1081 ± 614		1383 ± 428
WEEK 14 MALES							
AST (u/L)	142 ± 27	155 ± 60	155 ± 57	210 ± 99	271 ± 114*	317 ± 328	333 ± 220*
ALT (u/L)	66 ± 18	64 ± 33	65 ± 33	114 ± 64	219 ± 169*	408 ± 753*	226 ± 148*
ALK P (u/L)	59 ± 26	83 ± 47	87 ± 19	211 ± 190*	192 ± 45*	258 ± 237*	170 ± 76*
LDH (u/L)	1076 ± 189	1005 ± 121	1052 ± 282	1130 ± 384	1290 ± 207	1447 ± 871	1300 ± 415
WEEK 14 FEMALES							
AST (u/L)	365 ± 455	257 ± 116	235 ± 135	246 ± 129	203 ± 60	559 ± 521	550 ± 753
ALT (u/L)	79 ± 44	63 ± 17	83 ± 47	105 ± 56	99 ± 43	247 ± 118*	216 ± 116*
ALK P (u/L)	71 ± 15	112 ± 35*	127 ± 36*	122 ± 27*	131 ± 23*	119 ± 36*	176 ± 94*
LDH (u/L)	1242 ± 973	1157 ± 427	1019 ± 388	1096 ± 358	1027 ± 388	1894 ± 1616	2633 ± 3766

[a]Data extracted from Study No. HWA 483-287, Tables 9 A and 9 B
[b]Values not determined for satellite groups at 75, 200, and 750 mg/kg/day
*Significantly different from control, p≤0.05

Digestive	Cardiovascular/Hematologic	Neurologic
X Tongue	X Aorta*	XX Brain*+
XX Salivary glands*	XX Heart*	X Peripheral nerve* (sciatic nerve)
X Esophagus*	X Bone marrow*	X Spinal cord* (3 regions)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	XX Spleen*	X Eyes (w/optic nerve*)
X Jejunum*	XX Thymus*	
X Ileum*		Glandular
X Cecum	Urogenital	
X Colon*		XX Adrenal gland*
X Rectum*	XX Kidneys*+	Submaxillary gland
XX Liver*+	X Urinary bladder*	X Mammary gland*
XX Gall bladder*	XX Testes*+	X Parathyroids*++
X Pancreas*	XX Epididymides	X Thyroids*++
	XX Prostate	X Lacrimal gland
Respiratory	Seminal vesicles	
	XX Ovaries*+	Other
X Trachea*	XX Uterus*	
XX Lung*	X Vagina	X Skeletal muscle*
Bronchus		X Bone (femur)*
Pharynx		X Sternum
Larynx		X Skin
		X Gross lesions*

* Required for subchronic and chronic studies.
\+ Organ weight required for subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

Results

Organ Weights

Summaries of affected organ weights are presented in Tables 7 A (main groups, males), 7 B (main groups, females), and 7 C (satellite groups, males and females). Significant compound-related effects on the liver/gallbladder were observed at ≥75 mg/kg/day in the main groups and at ≥350 mg/kg/day in the satellite groups. Additional treatment-related effects were noted on kidney, adrenal, salivary gland, and uterine weights at 350, 750, and/or 900 mg/kg/day (see details below).

Significant increasing trends were observed in absolute and relative (to brain and body weights) liver/gallbladder weights in males and females. In the main groups at dose levels of ≥75 mg/kg/day, significant increases in absolute and relative liver/gallbladder weights ranged from 123% to 310% of controls. In the satellite groups at dose levels of ≥350 mg/kg/day, significant increases in absolute and relative (to body weight) liver/gallbladder weights ranged from 188% to 309% of controls.

Significant decreasing trends were observed in absolute and relative (to body weight) kidney weights among males and females. In males, at dose levels of ≥350 mg/kg/day, significant decreases in absolute and relative kidney weights ranged from 79% to 89% of controls. In females, decreased kidney weights reached a significant level only at 900 mg/kg/day for absolute weights (87%) which were probably not treatment related.

Absolute and relative (to body weights) salivary gland weights decreased significantly (73%-83% of controls) in both sexes at 750 and 900 mg/kg/day.

Absolute and relative (to body and brain weights) adrenal weights increased significantly (≈134% of controls) in males at 750 and 900 mg/kg/day. A similar effect was not observed in females.

