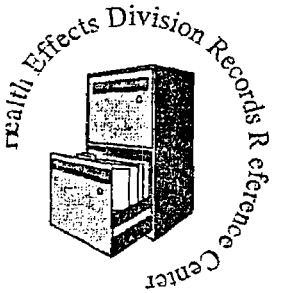
HIARC Briefing Packages

PC Code

054901

Date of Package

3-10-98



Signature & Date

B Goblad 8-4-00

3/10/1998

THE HAZID MEETING FOR **TRICLOSAN** WILL BE HELD ON TUESDAY, MARCH 10, IN ROOM 817 FROM 9:00 TO 12:00.

K. Baetcke
W. Burnam
R. Fricke
K. Hamernik
S. Makris
M. Metzger
M. Morrow
J. Redden (Rm. 246)
J. Rowland
C. Swentzel

Reviewer/Presenter: T. McMahon

Other: S. Diwan

(2)

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

Memorandum

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: TOX-SAC report on Trichlosan

From: Joycelyn E. Stewart, Ph.D., Chair,
TOX-SAC, HED

To: George Ghali, Ph.D., Executive Secretary
HAZ ID SARC

To: K. Clark Swentzel, Chair
HAZ ID SARC

The Toxicology Science Advisory Council has evaluated the available DER's for the chemical Trichlosan, and has concluded the following:

Developmental toxicity-rabbit DER Acceptable with revisions Study Acceptable	MRID 43022607	See comments
Developmental toxicity-rabbit DER Acceptable with revisions Study Acceptable	MRID 43820401 43787101	See comments
Developmental toxicity-rats DER Acceptable with revisions Study Unacceptable	MRID 43022606	See comments
Multigen. Repro study-rats DER Acceptable with revisions Study Unacceptable	MRID 41418501	See comments
Developmental toxicity-rats DER Acceptable with revisions Study Acceptable	MRID 43825301 43820402	See comments
Developmental toxicity-rats DER Acceptable with revisions Study Acceptable	MRID 43817502 43817503	See comments

Chronic/onco-rat DER Acceptable with revisions Study Acceptable	MRID 42027906 Acc 263791	See comments
90 day dermal-rats DER Acceptable Study Acceptable	MRID 43328001	
90 day oral study-dogs DER Unacceptable Study Acceptable	MRID 001958 001968	See comments
Chronic Oral Study-baboons DER Unacceptable Study Acceptable	MRID 257773	See comments
Subchronic study-mice DER Acceptable Study Acceptable	MRID 43022605	
28 day oral toxicity-mice DER Acceptable Study Acceptable	MRID 44389707	See comments
Biochemical Studies DER's Acceptable with revisions Studies Acceptable	MRID 44389701	See comments

The TOX-SAC did not review the DER's for the acute studies

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-3a Developmental Toxicity - Rabbit Biodynamics 91-3666 4/16/92 Dose Levels: 0, 15, 50, 150 mg/kg/day Administered Gestation Days 6-18 inclusive	43022607	Mat.Tox.NOEL = 50 mg/kg/day Mat.Tox.LOEL = 150 mg/kg/day based on decreased body weight gains and food consumption. Dev.Tox.NOEL ≥ 150 mg/kg/day Dev.Tox.LOEL > 150 mg/kg/day	Acceptable with revisions to Executive Summary: study should be classified as Acceptable - Guideline. This is an earlier review of the review of Biodynamics 91-3666 and 91-3655, 4/16/92 and 5/6/92 combined	Acceptable.	Study should be classified as Acceptable - Guideline. Changes can be made to one-liners only
TOX SAC Member Signature: Stephen C. Dapson				Date: 1/8/98	

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-3a Developmental Toxicity - Rabbit Biodynamics 91-3666 and 91-3655 4/16/92 and 5/6/92 Dose Levels: 0, 15, 50, 150 mg/kg/day Administered Gestation Days 6-18 inclusive	43820401 43787101	Mat.Tox.NOEL = 50 mg/kg/day Mat.Tox.LOEL = 150 mg/kg/day based on decreased body weight gains and food consumption. Dev.Tox.NOEL ≥ 150 mg/kg/day Dev.Tox.LOEL > 150 mg/kg/day	Acceptable with revisions to Executive Summary: study should be classified as Acceptable - Guideline.	Acceptable.	Study should be classified as Acceptable - Guideline. Changes can be made to one-liners only
TOX SAC Member Signature: Stephen C. Dapson					Date: 1/8/98

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-3a Developmental Toxicity - Rat Bio/dynamics 91-3665 4/16/92 Dose Levels: 0, 15, 50, 150 mg/kg/day Administered Gestation Days 6-15 inclusive	43022606	Mat.Tox.NOEL ≥ 150 mg/kg/day Mat.Tox.LOEL > 150 mg/kg/day Dev.Tox.NOEL ≥ 150 mg/kg/day Dev.Tox LOEL > 150 mg/kg/day.	Acceptable with revisions to Executive Summary: study should be classified as Unacceptable - Guideline. This is an earlier review of the review of Bio/dynamics 91-3665 and 91-3654, 4/16/92 and 5/6/92 combined	Unacceptable but unacceptable study is available (see Environmental Safety Laboratory, RT/3/84, 12/92 MRID# 43817502 and 43817503	Study should be classified as Unacceptable - Guideline. Changes can be made to one-liners only.
TOX SAC Member Signature: Stephen C. Dapson				Date: 1/8/98	

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TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-4 Multi Generation Reproduction -Rat Hazleton Laboratories America, Inc. HLA No. 2386-100 3/18/88 Dose Levels: 0, 300, 1000, 3000 ppm (assume 0, 15, 50, 150 mg/kg/day, need to be added to DER or one-liners)	41418501	Mat/Pat/Systemic Tox NOEL = 1000 ppm (50 mg/kg/day) Mat/Pat/Systemic Tox LOEL = 3000 ppm (150 mg/kg/day) based on reduced body weights. Repro Tox NOEL = 1000 ppm (50 mg/kg/day) Repro Tox LOEL = 3000 ppm (150 mg/kg/day) based on reduced viability. Dev.Tox NOEL = = 1000 ppm (50 mg/kg/day) Dev.Tox LOEL = = 3000 ppm (150 mg/kg/day) based on reduced body weights.	Acceptable with revisions to Executive Summary: format should be new version, calculated doses in mg/kg/day need to be added to DER (new executive summary), study should be classified as Unacceptable - Guideline.	Unacceptable, there is no indication if clarification asked for in review was provided.	Study should be classified as Unacceptable - Guideline. See DER section.
TOX SAC Member Signature: Stephen C. Dapson				Date: 1/8/98	

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-3a Developmental Toxicity - Rat Environmental Safety Laboratory RT/3/84 12/92 Dose Levels: 0, 30, 100, 300 mg/kg/day Administered Gestation Days 6-15 inclusive	43817502 43817503	Mat.Tox.NOEL = 100 mg/kg/day Mat.Tox.LOEL = 300 mg/kg/day based on increased clinical signs, decreased body weight gains, and food consumption and increased water consumption. Dev.Tox.NOEL ≥ 300 mg/kg/day Dev.Tox LOEL > 300 mg/kg/day.	Acceptable with revisions to Executive Summary: study should be classified as Acceptable - Guideline.	Acceptable	Study should be classified as Acceptable - Guideline. Changes can be made to one-liners only.
TOX SAC Member Signature: Stephen P. Dapson					Date: 1/8/98

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TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Triclosan					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
§83-3a Developmental Toxicity - Rat Bio/dynamics 91-3665 and 91-3654 4/16/92 and 5/6/92 Dose Levels: 0, 15, 50, 150 mg/kg/day Administered Gestation Days 6-15 inclusive	43825301 43820402	Mat.Tox.NOEL ≥ 150 mg/kg/day Mat.Tox.LOEL > 150 mg/kg/day Dev.Tox.NOEL ≥ 150 mg/kg/day Dev.Tox LOEL > 150 mg/kg/day.	Acceptable with revisions to Executive Summary: study should be classified as Unacceptable - Guideline.	Unacceptable but unacceptable study is available (see Environmental Safety Laboratory, RT/3/84, 12/92 MRID# 43817502 and 43817503	Study should be classified as Unacceptable - Guideline. Changes can be made to one-liners only.
TOX SAC Member Signature: Stephen C Dapson					Date: 1/8/98

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Irgasan DP-300					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments

90 Day Subacute Oral Toxicity Study with Irgasan DP-300 in Beagle Dogs	00195 8, 00196 8	NOEL = 12.5 mg/kg/day by gavage; LOEL = 25 mg/kg/day based on liver histopath ology consistin g of focal acidophil ic to granular degenerat ion of the cytoplasm of adjacent hepatocyt es.	Unacceptable Executive Summary is needed	Acceptable	Tables of Individual Results taken from the study would assist HAZARD ID Committee in reaching conclusion regarding NOEL and LOEL
TOX SAC Member Signature: William Dykstra					Date: 11/17/98

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TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Irgasan 054901					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments

90-Day Dermal Toxicity Study in Rats; Exxon No. 139910b; 1994	433280 01	NOEL = 40 mg/kg/day and LOEL = 80 mg/kg/day *[HDT] based* on occult blood in the urine in males of both regular [2/9] and satellite [3/9] groups in comparison to controls [0/10]. Additionally, 1/10 females of the 80 mg/kg/day group had occult blood in the urine in comparison to zero in the controls	Acceptable	Acceptable	Test material was dissolved in PPG
TOX SAC Member Signature: William Dykstra				Date: 1/12/98	

TOX SAC REVIEW ASSESSMENT

CHEMICAL NAME: Triclosan (PC Code: 054901)

Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments
Subchronic oral toxicity-mice	43022605	LOEL(mg/kg/day) = 25 (LDT)	Acceptable	Acceptable	
28-day oral toxicity-mice	44389707	NOEL (mg/kg/day) =6.48(M)/8.25(F) LOEL (mg/kg/day) =135.59(M)/168.78(F)	Acceptable (Nonguideline)	Acceptable (Nonguideline)	The study was conducted according to OECD guideline.
Nonguideline studies	1.No # 2.44389701 3. No # 4. No #	Supplementary info.	Acceptable (Nonguideline)	Acceptable (Nonguideline)	Need executive summaries for oneliner.

TOX SAC Member Signature: Yung G. Yang **Date:** 1/14/98

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TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Irgasan, DP-300, FAT 80 023/A					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments

1 Yr. Oral Toxicity Study in Baboons with Compound FAT 80 023/A; A.J.C. Drake and A. Buxtorf; Geigy Pharmaceuticals, Toxicology Department, Stamford Lodge, Wilmslow, Cheshire, June 28, 1976.	25777 3	NOEL = 30 mg/kg/day ; LOEL = 100 mg/kg/day based on vomiting, failure to eat, and diarrhea	Unacceptable Executive Summary is needed.	Acceptable	Tables of Individual Results taken from the study would assisst the Hazard ID Committee in assessing the NOEL and LOEL for this study.