TABLE 7 A. Main Groups - Selected Organ Weights in Males (± S.D.)[a,b]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
Salivary Gland							
Absolute (g)	0.22 ± 0.04	0.21 ± 0.04 (95)	0.21 ± 0.03 (95)	0.21 ± 0.02 (95)	0.20 ± 0.04 (91)	0.16 ± 0.03* (73)	0.16 ± 0.04* (73)
Relative to body weight	0.82 ± 0.15	0.78 ± 0.16 (95)	0.75 ± 0.11 (91)	0.74 ± 0.06 (90)	0.69 ± 0.12* (84)	0.60 ± 0.11* (73)	0.61 ± 0.16* (74)
Relative to brain weight	0.46 ± 0.08	0.44 ± 0.10 (96)	0.44 ± 0.07 (96)	0.44 ± 0.06 (96)	0.41 ± 0.08 (89)	0.33 ± 0.07* (72)	0.34 ± 0.09* (74)
Kidney							
Absolute (g)	0.53 ± 0.07†	0.51 ± 0.06 (96)	0.49 ± 0.08 (92)	0.49 ± 0.04 (92)	0.47 ± 0.03* (89)	0.43 ± 0.04* (81)	0.45 ± 0.05* (85)
Relative to body weight	1.96 ± 0.28†	1.83 ± 0.19 (93)	1.74 ± 0.27* (89)	1.70 ± 0.18 (87)	1.62 ± 0.14* (83)	1.62 ± 0.13* (83)	1.66 ± 0.12* (85)
Relative to brain weight	1.11 ± 0.15	1.01 ± 0.09 (91)	1.01 ± 0.18 (91)	1.02 ± 0.13 (92)	0.96 ± 0.09* (86)	0.88 ± 0.10* (79)	0.93 ± 0.11* (84)
Liver/Gallbladder							
Absolute (g)	1.25 ± 0.12†	1.32 ± 0.12 (106)	1.63 ± 0.15* (130)	2.08 ± 0.29* (166)	2.61 ± 0.32* (209)	3.52 ± 0.42* (282)	3.70 ± 0.68* (296)
Relative to body weight	4.68 ± 0.56†	4.77 ± 0.31 (102)	5.75 ± 0.47* (123)	7.16 ± 0.85* (153)	8.97 ± 0.95* (192)	13.14 ± 1.43* (281)	13.72 ± 1.71* (293)
Relative brain weight	2.65 ± 0.27†	2.66 ± 0.32 (100)	3.35 ± 0.43* (126)	4.31 ± 0.86* (163)	5.36 ± 0.76* (202)	7.22 ± 1.18* (272)	7.70 ± 1.41* (291)
Adrenal							
Absolute (g)	0.009 ± 0.002	0.007 ± 0.003 (78)	0.007 ± 0.002 (78)	0.009 ± 0.002 (100)	0.011 ± 0.003 (122)	0.012 ± 0.003* (133)	0.012 ± 0.002* (133)
Relative to body weight	0.034 ± 0.010	0.024 ± 0.011 (71)	0.026 ± 0.009 (76)	0.029 ± 0.007 (85)	0.039 ± 0.011 (115)	0.045 ± 0.012* (132)	0.046 ± 0.008* (135)
Relative to brain weight	0.019 ± 0.005	0.013 ± 0.006* (68)	0.015 ± 0.006 (79)	0.018 ± 0.005 (95)	0.023 ± 0.006 (121)	0.025 ± 0.007* (132)	0.025 ± 0.004* (132)

[a]Data extracted from Study No. HWA 483-287, Tables 11 A, 12 A, and 13
[b]Percentage of control within parenthesis
*Significantly different from control, p≤0.05
†Significant decreasing or increasing trend, p≤0.05