TOX SAC Member Signature: William Dykstra	Date: 11/7/98

1 Yr. Oral Toxicity Study in Baboons with Compound FAT 80 023/A; A.J.C. Drake and A. Buxtorf; Geigy Pharmaceuticals, Toxicology Department, Stamford Lodge, Wilmslow, Cheshire, June 28, 1976.	25777 3	NOEL = 30 mg/kg/day ; LOEL = 100 mg/kg/day based on vomiting, failure to eat, and diarrhea	Unacceptable Executive Summary is needed.	Acceptable	Tables of Individual Results taken from the study would assisst the Hazard ID Committee in assessing the NOEL and LOEL for this study.

TOX SAC Member Signature: William Dykstra	Date: 11/7/98

TOX SAC REVIEW ASSESSMENT					
CHEMICAL NAME: Irgasan 054901					
Study Type	MRID#	Results (NOEL/LOEL)	DER (Acceptable/ Not Acceptable)	Study (Acceptable/ Not Acceptable)	Comments

Combined chronic toxicity/carcinogenicity-rat	Acc. # 263791 MRID # 42027906	NOEL = 1000 ppm; LOEL = 3000 ppm based on significantly decreased body weights in males & females & non-neoplastic liver changes in males	Acceptable	Acceptable	Executive Summary should be prepared using the format described in January 1996 guidance from Stephanie Irene
TOX SAC Member Signature: Virginia Dobozy					**Date:** 1/13/98

(2)

PROPOSED DATA PRESENTATION TO THE HAZARD I.D. SARC

I. DIETARY

1. ACUTE DIETARY (ONE DAY)*

Type of Study Proposed: Developmental toxicity MRID #: 43820401

Guideline: §83-3(b)

Executive Summary: 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 NOEL = 150 mg/kg/day, based on decreased body weight gain and food consumption during treatment. The Maternal NOEL = 50 mg/kg/day. No developmental toxicity was observed under the conditions of this study. The Developmental LOEL = not determined; the developmental NOEL = 150 mg/kg/day.

Dose and Endpoint Proposed for Consideration: NOEL of 50 mg/kg/day, based on maternal effects (decreased bodyweight gain and food consumption) observed at the 150 mg/kg/day dose.

Comments about Study/Endpoint: The significance of the cesarean section observations should be verified in this study to determine that the effects are in fact not related to developmental toxicity.

Risk assessment for Acute Dietary Required: __x__ YES _____NO

**this risk assessment may be necessary for types of population groups [Females 13+ years as well as General Population (including infants and kids) depending upon the type of study/dose/endpoint used.*

2. CHRONIC DIETARY [Reference Dose (RfD)]

Type of Study Proposed: Chronic Toxicity - Baboon MRID #: 00251773
Guideline: §83-1

Executive Summary: In a chronic toxicity study, groups of 7 baboons/sex/dose received Irgasan DP 300 orally at doses of 30, 100, and 300 mg/kg/day by capsule for 52 weeks. At 100 and 300 mg/kg/day, the 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 NOEL was determined to be 30 mg/kg/day, and the systemic LOEL was determined to be 100 mg/kg/day, based on clinical signs of toxicity.

Dose Proposed for Consideration: NOEL of 30 mg/kg/day, based on observed clinical toxicity at 100 mg/kg/day in baboons.

Uncertainty Factor(s) Proposed for Consideration: Based on the apparent lack of sensitivity of infants and children to the toxicity of Irgasan, an uncertainty factor of 100 is proposed for consideration.

Proposed RfD: 0.3 mg/kg/day

Comments about Study: The above study provides results in a non-human primate species, which may be more relevant to the human response. However, the effect identified in this study relating primarily to the stomach is likely an effect resulting from contact of the test material with the stomach itself. In addition, a two-year rat study showed a NOEL of 52 mg/kg/day, and was based on decreased body weight as well as non-neoplastic changes in the liver of male and female rats, which is more consistent with the response observed in all of the other animal toxicity studies.

PROPOSED DATA PRESENTATION TO THE HAZARD I.D. SARC

II. OCCUPATIONAL / RESIDENTIAL EXPOSURE - DERMAL

DERMAL ABSORPTION (if available)

Type of Study Proposed: Dermal absorption study not available

MRID #: N/A

Executive Summary:

Percentage (%) Dermal Absorption Proposed for Consideration:

Assume 100% absorption. It is noted that the NOEL from a subchronical oral study in rats (Accession number 252003) was 50 mg/kg/day, while the NOEL from a 90-day dermal toxicity study in rats (MRID # 43328001) was 40 mg/kg/day, supporting the conclusion of significant dermal absorption.

PROPOSED DATA PRESENTATION TO THE HAZARD I.D. SARC

1. SHORT-TERM (1-7 days)

Type of Study Proposed: Liver Biochemistry effect study in Mice.

MRID#: 44389702 Guideline: § N/A

Executive Summary: In this study, Irgasan DP 300 technical was administered to five groups of young adult male CD-1 mice in a pelleted standard rodent diet at doses of 0, 100, 300, 1200, and 3000 ppm (approximately 0, 25, 75, 350, and 900 mg/kg/day) for 14 days. Two additionhal groups of nine male mice received either 0 or 900 mg/kg/day Irgasan for 14 days followed by a 4 week recovery period. Groups of female mice received Irgasn in the diet at doses of 0, 25, 350, and 900 mg/kg/day for 14 days. Livers were immediately frozen at necropsy in liquid nitrogen for biochemical and immunochemical measurements. Liver weight was significantly increased in male mice at 75 mg/kg/dayand above and in female mice at 350 mg/kg/day and above. At the lowest dose in male mice (18.4 mg/kg/day), signficant increases were observed in microsomal protein (25%), EROD activity (82%), and PROD activity (431%). Total microsomal hydroxylation of testosterone was significantly increased at the low dose in male mice, as was stereoselective hydroxylation of the 6ß-hydroxylated metabolite. In female mice, significant induction of CYP 3A1 and CYP 3A2 as well as CYP4A was observed at the lowest dose in female mice (19.8 mg/kg/day).

Dose and Endpoint Proposed for Consideration: Low dose of 18.4 mg/kg/day, based on significant increases in serum biochemistry parameters observed in the liver of male mice.

Comments about Study/Endpoint: Although a NOEL was not identified in this study, the effects observed are supported by the results of a cell proliferation study (MRID # 44389701) conducted on liver tissue from the same mice used in the above study, where a NOEL of 18.4 mg/kg/day was identified for cell proliferation in male mouse liver. In addition, a NOEL of 12.5 mg/kg/day was identified in a subchronic toxicity study in dogs (HED document # 001968) based on increases in liver enzymes observed at 25 mg/kg/day, and a NOEL of 25 mg/kg/day identified in a 91-day toxicity study in dogs based on liver effects observed at 50 mg/kg/day.

Risk Assessment for Short-Term Dermal Exposure Required: _x__YES ___NO

PROPOSED DATA PRESENTATION TO THE HAZARD I.D. SARC

2. INTERMEDIATE TERM (1 WEEK TO SEVERAL MONTHS)

Type of Study Proposed: Subchronic toxicity in Dogs.
MRID #: 43982701 Guideline: §83-1

Executive Summary: In a subchronic toxicity study in dogs (document # 001968), male and female beagle dogs (4/sex) received Irgasan DP-300 by gelatin capsule seven days per week for 13 weeks at doses of 0, 12,5, 25, 50, and 100 mg/kg/day. A group of 2 dogs/sex received 50 mg/kg/day Irgasan DP 300 and was allowed a four week recovery period. Mortality of 2 male dogs was observed at 100 mg/kg/day after 23 and 26 days on test, respectively. A single female dog was sacrificed at 50 mg/kg/day after 57 days on test. All animals sacrificed and/or found dead diplayed weight loss, anorexia, and symptoms of jaundice. The cause of death in all three dogs was attributed to hepatotoxicity which resulted in obstructive jaundice. Increases in serum alkaline phosphatase were observed at 100 mg/kg/day and 50 mg/kg/day. Treatment-related morphologic changes in the liver were observed at the 25, 50, and 100 mg/kg/day dose levels. These changes consisted of focal acidophilic to granular degeneration of the cytoplasm of a few individual helatocytes or small numbers of hepatocytes. No signs of toxicity were observed in those dogs receiving 12.5 mg/kg/day or in those dogs receiving 50 mg/kg/day test material and allowed a four week recovery. The systemic NOEL was determined to be 12.5 mg/kg/day, and the systemic LOEL was determined to be 25 mg/kg/day, based on the morphologic changes observed in the liver.

Dose Proposed for Consideration: NOEL of 12.5 mg/kg/day, based on histopathological changes observed in the liver at 25 mg/kg/day.

Comments about Study/Endpoint: Although this is an older study, the effects of Irgasan on the liver are consistent with other subchronic toxicity studies conducted with this chemical, where the primary effect is observed in the liver. The LOEL is also similar to LOELs observed in other subchronic toxicity studies. The jaundice observed in the dog study is related either to a primary hepatotoxic effect or competition of Irgasan with bilirubin for glucuronidation in the liver.

CHEMICAL: TRICLOSAN
PC CODE: 054901

TOXICITY PROFILE FOR TRICLOSAN

DER #	STUDY TYPE - DOSE LEVELS	NOEL (mg/kg/day)	LOEL (mg/kg/day)
1	2-YR FEED/CARCINOGENIC RAT (1989)	168 mg/kg/d [M]; 217 mg/kg/d [F]	415 mg/kg/d [M]; 519 mg/kg/d [F]
2	1-YR FEEDING BABOON (1976)	30 mg/kg/day	100 mg/kg/day
3	2-GEN REPRODUCTION RAT (1988)	50 mg/kg/day	150 mg/kg/day
4	DEVELOPMENTAL TOX RAT (1992)	Mat NOEL =100 mg/kg/day; Devel. NOEL ≥ 300 mg/kg/day	Mat LOEL = 300 mg/kg/day; Devel. LOEL > 300 mg/kg/day
5	DEVELOPMENTAL TOX RABBIT (1992)	Mat NOEL = 50 mg/kg/day; Devel. NOEL = 150 mg/kg/day	Mat LOEL = 150 mg/kg/day; Devel LOEL > 150 mg/kg/day
6	DEVELOPMENTAL TOX RAT (1992) (same study as #5)	Mat NOEL = 50 mg/kg/day; Devel. NOEL = 150 mg/kg/day	Mat LOEL = 150 mg/kg/day; Devel. LOEL > 150 mg/kg/day
7	13-WEEK DERMAL RAT (1993)	NOEL = 40 mg/kg/day	LOEL = 80 mg/kg/day
8	13-WEEK FEEDING DOG (1972)	NOEL = 12.5 mg/kg/day	LOEL = 25 mg/kg/day
9	13-WEEK FEEDING MOUSE (1993)	NOEL < 25 mg/kg/day	LOEL = 25 mg/kg/day (LDT)
10	28-DAY ORAL TOXICITY MOUSE (1987)	NOEL = 6.48 mg/kg/day [M}; 8.25 mg/kg/day [F}	LOEL = 135.59 mg/kg/day [M]; 168.78 mg/kg/day [F]

Acute Toxicity Profile

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral	43206501 42306901	LD_{50} > 4 g/kg	IV
81-2	Acute Dermal	42306902	LD_{50} > 9.3 g/kg	III
81-3	Acute Inhalation	42306902 43310501	LC_{50} > 0.15 mg/L	II
81-4	Primary Eye Irritation	no MRID available	no irritation reported	
81-5	Primary Skin Irritation	42306903	moderately irritating at 72 hours	III
81-6	Dermal Sensitization	41008909	not a sensitizer	

1/7/98

James O. Pepping 1/7/98

1) Citation: Molitor, E.; Persohn, E.; Thomas, H. (1995): The Effect of FAT 80'023/Q (IRGASAN DP 300) on Selected Biochemical Liver Parameters Following Subchronic Dietary Administration to Male and Female Mice. Ciba-Geigy Limited, Toxicology Services, Basel, Switzerland. Submitted to EPA (no MRID). Unpublished.

In this study, Irgasan DP 300 technical (purity not stated; batch No. EN 91390.76) was administered to five groups of young adult male CD-1 mice (22-28 g, nine mice per group) in a pelleted standard rodent diet (Nafag 890) at concentrations of 0, 100, 300, 1200, and 3000 ppm (approximately 0, 25, 75, 350, and 900 mg/kg/day) for 14 days. Two additional groups of nine male mice received either 0 or 900 mg/kg/day for 14 days followed by a 4-week recovery period. Groups of female mice (four groups of three mice per group) received Irgasan DP 300 in the pelleted diet at doses of 0, 25, 350, and 900 mg/kg/day.

Each group of nine male mice above was subdivided at termination into six mice for biochemical determinations and three mice for electron microscopic evaluation. In addition, one group of nine control mice (group 2) and one group of nine high dose mice (group 7) were administered test material for 2 weeks and allowed to recover for four weeks (by feeding of non-treated diet).

The following measurements were made: Individual body weights were recorded daily during the treatment period, and twice per week during the recovery period. Food consumption and daily dose of test chemical were recorded. At the end of treatment or recovery, mice were fasted for 20 hours and then killed under carbon dioxide anesthesia. Livers were removed, weighed, and after sampling for electron microscopy, were immediately frozen in liquid nitrogen for biochemical and immunochemical measurements. Microsomal and cytosolic liver fractions were prepared by differential centrifugation, and biochemical measurements performed (total protein, cyanide-insenitive peroxisomal ß-oxidation, microsomal lauric acid hydroxylation, cytochrome P-450, microsomal 7-EROD and 7-PROD de-alkylase activities, microsomal testosterone hydroxylation, and cytosolic glutathione-S-transferase). Monoclonal antibodies against rat liver cytochromes CYP1A1 and CYP1A2, CYP3A1, and gene family CYP4A were used for immunoblot analysis. Electron microscopy was performed on double stained (uranyl-acetate and lead citrate) thin sections of three randomly selected male mice of all groups, as well as control and high dose female mice.

Results of dietary analyses (stability data) showed the test material to be stable in the pelleted diet up to 25 days at room temeperature. No data were presented showing homogeneity of the test material within the pellets, although this claim was made in the report. Mean daily intake of test chemical for male mice used in biochemical measurements is as follows:

Test Group	Actual mg/kg/day	Nominal mg/kg/day
1M-BI	0	0
3M-BI	18.4	25.0
4M-BI	53.2	75.0
5M-BI	249.3	350
6M-BI	793.8	900

For male mice used in electron microscopy measurements, intake of test article was as follows:

Test Group	Actual mg/kg/day	Nominal mg/kg/day
1M-EM	0	0
3M-EM	17.4	25.0
4M-EM	54.7	75.0
5M-EM	267.6	350
6M-EM	1346.8	900

For female mice (used for both biochemical and electron microscopy measurements), the following compound intake data were provided:

Test Group	Actual mg/kg/day	Nominal mg/kg/day
8F	0	0
9F	19.8	25.0
10F	271.3	350
11F	1105.6	900

As shown above, the actual test article intake in male mice used for either biochemical or electron microscopic determinations was similar, except for the high dose, where male mice used for electron microscopic determinations received a higher dose (1346.8 mg/kg/day), on average, than male mice used in biochemical determinations (793.8 mg/kg/day).

Decreased absolute body weight and body weight gain was observed in male mice at the 900 mg/kg/day dose level after 14 days of test article administration. Weight gain for high dose male mice was decreased 75% vs. control, and absolute group mean body weight decreased by 16% vs control. No effect on body weight or body weight gain was observed in female mice for the 14 day treatment period. Male mice in the recovery group showed depressed body weight gain for approximately the first 7 days after cessation of treatment, and then began demonstrating bodyweight gain such that by day 15 post-recovery, body weight was equivalent to control. Food consumption (mean daily consumption over the two-week period) appeared increased at the 350 and 900 mg/kg/day dose levels compared to control (food consumption of 0.178, 0.184, 0.178, 0.208, and 0.265 at the 0, 25, 75, 350, and 900 mg/kg/day dose levels, respectively). By inference, food efficiency would be decreased at the high dose, based on the decrease in body weight gain at the 900 mg/kg/day dose. In female mice, group mean body weight gain was increased at the 350 and 900 mg/kg/day dose levels (gains of 3.9 and 3.1 grams, respectively, vs. 2.6 and 2.4 in the control and low dose, respectively.

Liver weight in male mice receiving 75 mg/kg/day and above and in female mice at 350 and 900 mg/kg/day was significantly increased in comparison to the respective controls. Data are shown as follows (from page 28 of the report):

Test Group	**Liver Weight (g)**
1M	1.33±0.15
3M	1.38±0.36
4M	2.07±0.22***
5M	3.15±0.46***
6M	3.60±0.46***
8F	0.99±0.12
9F	1.14±0.07
10F	2.74±0.39***
11F	3.18±0.31***

It is noted that in the high dose male recovery group, the effect of Irgasan DP 300 on liver weight was reversible, with a final liver weight in this group of 1.49±0.10 grams after a 4-week recovery period.

The effects of Irgasan DP 300 treatment on measured enzyme activities in this study are shown below:

The Effect of Irgasan DP 300 on Selected Biochemical Parameters in **Male** Mouse Liver								
Test Group	Prot.	P-450	GST	FAO	11-OH	12-OH	EROD	PROD
1M	22.0± 4.7	11.4± 4.3	332± 52	963± 103	25.0± 6.6	62.4± 17.8	1.49± 0.63	0.76± 0.43
3M	27.6± 2.1*	17.6± 6.9	403± 101	974± 103	36.9± 7.3#	87.6 ±19.5	2.72± 0.92#	3.28± 1.66†
4M	27.6± 1.8*	19.2± 6.6*	311± 75	1568± 432†	56.7± 16.5†	186.5± 90.7†	3.52± 0.76†	4.57± 1.13†
5M	31.0± 3.4†	38.1± 4.5†	527± 80†	2381± 386†	92.5± 12.8†	391.3± 66.2†	5.63± 1.19†	10.50± 1.94†
6M	34.7± 3.5†	49.9± 10.9†	867± 111†	3269± 292	110.4 ±19.9 †	519.5± 69.7†	7.48± 1.97†	18.15± 5.66†

Data from pages 29-32 of the report.

Prot. = microsomal protein (mg/g liver); P-450 = cytochrome P-450 (nmol/g liver); GST = glutathione-S-transferase (nmol/min/g liver); FAO = fatty acid ß-oxidation (nmol/min/g liver); 11-0H and 12-OH = lauric acid hydroxylation (nmol/min/g liver); EROD = ethoxyresorufin O-de-ethylase (nmol/min/g liver); PROD = pentoxyresorufin O-depentylase (nmol/min/g liver).
* $p<0.05$; # $p<0.01$; † $p<0.001$ vs. control by Dunnett's test.
Test groups: 1M = control; 3M = 18.4 mg/kg/day; 4M = 53.2 mg/kg/day; 5M = 249.3 mg/kg/day; 6M = 793.8 mg/kg/day.

As the above data show, significant increases were observed in the activities of all bioche.0mical parameters listed above at the top dose, and significant increases were observed for microsomal protein, lauric acid hydroxylation, and EROD and PROD activities at the lowest dose tested in males (18.4 mg/kg/day). Specifically, microsomal protein content was increased by 25%, EROD activity by 82%, and PROD activity by 431%. Male mice which had received the high dose of 793.8 mg/kg/day and were allowed to "recover" for four weeks by feeding of untreated diet were observed with liver biochemistry not significantly different than control, indicating reversibility of the inducing effects of Irgasan DP 300.
In female mice, there were no significant increases in liver biochemical parameters at the low dose with the exception of PROD activity, which was induced by 268% over control. Increases in liver biochemical parameters were observed for female mice at the mid dose of 1200 ppm (271.3 mg/kg/day) and above.

Regioselective testosterone hydroxylation in response to treatment with Irgasan DP 300 was also investigated, and is presented as a separate table due to the many metabolites detected from this assay. Determination of the regio- and stereo-selective hydroxylation of testosterone allows the determination of the effects of treatment with a test chemical on the activities of several constitutively and/or inducibly expressed isoenzymes of the microsomal P-450 family using a single substrate. Results are shown below:

Metabolites	Effect of Irgasan DP 300 Treatment on P-450 Dependent Testosterone Hydroxylation					
nmol/g /min	0 mg/kg /day	18.4 mg/kg /day	53.2 mg/kg /day	249.3 mg/kg /day	793.8 mg/kg /day	High dose recovery
2ß-OH	2.5±0.5	4.9±2.9*	5.7±2.9*	20.6± 5.2***	29.1± 3.5***	1.5± 0.2***
6ß-OH-T	30.0± 8.3	61.3± 40.8*	57.4± 26.8*	259.5± 59.1***	327.8± 31.7***	13.0± 1.8***
15ß-OH-T	3.1±0.8	4.4±2.0	5.5±2.7*	16.6 ±4.4***	22.7± 2.5***	1.6±0.9*
6 -OH-T	4.6±2.2	4.3±1.2	3.0±0.6	5.0±2.0	6.0±3.0	1.9±0.2*
7 -OH-T	10.6±4.8	14.7±6.9	15.6±6.8	20.7 ±7.5*	36.4± 8.7***	6.9±2.4
16 -OH-T	8.8±2.7	12.7±6.9	15.5 ±2.6*	19.1 ±3.7***	19.3 ±5.6**	3.7±1.2
androstene-dione	17.3 ±4.0	27.0 ±7.2	17.1 ±4.7	28.6 ±6.7	31.1 ±9.6	10.9 ±2.1
16ß-OH-T	2.2±1.8	3.5±4.2	3.9±1.8	9.5±5.3	16.8 ±2.5*	0.6±0.5
Total Activity	79.0 ±15.8	132.8 ±64.8*	123.7 ±41.2*	379.4 ±66.1***	489.1 ±51.0***	40.0 ±5.3***

As shown, total microsomal hydroxylation of testosterone was significantly increased at all dose levels tested in male mice, and the increases were dose-dependent. Formation of the 2ß-, 6ß-, 15ß-, and 16ß- metabolites were increased 11.6-fold, 10.9-fold, 7.3-fold, and 7.6-fold, respectively, at the high dose level. The prominent induction of formation of these metabolites is similar to that observed after treatment with the model inducers phenobarbital and pregnenolone 16-carbonitrile. Hydroxylation of testosterone at the 7 position (alpha configuration) is associated with CYP2A1 and isoenzymes of the peroxisome proliferator

inducible P-450 gene family CYP4A in the rat. The study results showing a 3.4-fold induction of 7-alpha-hydroxylation by dietary administration of Irgasan DP 300 in male mice supports the conclusion of a peroxisome-proliferator effect of the chemical. Previously mentioned induction of fatty-acid hydroxylation by Irgasan also supports this conclusion.