TABLE 7 B. Main Groups - Selected Organ Weights in Females (± S.D.)[a,b]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
Salivary Gland							
Absolute (g)	0.15 ± 0.02	0.14 ± 0.03 (93)	0.16 ± 0.03 (107)	0.13 ± 0.02 (87)	0.14 ± 0.02 (93)	0.12 ± 0.03* (80)	0.11 ± 0.02* (73)
Relative to body weight	0.63 ± 0.09	0.60 ± 0.11 (95)	0.66 ± 0.11 (105)	0.54 ± 0.08 (86)	0.58 ± 0.09 (92)	0.51 ± 0.12* (81)	0.52 ± 0.10* (83)
Relative to brain weight	0.29 ± 0.05	0.27 ± 0.06 (93)	0.32 ± 0.07 (110)	0.26 ± 0.04 (90)	0.28 ± 0.05 (97)	0.24 ± 0.06 (83)	0.24 ± 0.06 (83)
Kidney							
Absolute (g)	0.38 ± 0.03†	0.38 ± 0.04 (100)	0.37 ± 0.04 (97)	0.38 ± 0.04 (100)	0.38 ± 0.05 (100)	0.35 ± 0.05 (92)	0.33 ± 0.04* (87)
Relative to body weight	1.58 ± 0.10†	1.60 ± 0.16 (101)	1.54 ± 0.15 (97)	1.53 ± 0.16 (97)	1.51 ± 0.15 (96)	1.50 ± 0.15 (95)	1.49 ± 0.17 (94)
Relative to brain weight	0.72 ± 0.05	0.73 ± 0.09 (101)	0.74 ± 0.09 (103)	0.73 ± 0.08 (101)	0.73 ± 0.09 (101)	0.72 ± 0.10 (100)	0.69 ± 0.08 (96)
Liver/Gallbladder							
Absolute (g)	1.08 ± 0.13†	1.16 ± 0.12 (107)	1.40 ± 0.23* (130)	1.94 ± 0.25* (180)	2.41 ± 0.44* (223)	3.03 ± 0.36* (281)	3.04 ± 0.44* (281)
Relative to body weight	4.51 ± 0.32†	4.91 ± 0.35 (92)	5.73 ± 0.66* (127)	7.87 ± 0.92* (175)	9.61 ± 1.37* (213)	12.96 ± 1.42* (287)	13.76 ± 1.62* (305)
Relative to brain weight	2.06 ± 0.27†	2.24 ± 0.26 (109)	2.77 ± 0.58* (134)	3.74 ± 0.49* (182)	4.64 ± 0.90* (225)	6.26 ± 0.85* (304)	6.39 ± 0.99* (310)
Adrenal							
Absolute (g)	0.013 ± 0.002	0.011 ± 0.003 (85)	0.013 ± 0.003 (100)	0.013 ± 0.003 (100)	0.012 ± 0.003 (92)	0.013 ± 0.003 (100)	0.011 ± 0.002 (85)
Relative to body weight	0.055 ± 0.008	0.048 ± 0.012 (87)	0.056 ± 0.015 (102)	0.054 ± 0.013 (98)	0.048 ± 0.013 (87)	0.056 ± 0.012 (102)	0.051 ± 0.009 (93)
Relative to brain weight	0.025 ± 0.003	0.022 ± 0.005 (88)	0.026 ± 0.006 (104)	0.026 ± 0.007 (104)	0.023 ± 0.007 (92)	0.027 ± 0.005 (108)	0.024 ± 0.005 (96)
Uterus							
Absolute (g)	0.22 ± 0.06	0.25 ± 0.09 (114)	0.22 ± 0.10 (100)	0.19 ± 0.06 (86)	0.18 ± 0.07 (82)	0.12 ± 0.05* (55)	0.13 ± 0.06* (59)
Relative to body weight	0.91 ± 0.27	1.07 ± 0.35 (118	0.92 ± 0.41 (101)	0.76 ± 0.23 (84)	0.71 ± 0.27 (78)	0.50 ± 0.21* (55)	0.57 ± 0.28* (63)
Relative to brain weight	0.42 ± 0.12	0.49 ± 0.18 (117)	0.43 ± 0.17 (102)	0.36 ± 0.12 (86)	0.34 ± 0.13 (81)	0.24 ± 0.09* (57)	0.26 ± 0.13* (62)
Ovary							
Absolute (g)	0.04 ± 0.02	0.05 ± 0.01 (125)	0.04 ± 0.01 (100)	0.04 ± 0.02 (100)	0.05 ± 0.02 (125)	0.04 ± 0.01 (100)	0.03 ± 0.01* (75)
Relative to body weight	0.18 ± 0.07	0.21 ± 0.05 (117)	0.18 ± 0.05 (100)	0.17 ± 0.06 (94)	0.18 ± 0.06 (100)	0.15 ± 0.04 (83)	0.13 ± 0.04 (72)
Relative to brain weight	0.08 ± 0.03	0.09 ± 0.02 (113)	0.09 ± 0.02 (113)	0.08 ± 0.03 (100)	0.09 ± 0.03 (113)	0.07 ± 0.02 (88)	0.06 ± 0.02 (75)