Immunoblot Analysis

In this experiment, mixtures of equal volumes of microsomal suspensions of all mice per treatment group were subjected to SDSPAGE, and monoclonal antibodies d15 (against rat liver cytochrome CYP3A1), clo4 (against rat liver cytochrome gene family CYP4A) and p6 (against rat liver cytochrome CYP3A1) generated and used for immunoblot analysis.

Monoclonal antibody d15 is specific for the 3-methylchlanthrene inducible rat liver cytochromes CYP1A1 and CYP1A2. Monoclonal antibody p6 is specific for steriod- and barbiturate inducible rat liver cytochromes CYP3A1 and CYP3A2. Monoclonal antibody clo4 is diagnostic for the peroxisome-proliferator inducible rat liver isoenzymes of the CYP4A gene family. The results of this experiment indicated substantial induction of a monooxygenase protein similar to rat liver isoenzyme CYP3A1 and induction of CYP4A3 of the CYP4A gene family. Induction of cytochromes which are indicative of 3-methylcholanthrene type induction was not shown in this study, as d15 did not result in increases over control but showed decreases in treated mice.

Effect of Irgasan DP 300 and its Reversibility on Immunochemically Detectable Relative Protein Content of Female Mouse Liver Cytochromes P-450 Cross-reactive with monoclonal antibodies against purified rat liver cytochromes P-450 of the gene families CYP1A, CYP3A, and CYP4A.

Microsomal Fraction, female	CYP Detected by antibody d15 (1A1 and 1A2)	CYP Detected by antibody p6 (3A1 and 3A2)	CYP Detected by antibody clo4 (4A)
0 mg/kg/day	100	100	100
19.8 mg/kg/day	70	540	241
271.3 mg/kg/day	48	3293	703
1105.6 mg/kg/day	70	5851	852

Results are expressed as relative area units obtained from densitometric scans of single Western-blots containing 100, 30, and 5 grams liver tissue equivalents of microsomal protein per

lane for determination of relative monoclonal antibody d15, p6, and clo4 cross-reactive protein levels, respectively.

It is noted that in the methodology presentation of this report, it was stated that microsomal suspensions of "all animals per treatment group" were subjected to SDS-PAGE. In the results, however, only data for female mouse liver was presented. It is unclear whether male liver was used for immunoblot analysis. If so, these data are missing and should be included in the results of this study.

Electron Microscopy

A summary of observations made from electron microscopic examination of the livers of male and female mice in this study was presented on pages 36 and 37 of the report, showing the severity (but not incidence) of liver lesions. The following is taken from page 19 of the report:

In male mice, marginal (at 17.4 and 54.7 mg/kg/day) to moderate (267.6 and 1346.8 mg/kg/day) proliferation of smooth endoplasmic reticulum was observed. Rough endoplasmic reticulum membranes were distinctly reduced and disorganized at 54.7 mg/kg/day and above, leading to a mixture of rough and smooth ER membranes, mostly in vesicular form. Peroxisomes showed a moderate (at 54.7 mg/kg/day) to striking (1346.8 mg/kg/day) proliferation. At these same doses, peroxisomes were increased in size. At the 54.7 mg/kg/day dose and above, lipid vacuoles were encountered in hepatocyte nuclei, and at the top dose, nearly all nuclei contained numerous lipid vacuoles of various sizes.

In female mice, the same morphological alterations were observed at the top dose (in this case, 1105.6 mg/kg/day) as were observed in male mice. Reversibility of the morphological effects was demonstrated in the recovery group of male mice receiving the high dose of test material (in this case, 906.8 and 757.4 mg/kg/day for the biochemical and electron microscopy group of mice, respectively).

The report stated that "the reversible proliferative effect of (Triclosan) on the hepatocyte peroxisome compartment allows the classification of the test article as a peroxisome proliferator. The report noted, however, that the mechanism whereby lipid droplets arise or their toxicological implication has not been investigated.

From these data it is apparent that administration of Triclosan to the mouse results in significant hepatic effects. The biochemical alterations observed appear to support the conclusion of a barbiturate -type induction with peroxisome proliferation effects. Induction of certain liver enzyme activities as measured in this study appear to occur at the lowest dose tested in male mice (18.4 mg/kg/day), including significant increases in microsomal protein, lauric acid hydroxylation, and EROD and PROD activities Specifically, microsomal protein content is increased by 25%, EROD

activity by 82%, and PROD activity by 431%. Increases in liver weight and significant changes in liver morphology occur at doses of 54.7 mg/kg/day and above in male mice. Female mice appear to be less sensitive than males to the hepatic effects of dietary Triclosan administration.

2) Citation: Eldridge, S. (1993): Cell Proliferation in Rodent Liver. Study performed by Pathology Associates, Inc. for Ciba-Geigy Corporation. MRID # 44389701. Unpublished.

This study was conducted as supplemental to a previously reviewed subchronic feeding study in mice (MRID # 43022605) to determine whether cell proliferation was induced in the liver of male and female mice after 45 and 90 days' dietary administration of triclosan. Liver tissue from mice receiving 0, 25, 75, 200, 350, or 900 mg/kg/day was obtained as formalin-fixed wet tissue. Slides prepared from paraffin embedded tissue were stained with hematoxylin and eosin for histopathology evaluation, or stained for proliferating cell nuclear antigen (PCNA) using immunohistochemical methods.

Positive staining for PCNA was identified by uniform dark red nuclear staining of hepatocytes in the S-phase of the cell cycle. Homogeneity of a cell proliferative response was evaluated by perusing liver sections from each individual animal, and found to be similar among the lobes examined from each animal. At least 1000 hepatocellular nuclei were counted in six fields using a 20X objective and an eyepiece containing a 10x10 mm grid. Labeling index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of nuclei counted, and expressing the result as a percentage.

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

GROUP	Lipofus-cin	Hepato-cyte hyper-trophy	Bile reten-tion	Necrosis	Lipid vacuol-ization	Biliary hyper-plasia
01 MM	0	0	0	0	0	0
02 MM	0	0	0	0	0	0
03 MM	0.2	1	0.2	0.2	0.2	0.2
04 MM	1	1	0.8	0.6	0.4	0.8
05 MM	1	1	2	1.2	0.6	0.4
07 MM	1.4	2	1.2	1.8	0.8	1
08 MM	0	0	0	0	0	0

09 MM	0	1.6	0	0	0	0
10 MM	1.2	3.6	0.4	1.2	0.4	0.8
11 MM	2.2	3.4	1.2	3	0.8	2
01 FM	0.2	0	0	0	0	0
02 FM	0	0	0	0	0	0
03 FM	0	0.4	0	0	0	0
04 FM	0.2	0.8	1	1.2	0.4	0.6
05 FM	1.4	1	1.4	0.6	0.4	1
07 FM	1	1.4	1	1.6	0.8	1.6
08 FM	0	0	0	0	0	0
09 FM	0	1	0	0	0	0
10 FM	1	3	0.4	1	0	0.8
11 FM	1.8	3.4	1	2.4	1.2	2.2

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

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

At the 90 day time point, similar liver lesions were observed. Hepatocellular hypertrophy was observed at 75 mg/kg/day and above in male and female mice. With increasing dose, severity of this lesion also increased. In addition to hypertrophy, necrosis of hepatocytes was observed at 75 mg/kg/day and above in male mice

and at 200 mg/kg/day and above in female mice, again with a dose-related increase in severity. The report stated that with increasing dose, necrosis became more severe, involving groups of cells, and in the most severe cases, involved all cells of individual lobules (panlobular). Hepatocytes were also swollen and many had cytoplasmic yellow-brown granules at the periphery, near bile canaliculi. This lesions was classified as bile stasis.

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

Results of cell proliferation experiments are shown below:

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

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

According to the report, cell proliferation was significantly increased over control in male mouse liver at 200 mg/kg/day and higher, and the increase was sustained from 45 to 90 days. The reviewer would agree with the sustained increase, but it appears that cell proliferation (as judged by labeling index and fold increase over control) is also increased significantly at the 75 mg/kg/day dose level for male mice. This result is consistent with the apparent differences in sensitivity to the hepatic effects of triclosan between male and female mice, as cell proliferation in

female mice was not affected at 75 mg/kg/day, but was increased at 200 mg/kg/day, consistent with the observed difference in liver histopathology.

According to the report, the distribution of hepatocellular labeling was panlobular in both sexes.

The results of this study support a mode of action consistent with cellular regeneration as a result of hepatocellular cytotoxicity. The **25 mg/kg/day** dose level was identified as the **No Observed Effect Level (NOEL)** for **male** mice in this study, while the **75 mg/kg/day** dose level was considered the **NOEL for female mice** by the authors. The reviewer agrees with this interpretation, as liver responses in female mice, while evident at 75 mg/kg/day, were not significant enough to support a true effect level. Males, by contrast, did show hepatic responses at the 75 mg/kg/day dose which would support an effect level.

3. 2) Citation: Elridge, S. (1995): Cell Proliferation in Rodent Liver. Study conducted by Pathology Associates, Inc. Submitted to EPA (no MRID). Unpublished.

The purpose of this study was to examine whether cell proliferation was induced in male and female mice which had been the subject of an earlier subchronic toxicity study in mice exposed to dietary Irgasan at dose levels of 0, 25, 75, 200, 350, 750, or 900 mg/kg/day for 7 or 13 weeks (MRID # 430026-05).

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

Positive staining for PCNA was identified by uniform dark staining of hepatocytes in the S-phase of the cell cycle. Homogeneity of a cell proliferative response was evaluated by examining liver sections from each individual animal and was found to be similar among the lobes examined, although which lobes were examined was not detailed in this report. A labelinf index was calculated by dividing the number of labeled hepatocyte nuclei by the total number of nuclei counted. Student's t-test for the inequality of unpaired data sets was used to determine significant differences in labelinf index between controls and treated groups.

The results of this study are shown below. It is noted that the

study report itself appeared to be an abbreviated version with no detail on procedures for cell proliferation analyses.

Cell Proliferation in Male and Female Mice Administered Dietary Irgasan for 90 Days							
Group (males, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls
0	0.035	0.016	-	0	0.042	0.036	-
25	0.008	0.008	0.0	25	0.046	0.015	1.1
75	0.167	0.082	4.8	75	0.065	0.009	1.5
200	0.124	0.031	3.5	2 0 0^^^Document Error^^^ Error^^^	0.140	0.050	3.3
350	0.398	0.063	11.0	350	0.256	0.218	6.1
900	0.536	0.195	15.0	900	0.300	0.060	7.1
Cell Proliferation in Male and Female Mice Administered Irgasan in the Diet for 45 Days							
Group (males, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls	Group (females, mg/kg /day)	Mean Labeling Index	SEM	Fold Increase Over Controls
0	0.090	0.018	-	0	0.058	0.029	-
25	0.112	0.089	1.2	25	0.064	0.021	1.1
350	0.292	0.096	3.2	350	0.242	0.072	4.2
900	0.726	0.218	8.0	900	0.380	0.080	6.6

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

Although it appears that not all of the animals from the subchronic toxicity study were evaluated (in the subchronic study, groups of 10-20 mice/sex/dose were used), an increase in the

labeling index was apparent at 200 mg/kg/day and above for male and female mice. The labeling index was increased at day 45 in both sexes at 350 and 900 mg/kg/day, and as noted, this continued at these dose levels at 90 days as well as the increase observed at 200 mg/kg/day at 90 days for both sexes. The observation of an increase in labeling index from this study, in conjunction with other data which show toxicity to the liver of rats and mice, indicate cytolethality of Irgasan which is followed by induced cellular regeneration. However, there may be species differences in the response to Irgasan hepatotoxicity. Hepatic necrosis was observed in a two-year rat chronic toxicity / carcinogenicity study at 300 ppm, 1000 ppm, and 3000 ppm, but there was no significant increase in tumor incidence. The mouse also shows evidence of hepatic necrosis, but tumor data are missing with which to make a comparison to the rat.

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

4 8) Citation: Molitor, E. and Persohn, E. (1995): The Effects of FAT 80'023/Q (Irgasan DP 300) On Selected Biocehmical Liver Parameters Following Dietary Administration to Male Rats. Study performed by Ciba-Geigy Ltd., Switzerland. Submitted to USEPA (no MRID). Unpublished.

The present study was conducted to provide insight into the mechanistic properties and potential species-specificity of Triclosan through characterization of effects on the rat liver. Young adult male Sprague-Dawley rats (230-260 g body weight) were divided into groups of five rats. Four groups (groups 7-10) received the test article in the diet for 14 days at concentrations of 0, 300, 1500, and 6000 ppm. Two additional groups (groups 11 and 14) received 0 and 6000 ppm of test chemical for 42 days. Reversibility of test article effects was assessed in a single group of five animals, who received 6000 ppm in the diet for 14 days, followed by a 28 day recovery period.

Test article was prepared as a homogenous blend in pelleted diets (Nafag 890, Nafag, Switzerland). Analysis for stability of the test article was performed at the end of the 42-day treatment period. Contant and homogeneity of the test article in the diet was analyzed prior to dosing. Data on the concentration of the test article in the diet were presented on page 28 of the report and showed that for each dose level tested, the actual concentration of test article was 98-101% of nominal. Measurements of test article concentration after 42 days showed that the test article was stable in the pelleted feed (89-100% of

nominal across all dose groups).

The following measurements were made in this study: individual body weight and food consumption (daily up to day 14 and twice per week from days 15-42); electron microscpoic evaluation of liver tissue from all dose groups; biocehmical parameters in the liver of all group0s, including protein content, cyanide insensitive peroxisomal beta-oxidation, microsomal lauric acid hydroxylation, cytochrome P-450, 7-ethoxyresorufin and 7-pentoxyresorufin-O-dealkylase activity, microsomal testosterone hydroxylation, cytosolic glutathione-S-transferase, and immunoblot analysis for the presence of the P-450 isoenzymes CYP1A, CYP2B, CYP3A, and CYP4A.

Actual intake of test article was calculated from food consumption data and is presented below:

Dose Group	Test Article Concentration (ppm)	Mean Daily Dose (mg/kg/day)
14-Day Trtmt.		
07	0	0
08	300	23
09	1500	108
10	6000	518
14/28 Day Trtmt.		
11	0/0	0
14	6000/0	463
15	6000/6000	409

data from page 30 of the report.

Clinical Toxicity - There were no reported signs of toxicity in this study. Food consumption at the 6000 ppm dose level.

Food consumption - At the 6000 ppm dose level for both test groups (14 day continuous treatment and 14 day treatment followed by 28 day recovery), food consumption was decreased during the first day by approximately 50%. Lower dose levels did not show any effect on day 1 of the study. From days 3-7, the 300 and 1500 ppm dose levels showed decreases in food consumption of 10% and 6% respectively, while at the 6000 ppm dose level, food consumption rebounded such that intake was almost 3-fold over the intake at day 1. From days 8-14, decreases in food consumption of 18% and 15% were observed at the 300 and 1500 ppm dose levels, but a 15% decrease in food consumption was also observed in the control group. Food consumption at the 6000 ppm dose group remained

increased almost 3-fold over control from days 8-14. In those rats receiving 6000 ppm for 14 days followed by a 28 day recovery period, food consumption patterns were similar during the first 14 days to those of the 14 day non-recovery group, with increased food consumption after day 1 up to an intake approximately 3-fold higher than day 1 intake by day 14.

Body weight - At the 300 and 1500 ppm dose levels, group mean body weight was not significantly affected over the course of the study. At the 6000 ppm dose, group mean body weight was slightly decreased (4-8%) over the first 8 days of the study. Group mean body weight at the 6000 ppm dose level from day 10 onward was decreased by only 2-3%. In the group receiving test article for 14 days followed by a 28 day recovery period, group mean body weight was decreased by approximately 6% during the treatment period. Following withdrawal of test article from the food, group mean body weight was decreased by 8-13% until study termination compared to the control group. The reason for decreased group mean body weight following withdrawal of test material is not clear from the present data.

Organ weights - At the end of the treatment period, the rats receiving the 6000 ppm dietary treatment showed a significantly increased absolute and relative liver weight. Absolute liver weight was increased from 10.4±0.6 grams in control to 15.9±2.7 grams at the high dose, an increase of 52%. Relative liver weight was increased from 2.92±0.12 in control to 4.46±0.51 at the high dose, an increase of 52%. In those rats receiving the test article for 42 days at 6000 ppm, there was a significant increase in relative liver weight of 19% over control, but not absolute liver weight. The lack of increase in absolute liver weight for this group could be based upon the decrease in body weight observed for this group at study termination. In those rats receiving the test article for 14 days at 6000 ppm followed by a 28 day recovery period, liver weight was decreased by 18%, but relative liver weight was not affected. It is still of interest why a 14 day treatment produces a 52% increase in absolute and relative liver weight without a significant decrease in body weight, while essentially what should be the same regimen (with the exception of a 28 day recovery period) results in decreased body weight and decreased absolute liver weight. Those rats remaining on the test chemical for 42 days show decreased body weight, no increase in absolute liver weight, but an increase in relative liver weight.

Liver Biochemistry - The effects of Irgasan DP300 (FAT 80023/Q) on liver biochemical parameters in male Sprague-Dawley rat liver are summarized below:

The Effect of FAT 80'023/Q on Selected Biochemical Parameters in **Male** Rat Liver								
Test Group	Prot.	P-450	GST	FAO	11-OH	12-OH	EROD	PROD
07 0ppm	27.8± 3.2	23.3± 2.0	110± 21	756± 70	10.8± 5.8	6.9± 4.1	4.80± 1.91	5.14± 2.04
08 300ppm	25.0± 3.1	20.9± 2.9	110± 21	901± 124	4.8± 2.0	4.7± 2.3	2.10± 0.55#	6.58± 2.98†
09 1500ppm	27.0± 2.6	28.6± 6.4	126± 18	868± 69	7.3± 2.3	6.9± 2.9	2.39± 0.42#	14.86± 6.04#
10 6000ppm	29.9± 5.2	53.0± 12.2†	182± 30#	792± 60	20.9± 9.9	19.4± 6.7*	2.29± 0.60#	58.76± 18.52†
11 0/0ppm	24.9± 3.3	19.6± 10.8	149± 20	825± 75	11.6± 6.4	7.5± 3.7	4.59± 1.97	7.20± 2.17
14 6000/ 0ppm	26.7± 3.3	23.9± 4.3	148± 19	876± 185	13.0± 4.7	8.3± 4.8	4.47± 0.87	7.91± 1.56
15 6000/ 6000ppm	30.9± 1.8*	54.8± 5.4*	237± 26†	891± 143	30.0± 11.5#	19.8± 3.2#	2.72± 0.65	46.82± 8.45†

Prot. = microsomal protein; P-450 = cytochrome P-450; GST = glutathione-S-transferase; FAO = fatty acid ß-oxidation; 11-OH and 12-OH = lauric acid hydroxylation; EROD = ethoxyresorufin O-de-ethylase; PROD = pentoxyresorufin O-dependentylase.

*p < 0.05; #p < 0.01; †p < 0.001. Data from pages 35-38 of the report.

As the above data show, significant effects were observed for several biochemical parameters in the liver at the 6000 ppm (~ 300 mg/kg/day) dose level. Cytochrome P-450 content was approximately doubled in the high dose group, while activity of glutathione-S-transferase was increased by 65%. In contrast to mice, where significant induction of cyanide-insenitive peroxisomal fatty acid ß-oxidation was observed at doses of 75 mg/kg/day and above, this enzyme activity was not induced in rat liver at doses up to 300 mg/kg/day nominal. Thus, as a measure of peroxisomal proliferation, Triclosan did not appear to have an effect in rat liver as it had in mouse liver. It is noted that recent research in the area of peroxisome proliferation and hepatocarcinogenesis (CIIT Activities, 17:1, January 1997) indicates that while several classes of chemicals can produce a proliferation of peroxisomes

in rats and mice, humans appear relatively insensitive to equivalent doses. Thus, humans may be relatively resistant to the hepatocarcinogenic effects of peroxisome proliferators.
Other enzymes affected at the 6000 ppm dose level included lauric acid hydroxylation (12-position hydroxylation induced almost 3-fold; 11-position hydroxylation increased but not significantly); and EROD activity (decreased almost 50%). PROD activity by contrast was increased over 10-fold at the 6000 ppm dose level. For PROD activity, a dose-response in induction of this enzyme was observed, with increases of 128%, 289%, and 1143% at the 300, 1500, and 6000 ppm dose levels, respectively. The type of induction observed with Triclosan in this study is similar to that observed after phenobarbital administration and not 3-methylcholanthrene induction.
In general, those rats allowed to recover for 28 days following the 14-day administration of test chemical showed no significant induction or inhibition of enzyme activities, while for those maintained for 42 days on the 6000 ppm test diet continued to show enzymatic changes consistent with those observed at the 6000 ppm dose level in the 14-day dose group.