[a]Data extracted from Study No. HWA 483-287, Tables 11 A, 12 A, and 13
[b]Percentage of control within parentheses
*Significantly different from control, p≤0.05
†Significant decreasing or increasing trend, p≤0.05

TABLE 7 C. Satellite Groups - Liver/Gallbladder Weights[a,b]

Observation	Dose Level (mg/kg/day) 0	25	350	900
		Males		
Liver/Gallbladder				
Absolute (g)	1.24 ± 0.08†	1.36 ± 0.14 (110)	2.51 ± 0.29* (202)	3.50 ± 0.56* (282)
Relative to body weight	4.71 ± 0.27†	5.26 ± 0.75 (112)	9.30 ± 0.65* (197)	14.55 ± 1.36* (309)
		Females		
Liver/Gallbladder				
Absolute (g)	1.05 ± 0.10†	1.10 ± 0.10 (105)	2.08 ± 0.13* (198)	2.96 ± 0.47* (282)
Relative to body weight	4.78 ± 0.25†	5.15 ± 0.38 (103)	9.01 ± 0.38* (188)	13.54 ± 2.05* (283)

[a]Data Extracted From Study No. 483287, Tables 11 B and 12 B
[b]Percentage of control within parenthesis
*Significantly different from control, p≤0.05
†Significant increasing trend, p≤0.05

Absolute and relative (to body and brain weights) uterine weights decreased significantly (55%-63% of controls) in females at 750 and 900 mg/kg/day. Absolute and relative (to body and brain weights) ovarian weights also decreased at these dose levels but reached a significant level only at 900 mg/kg/day for absolute weights (75%).

Gross Pathology

Selected gross findings are presented in Table 8. Compound-related effects were observed at ≥75 mg/kg/day as evidenced by liver changes including pale areas, dark livers, and/or enlarged livers. In addition, the incidence of males (but not females) with dark areas in the stomach was greater than control in both groups (main and satellite) at ≥350 mg/kg/day.

Microscopic Pathology

A summary of histopathology findings is presented in Tables 9 A (males) and 9 B (females). Compound-related effects were observed at all dose levels as manifested by findings in the liver. At 25 mg/kg/day, however, increases and severity of lesions indicated that effects may not have been adverse. Additional findings in the glandular stomach, female kidney, adrenal cortex, female mammary gland, uterus, and cervix, also believed to be treatment related, were noted at 200 mg/kg/day or higher doses (see below for details).

Liver changes were observed in a dose-related manner at ≥25 mg/kg/day in males (increased incidences of hypertrophic hepatocytes, vacuolization, and chronic inflammation); at ≥75 mg/kg/day in males (pigmented Kupffer cells/macrophages, and necrosis); at ≥200 mg/kg/day in males (pigmented bile and hepatocytes); and at ≥200 mg/kg/day in females (hypertrophic hepatocytes, vacuolization, pigmented Kupffer cells/macrophages and hepatocytes, and necrosis). Additional dose-related liver findings included increased incidences of chronic bile duct inflammation and mineralization at ≥200 mg/kg/day in males and chronic bile duct inflammation at ≥350 mg/kg/day in females.

In the spleen, the severity of increased extramedullary hematopoiesis was greater than controls at ≥200 mg/kg/day for males and at ≥750 mg/kg/day for females.

TABLE 8. Selected Gross Pathology Findings[a,b,c]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
MALES, 7 WEEKS							
Liver							
Pale area	1/10	0/10			7/10		10/10
Dark	0/10	0/10			5/10		9/10
Enlarged	0/10	0/10			3/10		8/10
Stomach							
Dark area	0/10	0/10			1/10		2/10
FEMALES, 7 WEEKS							
Liver							
Pale area	0/10	1/10			8/10		9/10
Dark	0/10	0/10			7/10		5/10
Enlarged	0/10	0/10			4/10		4/10
Stomach							
Dark area	0/10	0/10			0/10		0/10
MALES, 14 WEEKS							
Liver							
Pale area	0/15	0/15	0/15	4/15	8/15	9/15	13/15
Enlarged	0/15	0/15	1/15	6/15	13/15	15/15	12/15
Dark	0/15	1/15	2/15	11/15	14/15	15/15	13/15
Mass	0/15	0/15	1/15	0/15	0/15	0/15	0/15
Stomach							
Dark area	0/15	0/15	0/15	0/15	0/15	3/15	4/15
FEMALES, 14 WEEKS							
Liver							
Pale area	0/15	0/15	2/15	3/15	5/15	5/15	11/15
Enlarged	0/15	0/15	1/15	7/15	12/15	14/15	13/15
Dark	0/15	0/15	2/15	10/15	13/15	14/15	14/15
Mass	0/15	0/15	1/15	0/15	0/15	0/15	0/15
Dark area	0/15	0/15	1/15	0/15	0/15	0/15	0/15
Stomach							
Dark area	0/15	1/15	2/15	0/15	2/15	0/15	2/15

[a]Data extracted from Study No. HWA 483-287, Tables 10 A, B, and C
[b]Includes animals found dead and sacrificed moribund.
[c]Not examined for satellite groups at 75, 200, and 750 mg/kg/day

TABLE 9 A. Selected Histopathology Findings in Males[a,b,c]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
Spleen							
Extramedullary hemato-poiesis, increased	15/15 (1.8)	---[d]	8/10 (1.6)	8/10 (2.4)	14/15 (2.0)	15/15 (2.4)	14/15 (2.7)
Stomach							
Hyperplasia	0/14	---	0/10	0/9	0/15	1/15 (2.0)	3/15 (2.0)
Hyperplasia, cystic	1/14 (1.0)	---	1/10 (1.0)	6/9 (1.2)	7/15 (1.1)	7/15 (1.0)	5/15 (1.6)
Adrenal Cortex							
Hypertrophy, zona fasciculata	0/15	0/15	0/10	2/10 (1.0)	11/15 (1.0)	9/15 (1.0)	12/15 (1.5)
Kidney							
Inflammation chronic	9/15 (1.0)	---	---	0/2	1/1 (2.0)	---	10/15 (1.0)
Tubule, mineralization	0/15	---	---	0/2	1/1 (1.0)	---	2/15 (1.0)
Tubule regeneration	6/15 (1.0)	---	---	0/2	1/1 (2.0)	---	10/15 (1.6)
Hyperplasia, lymphoid	5/15 (1.0)	---	---	0/2	1/1 (1.0)	---	8/15 (1.0)
Liver							
Hepatocyte, hypertrophy centrilobular	3/15 (1.3)	6/15 (1.0)	12/15 (1.4)	13/14 (2.0)	15/15 (2.5)	15/15 (3.4)	15/15 (3.7)
Vacuolization	0/15	2/15 (1.0)	7/15 (1.3)	11/14 (1.7)	14/15 (1.7)	13/15 (1.5)	13/15 (1.1)
Pigment, bile	0/15	0/15	1/15 (1.0)	7/14 (1.0)	12/15 (1.0)	15/15 (1.4)	13/15 (1.8)
Kupffer cell/macrophage pigment	0/15	0/15	5/15 (1.0)	13/14 (1.2)	15/15 (1.1)	15/15 (1.3)	13/15 (1.8)
Hepatocyte pigment	0/15	0/15	0/15	12/14 (1.5)	14/15 (1.2)	15/15 (1.8)	13/15 (1.8)
Necrosis	0/15	0/15	1/15 (1.0)	5/14 (1.4)	10/15 (1.7)	7/15 (2.0)	8/15 (3.0)
Necrosis, indiv. cell	0/15	1/15 (1.0)	4/15 (1.0)	12/14 (1.3)	14/15 (1.3)	15/15 (1.7)	15/15 (1.7)
Inflammation, chronic	1/15 (1.0)	4/15 (1.0)	5/15 (1.0)	13/14 (1.2)	14/15 (1.5)	12/15 (1.3)	9/15 (2.2)
Bile duct inflammation	0/15	0/15	0/15	2/14 (1.0)	10/15 (1.0)	8/15 (1.6)	6/15 (1.2)
Mineralization	0/15	0/15	0/15	1/14 (1.0)	3/15 (1.0)	3/15 (1.0)	5/15 (1.3)
Mandibular Lymph Node							
Macrophages pigmented	3/13 (1.0)	---	---	0/2	---	---	8/15 (1.0)
Marrow of Femur							
Hypercellularity	0/15	---	---	0/2	---	---	4/15 present