Testosterone hydroxylation - The effect of Triclosan administration on microsomal testosterone hydroxylation is summarized below:

Metabolites	Effect of Irgasan DP 300 Treatment on P-450 Dependent Testosterone Hydroxylation					
nmol/g /min	0 mg/kg /day	23 mg/kg /day	108 mg/kg /day	518 mg/kg /day	14/28 day recovery	42 day high dose
2ß-OH	3.8±0.6	2.3±1.7	5.1±3.6	8.8±3.9	1.6±0.3	4.3±2.8
6ß-OH-T	23.0± 0.7	15.4± 8.4	30.9± 5.5	47.8± 31.7	12.0± 3.0*	23.5± 11.1
15ß-OH-T	23.2± 16.5	13.4± 9.0	13.9± 6.9	18.6± 18.0	10.6± 7.4	13.6 ±7.2
2-OH-T	64.9±6.5	48.4± 27.4	70.7± 11.2	53.0± 17.2	25.8± 9.5*	23.2 ±8.6*
7 -OH-T	5.7±2.0	4.8±2.7	4.8±1.2	10.7 ±3.5	3.4± 0.8	4.8±1.9
16 -OH-T	82.2± 7.1	58. 5±32.4	96.8 ±16.8	102.2 ±48.2	36.6 ±10.1*	44.0 ±13.3
androstene-dione	44.3 ±2.5	34.1 ±17.3	57.9 ±14.6	83.6 ±18.4	23.2 ±5.9#	33.1 ±6.6
16ß-OH-T	<0.1	<0.1	3.0±1.3†	27.6± 10.9†	1.4± 1.9*	9.3±2.8†
15-OH-T	<0.1	<0.1	<0.1	4.7±3.3†	1.5±0.4†	1.9±1.0†

Total Activity	260 ±25	187 ±100	299 ±56.4	375 ±160	124 ±26.0	166 ±50

*p <0.05; #p < 0.01; †p < 0.001. Data obtained from pages 40-41 of the report.

]According to the report, determination of the regio- and stereo-selective testosterone hydroxylation allows the assessment of treatment related effects on the activities of several constitutively and/or inducibly expressed enzymes of the P-450 enzyme family using a single substrate.

The most noticeable change as a result of treatment with Triclsan was an increase in the activity of the enzyme(s) responsible for production of the 15-ß and 16-ß hydroxy metabolites of testosterone. The report noted that the 16-ß hydroxylation of testosterone is catalyzed by the CYP2B family of P-450, a barbiturate inducible type of enzyme. A new metabolite, 15-alpha hydroxytestosterone, was quantitated at the high dose of test chemical, as well as in the recovery group and the 42-day continuous administration group. This metabolite is reported to be specific for the CYP2C12 and CYP2C13 cytochromes. Production of the 2-ß and 6-ß hydroxy metabolites of testosterone was increased at the high dose, indicative of an increased activity and/or expression of CYP3A. Total activity of testosterone hydroxylation was increased at both the 1500 and 6000 ppm dose levels, while total activity in the recovery group was not increased and illustrated the reversibility of the effect of treatment, although increased 15-alpha and 16-beta hydroxy metabolites were still reported for the 28 day recovery group and the 42 day continuous treatment group.
The results of this experiment support the barbiturate type of induction for the test chemical, and are also similar to the type of induction observed in the mouse after administration of Triclosan the diet.

Immunoblot analysis - In this study, monoclonal antibodies to cytochrome P-450 gene subfamilies CYP1A, CYP2B, CYP3A, and CYP4A were used. Mab d15 is specific for the major 3-methylcholanthrene-inducible liver cytochromes CYP1A1 and CYP1A2. After 14 days of treatment, a weak protein band was recognized which was though to represent a proteolytic fragment of CYP1A protein. The total protein content recognized by this antibody was increased approximately 2-fold after 14 days at 1500 ppm, and approximately 4-fold after 14 or 42 days of treatment at 6000 ppm.

The Mab be4 antibody is specific for barbiturate-inducible cytochromes CYP2B1 and CYP2B2. A marked dose-dependent increase in the CYP2B proteins was observed at the 1500 and 6000 ppm dose level after 14 days of treatment (650% and 2469% of control at 1500 and 6000 ppm. respectively). Treatment with 6000 ppm of

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Triclosan for 42 days also resulted in a similar increase as that observed after 14 days (2592% of control).

The Mab p6 monoclonal antibody was used to detect changes in the CYP3A subfamily. After 14 days of treatment at 1500 and 6000 ppm Triclosan in the diet, a slight dose-related induction of a single monooxygenase protein with a molecular weight identical to CYP3A1 was identified (131% and 209% of control, respectively). After 42 days of treatment at 6000 ppm, the effect on CYP3A1 persisted (306% of control in this dose group).

Mab Clo4 was used to identify the peroxisome proliferator inducible rat liver isoenzymes of the CYP4A gene family. A slight dose-dependent increase in the level of this protein (116% at 1500 ppm, 164% at 6000 ppm) was observed after 14 days of treatment. Treatment for 42 days at 6000 ppm produced a similar increase as for 14 days (172% of control).

The report claimed that the above finding for the CYP4A isoenzyme does not support the classification of Triclosan as a peroxisome proliferator type inducer in rat liver. The report regards the data as indicative of a co-inductive effect, based on the test article's barbiturate-like inducing properties.

Electron Microscopy - Electron microscopic analysis was performed on liver tissue of three selected rats of the control and high dose group (groups 07 and 10) as well as on three rats from groups 11, 14, and 15. The report noted a moderate to distinct proliferation of smooth endoplasmic reticulum after 14 days of treatment with 6000 ppm Triclosan, and a striking proliferation of smooth endoplasmic reticulum after 42 days of treatment with 6000 ppm Triclosan. In two rats each from the 14 and 42 day treatment groups, membranous whorls or "fingerprints" consisting of concentrically arranged smooth endoplasmic reticulum membranes were observed. The report stated that the metabolic function and ultimate fate of these structural derivatives is not known, but is tentatively interpreted as an adaptive response to "hepatotropic" agents. In this reviewer's opinion, the proliferation of smooth ER could be related to any of its general functions, including increased protein synthesis (particularly increased cytochrome content) or increased activity of detoxifying enzymes in response to administration of Triclosan.

In addition to proliferation of smooth ER, the number of cytoplasmic lipid vacuoles was increased after 14 and 42 days of treatment at 6000 ppm.

The proliferation of smooth ER as well as the increased cytoplasmic lipid vacuoles were demonstrated to be reversible conditions as shown by examination of liver from those rats allowed a 28 day recovery periof following 14 days of treatment.

Discussion - The results of the present study, like those of the mouse liver study, show the liver to be the target organ for Triclosan-induced toxicity. In terms of doses received, the rats in this study received actual doses of 23, 108, and 518 mg/kg/day Triclosan in the diet for either 14 or 42 consecutive days. Reversibility of the effects were assessed through recovery of a group of rats for 28 days following a 14 day administration of 463 mg/kg/day. Significant hepatic effects, similar to those observed in mice, were seen at the top dose of Triclosan in this study. These included a significant increase in liver weight, increases in biochemical enzyme parameters (P-450 content, glutathione-S-transferase activity, EROD and PROD activity), and proliferation of smooth endoplasmic reticulum and cytoplasmic lipid vacuoles. In contrast to mice, no significant increase in cyanide-insensitive peroxisomal fatty acid ß-oxidation was observed in this study, although induction of cytochrome CYP4A was observed. This indicates possibly that Triclosan is less effective as a peroxisome proliferating agent in the rat liver vs the mouse liver. The potency of Tri[illegible]san in the rat vs mouse liver may be equivalent, although the [illegible]are not conclusive based on the different doses employed i[illegible]e two studies. It appears that the low dose in mice (18.4 mg/k[illegible]y) resulted in biocehmical effects in the liver as well as inc[illegible]on of specific cytochrome P-450 isozymes. In the rat, the low dose of 23 mg/kg/day did not result in induction of cytochrome P-450 proteins, although induction of EROD and PROD was observed. In the mouse, Triclosan appears to function more potently as an inducer of peroxisome proliferation. However, as mentioned, the significance of peroxisome proliferation observed in rodent studies as predictive for human toxicity is inconclusive and may even be irrelevant based on research showing a potential lack of sensitivity of human liver to peroxisome proliferating agents.

Of interest are the results from mouse liver showing apparent cell proliferation after 14 days treatment at 54.7 mg/kg/day Triclosan. These data are likely one of the the relevant endpoints for use in risk [illegible]sment of this chemical, as cell proliferation is a compone[illegible] a mode of action for many chemicals which can lead to neoplasia. Although tumorigenicity was not actually observed in long-term studies with Triclosan, significant hepatotropic effects including cell proliferation should be used for risk assessment of this chemical, based on the strength of the hepatic response in both rats and mice. In fact, the 18.4 mg/kg/day dose can be selected as a relevant NOEL based on the cell proliferative response in male mice at a dose of 54.7 mg/kg/day.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS/HEALTH EFFECTS DIVISION
TOX ONELINERS

Print Date: 10-Mar-1998
Tox Chem No.: 186A
CAS: 3380-34-5
Last Updated: 10-Mar-1998

EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
Allergizing Effects Species: Guinea Pig	Irgasan		Did not cause irritation or allergizing effects. Doses tested: 0.10 % in saline injected i.c.	001955
Acute Intravenous LD50 Species: Rat	Irgasan		LD 50 = 29 mg/kg. Exophthalmos, twitching, dypsnea and apnea.	001956
Acute Intravenous LD50 Species: Rat Ciba Geigy, N.J. ; 30-Apr-1968	Irgasan		LD50 = 29.9 (26.0-34.4) mg/kg (M). LD50 = 28.3 (24.8-32.3) mg/kg (F)	001956
Acute Subcutaneous Species: Rat	Irgasan in 95 % alcohol		LD50 > 14700 mg/kg (females). Doses tested: 1000,4640, 6810, and14700 mg/kg.	001956
Acute Intraperitoneal LD50 Species: Cat Industrial Bio-Test Laboratories, Inc. 621-0477; 04-Jun-1974	Irgasan, DP 300		LD 50 < 500 mg/kg. (pupils dilated). Doses tested 0, 250, 500, 750, and 1000 mg/kg.	Supplementary 001966
Photosensitization Species: Guinea Pig	Irgasan 2.0 % w/v in alcohol		3 phase test.	Invalid 001958 Invalid 001968
Photosensitization Species: Human	Irgasan		No primary photosensitizing nor bio-sensitization capablilities. Doses tested: 1.25 - 2.0%.	001955
Acute Intravenous LD50 Species: Mouse	Irgasan		LD50 = 19 mg/kg. Cramps, exophthalmos, twitching, dypsnea and apnea.	001956