[a]Data extracted from Study No. MMA 483-287, Tables 14 A and 14 B and page 51
[b]Includes animals found dead and sacrificed moribund
[c]Within parenthesis, mean severity score (based on number of organs in which change was observed)
[d]Not examined

TABLE 9 B. Selected Histopathological Findings in Females[a,b,c]

Observation	Dose Level (mg/kg/day) 0	25	75	200	350	750	900
Spleen							
Extramedullary hemato-poiesis, increased	12/15 (1.8)	---[d]	---	---	11/15 (1.2)	14/14 (2.2)	14/15 (2.6)
Stomach							
Hyperplasia	0/15	0/1	1/2 (1.0)	0/10	1/15 (2.0)	0/15	2/15 (1.0)
Hyperplasia, cystic	2/15 (1.0)	0/1	1/2 (2.0)	3/10 (1.0)	5/15 (1.2)	10/15 (1.1)	4/15 (1.8)
Liver							
Hepatocyte, hypertrophy centrilobular	0/15	2/15 (1.0)	3/15 (1.0)	15/15 (1.3)	14/15 (2.1)	14/14 (2.0)	15/15 (3.0)
Vacuolization	0/15	1/15 (1.0)	2/15 (1.5)	8/15 (2.0)	7/15 (2.3)	13/14 (1.2)	14/15 (1.5)
Pigment, bile	0/15	0/15	0/15	1/15 (1.0)	7/15 (1.0)	14/14 (1.0)	14/15 (1.1)
Kupffer cell/macrophage pigment	2/15 (1.0)	0/15	2/15 (1.0)	8/15 (1.3)	14/15 (1.3)	14/14 (1.7)	15/15 (1.2)
Hepatocyte pigment	0/15	0/15	0/15	12/15 (1.1)	14/15 (1.3)	14/14 (2.1)	13/15 (1.9)
Necrosis	1/15 (1.0)	0/15	3/15 (1.0)	3/15 (1.0)	5/15 (1.4)	3/14 (2.7)	7/15 (1.9)
Necrosis, indiv. cell	0/15	3/15 (1.0)	2/15 (1.0)	8/15 (1.0)	11/15 (1.0)	14/14 (1.2)	13/15 (1.4)
Inflammation, chronic	7/15 (1.0)	7/15 (1.0)	9/15 (1.0)	10/15 (1.1)	12/15 (1.3)	11/14 (1.4)	7/15 (1.6)
Bile duct inflammation	2/15 (1.0)	0/15	0/15	2/15 (1.0)	4/15 (1.0)	12/14 (1.1)	10/15 (1.2)
Kidney							
Inflammation, chronic	5/15 (1.0)	---	3/9 (1.0)	2/10 (1.0)	6/15 (1.8)	9/14 (1.3)	8/15 (1.5)
Tubule regeneration	1/15	---	1/9 (1.0)	1/10 (1.0)	5/10 (1.0)	1/14 (1.0)	7/15 (1.4)
Hyperplasia, lymphoid	1/15 (1.0)	---	0/9	0/10	0/15	0/14	5/15 (1.0)
Inflammation, subacute	0/15	---	0/9	5/10 (1.2)	8/15 (1.0)	4/14 (1.0)	4/15 (1.0)
Uterus							
Hypoplasia	3/15 (1.7)	0/2	2/9 (1.0)	3/10 (3.0)	9/14 (2.7)	13/13 (3.4)	13/15 (3.9)
Uterus, Cervix							
Hypoplasia	1/14 (1.0)	---	0/8	1/9 (4.0)	2/14 (2.0)	2/13 (4.0)	7/15 (3.3)
Mammary							
Dilatation, cystic	0/15	---	---	---	0/12	5/14 (1.4)	8/15 (1.1)
Hypoplasia, epithelial	0/15	---	---	---	0/12	4/14 present	8/15 present
Heart							
Inflammation, chronic	1/15 (1.0)	---	---	---	---	---	6/15 (1.0)
Cecum							
Hyperplasia, lymphoid	0/15	---	---	---	---	---	8/15 present

[a]Data extracted from Study No. MHA 483-287, Tables 14 A and 14 B and page 51
[b]Includes animals found dead and sacrificed moribund
[c]Within parenthesis, mean severity score (based on number of organs in which change was observed)
[d]Not examined

The following incidences were also considered treatment related: cystic hyperplasia of the stomach (≥200 mg/kg/day for males and ≥350 mg/kg/day for females); hypertrophy of the adrenal cortex (≥200 mg/kg/day for males); chronic inflammation of the kidney (≥750 mg/kg/day for females); tubule regeneration of the kidney (900 mg/kg/day for females); uterine and cervical hypoplasia (≥350 mg/kg/day); and cystic dilatation and epithelial hypoplasia of the mammary gland (≥750 mg/kg/day for females).

Of less importance, but slightly increased, were the following incidences: pigmented macrophages in the mandibular lymph node and hypercellularity of the marrow in the femur (900 mg/kg/day for males); chronic inflammation of the heart (900 mg/kg/day for females); and hypoplasia of the cecum (900 mg/kg/day for females).

D. DISCUSSION

Reviews of the main and satellite groups with supporting data indicate that the conduct of the study was adequate and the reporting of the results was accurate. The target organ for triclosan toxicity appears to be the liver as manifested by corresponding changes in organ weight and enzyme levels as well as gross and histopathological lesions in the liver. Adverse effects, dose dependent and first appearing at the lowest dose level in both sexes, consisted of changes in enzyme levels (alanine aminotransferase and alkaline phosphatase, indicative of liver injury) and slight increases in incidences of selected liver lesions. Histological lesions generally became more severe at higher doses. Increased liver weights and gross findings appeared at 75 mg/kg/day; clinical signs appeared at 750 mg/kg/day; and finally, body weight gain was affected at the highest dose level.

At ≥25 mg/kg/day, dose-dependent changes in several hematology parameters (erythrocyte, hemoglobin, and hematocrit values) were also observed in both sexes. There were no accompanying gross findings in the spleen or effects on spleen weight. Few additional animals (compared to controls) were noted with increased extramedullary hematopoiesis. However, the severity of the increase was noticeable at ≥200 mg/kg/day in males and at ≥750 mg/kg/day in females, thus indicating that this effect was treatment related. Most likely related to these effects were the increased pigmentation of macrophages in the mandibular lymph node and hypercellularity of the marrow of the femur in males at the highest dose level.

Female reproductive effects in the uterus were manifested as decreased organ weight and increased hypoplasia at ≥200 mg/kg/day. The degree of severity of the uterine hypoplasia was especially noticeable. An increased incidence was also noted in mammary gland hypoplasia and cystic dilatation at ≥750 mg/kg/day (but severity of the lesions did not increase). The male reproductive system was not affected.

Dose-related effects on kidney weights were more prominent in males (absolute and relative weights at ≥350 mg/kg/day) than in females (absolute weight only at 900 mg/kg/day). However, the opposite was noted with regard to histopathological lesions of the kidney evident in both sexes at ≥350 mg/kg/day, but occurring at higher incidences in females and not always in a dose related manner.

Hyperplasia of the stomach increased slightly in both sexes (incidence and severity of lesion) at ≥200 mg/kg/day. These findings are equivocal, however, since there was no clear dose response.

Based on changes in clinical chemistry and hematology parameters and increased incidences of liver lesions (hepatocellular hypertrophy [males and females], inflammation [males], and necrosis [females]) at the lowest dose level, the LOEL for systemic toxicity was 25 mg/kg/day; the NOEL could not be determined.

E. STUDY DEFICIENCIES

011304

Results from the clinical chemistry data were limited by "quantity not sufficient", i.e., few parameters could be evaluated meaningfully. Also, marrow should have been examined at the lower doses since a slight effect was observed at the highest dose.

F. CLASSIFICATION

The study has been classified as Core Minimum and satisfies the minimum requirements for a subchronic study in mice. In spite of inadequate clinical chemistry data, several important hepatic indicators were measurable.