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
Phototoxicity Species: Human	Irgasan in petrolatum		No significant phototoxicity effects noted.	001955
Acute Intravenous LD50 Species: Rat	Irgasan		LD50 = 28.3 mg/kg.	001956
Phototoxicity Species: Human	CH 3565 (in petrolatum)		No significant phototoxicity effects noted.	001955
Photosensitization Species: Human	CH 3565		Ch 3565 has no primary photosensitizing nor bio-sensitization capabilities. Doses tested: 1.25 - 2.0%.	001955
Photosensitization Species: Guinea Pig	Wool containing 7200 ppm Nylon containing 18900ppm			Invalid 001958 Invalid 001968
Photosensitization Species: Human	CH 3565 (2.5% in petrolatum)		No reaction noted.	001958
Photoallergy Species: Human	CH 3565 (10% in petrolatum)		No reaction noted.	001958
Nephrotox. & Hepatotoxicity Species: Rat	Triclosan		Triclosan affects renal functions as indicated by the accumulation of p-aminohippurate and N-methyl nicotinamide. Not a hepatotoxic agent(affects only PAH in vivo, but both parameters in vitro). Doses tested: 0.025, 2.5 g/kg - in vivo; 10-5M or 10-4M in vitro.	Minimum 001965

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-1 Acute Oral Toxicity Species: Rat (Sprague-Dawley) Products Safety Labs 2800; 11-Mar-1994	Triclosan; Purity: 99.7% a.i.	43206501	Five male and 5 female rats received a limited dose (5,000 mg/kg) of Triclosan (99.7% a.i.) by gavage. Only one animal (female) died. LD50 for males and females >5,000 mg/kg (limit dose for acute oral toxicity study). Clinical signs of lethargy, abdominal distention, piloerection, ocular discharge, and irregular respiration were seen in all treated animals. By day 7, essentially all treated animals recovered except one male which continued to exhibit signs of ocular discharge and chemosis until the end of the study.	Acceptable 011304 24-Oct-1994
81-1 Acute Oral LD50 Species: Rat	Product A461 and GP1 Spray Cleaner, Irgasan 0.05 %		LD50 > 5000 g/kg (only dose tested). No deaths-lethargy and diarrhea. Toxicity Category IV.	001954
81-1 Acute Oral LD50 Species: Rat Economics Laboratory, Inc. ; 01-Jul-1970	Irgasan 33.3% (Soilax laundri Soft)		LD50 = 7.5 gm/kg	007350
81-1 Acute Oral LD50 Species: Rat	Diasof conc., 1.5 % Irgasan			Invalid 001961
81-1 Acute Oral LD50 Species: Cat Industrial Bio-Test Laboratories, Inc. 621-04720	Irgasan, DP-300		LD50 not determined (one animal died at all but the lowest dose). (2 cats/dose). Doses tested: 3000, 3250, 3500, 4000 & 6000 mg/kg.	001966
81-1 Acute Oral LD50 Species: Rat	Radene, 0.2 % Irgasan		LD50 > 5 ml/kg. Toxicity Category IV.	001959
81-1 Acute Oral LD50 Species: Rat	Touch up, 0.1 % Irgasan		LD50 > 5 ml/kg. Toxicity Category IV.	001960

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EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-1 Acute Oral LD50 Species: Dog Industrial Bio-Test Laboratories, Inc. ; 15-Dec-1966	Irgasan		LD50 > 5000 mg/kg. Emesis occurred at all doses tested. Doses tested 2500 and 5000 mg/kg. Toxicity Category III.	Supplementary 001955 Supplementary 001968
81-1 Acute Oral LD50 Species: Mouse	Irgasan in gum arabic		LD50 = 4530 g/kg. Doses tested: 2500 and 5000 mg/kg. Toxicity Category III.	001955
81-1 Acute Oral LD50 Species: Rat (Sprague-Dawley)	Irgasan in corn oil		LD50 = 4.4 (S.D. +- 0.4) g/kg (M). LD50 = 3.7 (S.D. +- 0.5) g/kg. Hypoactivity, diarrhea, diuresis, and bloody nasal discharge. Doses tested: 3.0, 4.6, 6.8 g/kg - Sprague-Dawley strain. Toxicity Category III.	001955
81-1 Acute Oral LD50 Species: Rat Industrial Bio-Test Laboratories, Inc. A1993; 29-Sep-1972	Irgasan (DP 300)			Invalid 001966
81-2 Acute Dermal LD50 Species: Rabbit Economics Laboratory, Inc.	Irgasan 33.3% (Soflax Laundri Soft)		LD50 = 4.0 cc/kg.	007350
81-2 Acute Dermal LD50 Species: Rabbit	Irgasan in propylene glycol		LD50 > 9.3 g/kg. Moderate erythema and edema at 24 hours; skin very dry or necrotic at 7 days. Doses tested 4.6, 6.8, and 10.2 g/kg. Toxicity Category III.	001956
81-2 Acute Dermal LD50 Species: Rabbit	Diasof conc. 1.5 % Irgasan			Invalid 001961

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EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-3 Acute Inhalation LC50 Species: Rat Ciba-Geigy Ltd. 254597; 18-May-1990	Triclosan; Purity: not specified	42306902 43310501	Five rats/sex received FAt 80'023/Q by inhalation for 4 hours at a gravimetric concentration of 0.15 mg/L. The test article did not produce any clinical signs or deaths in rats. It neither affected the body weights nor gross pathological changes. Based on these findings, the LC50 was >0.15 mg/L, and the category for the acute inhalation toxicity of this chemical was II. The information on the chemical purity and chemical analysis was missing. It does not meet the data requirements for an acute inhalation toxicity study (Guideline 81-3). However, it can be upgraded after submission and satisfactory evaluation of the missing information. Toxicity Category II.	Minimum 011367 23-Dec-1994 Supplementary 009903
81-3 Acute Inhalation LC50 Species: Rat	Irgasan		LC > 0.14 mg/L/4 hours(actual concentration). No deaths or toxic signs. Toxicity Category II.	001955
81-3 Acute Inhalation LC50 Species: Rat	Irgasan 0.1 %		Animals were exposed to 10 sprays during 5 hours. Each spray released 14.5 gm of the product. Generalized inactivity and weakness; No adverse effects on body weight and pathologic findings.	001956
81-3 Acute Inhalation LC50 Species: Rat	Diasof conc. 1.5 % Irgasan			Invalid 001961
81-4 Primary Eye Irritation Species: Rabbit	Product A461 and GP1 Spray Cleaner-Irgasan 0.05 %		Eye irritant. Dose tested: 0.1 ml-24 hour esp. then rinsed.	001954
81-4 Primary Eye Irritation Species: Rabbit	Irgasan 33.3% (Soilax Laundri Soft)		No irritation was noted.	007350
81-4 Primary Eye Irritation Species: Rabbit	Diasot conc. (1.5% Irgasan)			Invalid 001961

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-4 Primary Eye Irritation Species: Rabbit	Touch Up 0.1 % Irgasan		Corneal opacity and erythema with clearing by 48 hours. Toxicity Category III.	001960
81-4 Primary Eye Irritation Species: Rabbit	Radene, 0.2 % Irgasan		Corneal opacity present at 72 hours (observations not made on the 7th day). Toxicity Category II.	001959
81-4 Primary Eye Irritation Species: Rabbit	Irgasan		PIS = 59.3/110 (at 24 hours; washed eyes-2 second contact). PIS = 24.4/110 (at 7 days; washed eyes-4 seconds contact). PIS = 36.6/110 (at 24 hours; washed eyes- 4 seconds contact). PIS= 0 at 7 day washed eyes, 4 seconds contact). (Moderately irrit.). Dose: 100 mg.	001955
81-4 Primary Eye Irritation Species: Rabbit	Irgasan		PIS = 92/110(24 hours). 82/110 (72 hours). Severe irritation.	001955
81-5 Primary Dermal Irritation Species: Rabbit	Touch Up, 0.1 % Irgasan			Invalid 001960
81-5 Primary Dermal Irritation Species: Rabbit	Radene, 0.2 % Irgasan		PIS = 5.08/8.0. Toxicity Category III.	001959
81-5 Dermal Exposure Study Species: Rat Exxon Biomedical Sciences, Inc. 139910A; 03-Sep-1993	Irgasan; Purity: 99.7%	43251901	Groups of rats (2/sex/group) received Irgasan in propylene glycol (PPG) at dose levels of 0, 10, 25, 50, 100, and 200 mg/kg or in Drakeol at dose levels of 0, 25, and 200 mg/kg. The test animals were exposed to the test article for 6 hours. Skin irritation was seen in test animals that received 25 mg/kg or above, and that in 100 and 200 mg/kg groups was more marked. No dermal irritation was seen in the 10 mg/kg group. There was no systemic toxicity in any test groups. Based on these results, doses of 10, 40, and 80 mg/kg were selected for the 90-day dermal toxicity study.	Supplementary 011417 23-Feb-1995

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-5 Primary Dermal Irritation Species: Rabbit	Product A461 and GP1 Spray Cleaner, Irgasan 0.05 %.		PIS = 0.83/8.0. Minimally irritating to the skin. Dose tested: 0.5 ml- 24 hour exposure. Toxicity Category IV.	001954
81-5 Primary Dermal Irritation Species: Rabbit Economics Laboratory, Inc.	Irgasan 33.3% (Sollax laundri Soft)		No irritation was noted.	007350
81-6 Dermal Sensitization Study Species: Guinea Pig	Irgansan		Not a sensitizing agent. Doses tested: 0.05 Or 0.1 ml injected i.c.	001955
81-6 Dermal Sensitization Study Species: Human	Irgasan 0.05 %, Spray Cleaner		Slight to well defined erythema was noted in 10 of the 50 subjects during treatment. Not a skin sensitizing agent. Dose tested 0.1 ml -24 hour exposure, 3 times /week for 3 weeks.	001954
81-6 Dermal Sensitization Study Species: Human	Irgasan 0.5 % in soap solution		Very mild fatiguing agent. Dose tested: 0.5 ml-24 hour exposure (non-occlusive) 10 exposures, 2 weeks rest, then challenge.	001955
81-6 Dermal Sensitization Study Species: Human	Irgasan 2.5 % in soap solution		Is a fatiguing agent. Doses tested: 0.5 ml-24 hour exposure (non- occlusive). 10 exposures. Ten days free and then challenge.	001955
81-6 Dermal Sensitization Study Species: Human	Irgasan 10 % in petrolatum		Irgasan at 10 % concentration is highly irritating. Doses tested: 1, 2.5, and 10 % for 24 hours. Ten (10) occluded exposures.	001955
81-6 Dermal Sensitization Study Species: Human	Irgasan 25 % in petrolatum		Not a sensitizing agent. Dose tested: 25 %- Five 48 hour exposures 1 day between each exposure, challenge dose to new site.	

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
81-6 Repeated Insult Patch Test Species: Human	7200 ppm in wool, Nylon containing 18,900 ppm			Invalid 001956 Invalid 001968
81-6 Dermal Sensitization Study Species: Guinea Pig American Standards Bioscience Corpoation 88-470; 25-Oct-1988	Irgasan (formulation) Issue Plus II	41008909	Not a skin sensitizer; no positive control.	Supplementary 007097
81-6 Dermal Sensitization Study Species: Guinea Pig	Irgasan 0.05 % Spray Cleaner Product A461 and GP1		Not a sensitizing agent. Doses tested (injection): 0.1 % (v/v) in saline.	001954
81-6 Dermal Sensitization Study Species: Human	Irgasan 1 % soap solution		0.25 % and 0.5 % concentration caused moderate irritation. Doses tested: 0.25 % and 0.5 %-24 hour occluded exposure/10 exposures.	001955
81-8 Neurotoxicity- 14 Day Delayed Species: Rat	Irgasan DP 300 (in 2% carboxymethyl cellulose)		Some inhibition of movement, decreased muscular tone, polydypsia & polyuria at 300 mg/kg. Doses tested: 0, 100, 300, 1000, 2000 mg/kg.	Supplementary 001968
82-1(a) Feeding- 13 Week Species: Rat Ciba-Geigy Corp. Inc. ; 22-Jan-1968	GP 41353	00034333	Systemic NOEL < 125 mg/kg (LDT) (Nephrosis, small infiltrations of mononuclear cells). Levels tested: 0, 25, 50, 100, 200 mg/kg/day	Minimum 001958 Minimum 003655
82-1(a) Feeding- 3 Month Species: Rat (Sprague-Dawley) Laboratorium Pharm un Toxik;ge ; 27-Jul-1970	Irgasan tech	2517720008	NOEL > 1875 ppm (HDT). Doses tested: 187, 375, 937, and 1875 ppm Sprague-Dawley strain.	Minimum 003655
82-1(a) Feeding- 3 Month Species: Rat (CrL:COBS-CD(SD)) Bionetics Research Laboratories, Inc. 22188; 11-Oct-1983	Irgasan tech	00133545	NOEL = 1000 ppm (nonspecific liver), LEL = 3000 ppm (triglycerides decreased, creatinine increased (F), RBC decreased (M), increased spleen weight (M), increased heart weight (F), cytomegaly - considered in total as a non-specific liver toxicity). Doses tested: 0, 1000, 3000, 6000 ppm - CRL: COBS CD(SD) BR strain.	Minimum 003655

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Print Date: 10-Mar-1998
Tox Chem No.: 186A
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Last Updated: 10-Mar-1998

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
82-1(a) Feeding- 3 Month Species: Rat (Charles River CD(SD)) Sterling Winthrop Labs PD. 2-9028; 16-Mar-1973	Irgasan DP 300 (in 1% gum trayacanth)	00133229	Systemic NOEL = 50 mg/kg (LDT), Systemic LEL = 125 mg/kg (increased liver weight in males). Levels tested in Charles River CD strain: 0, 50, 125, and 315 mg/kg.	001968 Minimum 003655
82-1(a) Feeding- 13 Week Species: Mouse (CD-1) Hazleton Washington, Inc. HWA 483-287; 28-Jan-1993	Irgacare, Purity: 99.7%	43022605	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 of severity of histopathological lesions (especially in the liver). Clinical pathology included significantly decreased erythrocytes, hemoglobin, and hematocrit at > or equal to 25 mg/kg/day in males and > or equal to 75 mg/kg/day in females. Enzyme changes, indicative of liver injury, included increased alkaline phosphate 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.	Minimum 011304 24-Oct-1994
82-1(a) Feeding- 28 Day Species: Rat	Bacteriostat CH 3565		Systemic NOEL = 500 mg/kg, Systemic LEL = 1000 mg/kg (Death: 6/10). Doses tested: 50, 100, 200, 500, 1000 mg/kg by gavage.	001955
82-1(a) Feeding- 3 Month Species: Rat Industrial Bio-Test Laboratories, Inc. 622-4554; 23-Jul-1974	Irgasan		INVALID Clement Assoc. #68-01-5824 1/27/82.	Invalid 001963
82-1(a) Feeding- 28 Day Species: Monkey (Baboon)	GP 41353		Systemic NOEL > 100 mg/kg (HDT). Doses tested: 1.0, 10, 30, 100 mg/kg	001958
82-1(b) Feeding- 3 Month Species: Dog (Beagle) Laboratorium Pharm un Toxik;ge ; 10-Jul-1970	Irgasan tech	00133233	NOEL > 625 ppm (HDT). Doses tested in beagles - 125, 313 & 625 ppm.	Minimum 003655

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
82-1(b) Feeding- 13 Week Species: Rabbit (New Zealand White) Ciba Geigy Corp. ; 31-Mar-1969	GP 41353	00034332	Systemic NOEL = 3 mg/kg (LDT), Systemic LEL = 30 mg/kg (neutrophilia, lymphopenia, pulmonary infection, edema and lung necrosis). Levels tested: 3, 30, 150 mg/kg/day New Zealand white strain.	Minimum 001958 Minimum 003655
82-1(b) Feeding- 3 Month Species: Rabbit (New Zealand White) Laboratorium fuer Pharmakologie und Toc. ; 31-Jul-1970	Irgasan tech	00133234	NOEL > 2500 ppm (HDT). Doses tested: 250, 500, 1250, and 2500 ppm New Zealand white strain.	Minimum 003655
82-1(b) Feeding- 3 Month Species: Dog Ciba Geigy Corp. ; 21-Dec-1967	GP 41353	00034334	Systemic NOEL < 25 mg/kg (LDT; increased SAP, decreased hemoglobin and RBC values; dose related jaundice, increased liver weight). Levels tested: 0, 25, 50, 100, 200 mg/kg/day.	Minimum 001958 Minimum 003655
82-1(b) Feeding- 13 Week Species: Monkey (Baboon) Huntingdon Research Center ; 01-Jan-1969	GP 41353	00034331	Systemic NOEL > 3 mg/kg (single dose tested).	001958
82-1(b) Feeding- 3 Month Species: Dog (Beagle) Ciba Geigy Corp. 37/67/SL; 01-Jan-1967	Irgasan DP 300	00133232	Systemic NOEL = 12.5 mg/kg/day (LDT), Systemic LEL = 25 mg/kg/day (morphologic changes in liver - focal acidophilic granular degeneration of cytoplasm). Dose: 0, 17.5, 25, 50 & 100 mg/kg/day by gelatin capsule to beagle dogs.	001968
82-2 Dermal- 1 Week Species: Rat	CH 3565 (5% suspension in gum arabic)		No local irritant effects. Dose tested: 0.4 ml - 3 hr. exp. - 1 exp./ day	001955
82-2 Dermal- 28 Day Species: Rat	CH 3565 2.5%suspension (in gum arabic)		No local irritation or resorptive toxic effects. Dose tested: 0.4 ml. Exposure - 3 hrs/day for 4 weeks	001955
82-2 Dermal- 14 Day Species: Rabbit Industrial Bio-Test Laboratories, Inc. 601-03277; 03-May-1973	1% Triclosan	00096101	IBT - Invalid - Dynamac Corporation Accepted by EPA: 2/7/83	Invalid 002985

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
82-2 Dermal- 3 Week Species: Dog	Bacteriostat CH 3565 (0.1% solution)			Invalid 001956 Invalid 001968
82-2 Dermal- 14 Day Species: Rat (CrL:CD(SD)BR VAF/Plus) Exxon Biomedical Sciences, Inc 139910A; 03-Sep-1993	Irgasan; Purity: 99.7% [Batch No. 5.2.0211.0]	43251901	Groups of rats (2/sex/group) received Irgasan in propylene glycol (PPG) at dose levels of 0, 10, 25, 50, 100 and 200 mg/kg or in Drakeol at dose levels of 0, 25 and 25 mg/kg. The test animals were exposed to the test article for 6 hours. Skin irritation was seen in test animals whiched received 25 mg/kg or above, and that in 100 and 200 mg/kg groups was more marked. No dermal irritation was seen in the 10 mg/kg group. There was no systemic toxicity in any test groups. Based on these results dose of 10, 40 and 80 mg/kg were selected for the 90-day dermal toxicity study (MRID No. 43328001).	Supplementary 011417 23-Feb-1995
82-2 Dermal- 14 Day Species: Rat	CH 3565		No dermal effect noted. Dose tested: 2.0 ml (3% w/v in oil).	001958
82-2 Dermal- 10 Day Species: Rabbit	Ivory soap containing CH 3565		Mild edema which subsided promptly. Moderate erythema which subsided within 5 days. No pathological findings related to the test material were found. (These effects were seen in ALL animals including those exposed only to soap). Dose tested: 100 mg - Once daily for 10 days.	001955
82-2 Dermal- 14 Day Species: Rabbit Industrial Bio-Test Laboratories, Inc. A8434; 11-Jun-1970	Irgasan	00087999 00115191	INVALID Clement Assoc. #68-01-5824 Accepted by EPA: 10/27/81	Invalid 001964 Invalid 001674
82-2 Dermal- 14 Day Species: Rat Industrial Bio-Test Laboratories, Inc. A8434; 11-Jun-1970		00087999 00115191	IBT Invalid. Clement Associates Contract No. 68-01-5824. Accepted by EPA: 10/27/81	Invalid 001674

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
82-3 Dermal- 3 Month Species: Rat (CrL:CD(SD)BR VAF/Plus) Exxon Biomedical Sciences, Inc. 139910A; 03-Sep-1993	Irgasan; Purity: 99.7% [Batch No. 5.2.0211.0]	43328001	Groups of rats (10/sex/group) received Irgasan in PPG by dermal application at dose levels of 10, 40, and 80 mg/kg for 6 hours/day for 90 days. Dermal irritation at the application site was found in all dose groups, and the severity of the dermal irritation was dose-related. However, the dermal irritation was reversible after a certain recovery period. An increase in the incidence of occult blood in the urine of 80 mg/kg males and females was found. No additional systemic toxicity was seen. Under the conditions of this study, the LEL for this systemic toxicity was 80 mg/kg; NOEL, 40 mg/kg. This study meets the data requirements for a subchronic dermal toxicity study (82-3), and this study is classified as minimum.	Minimum 011417 23-Feb-1995
82-3 Dermal- 4 Month Species: Monkey Industrial Bio-Test Laboratories, Inc. 602-02220; 06-Oct-1974	Irgasan DP300 (soap solution)	00096114	INVALID (Dynamac Corporation Contract #68-01-6561; 5/14/82) Accepted by EPA: 5/24/82	Invalid 001957 Invalid 002982
82-3 Dermal- 3 Month Species: Rabbit (New Zealand White) Ciba-Geigy Corp. Inc. ; 07-Sep-1970	CH 3565 (3% solution)		Systemic NOEL = 0.5 ml/kg; LEL = 1.0 ml/kg (HDT) (Slight erythema and edema increasing in severity and accompanied by eschar formation and rhagades at 1.0 ml/kg). Doses tested: 0.1, 0.5, 1.0 ml/kg New Zealand white strain.	001968
82-4 Inhalation- 21 Day Species: Rat Ciba-Geigy Corp. Inc. ; 24-Jul-1974	Irgasan DP 300		LC50 < 1.3 mg/L/2 hrs. (5/9 M, 7/9 F died after exposure to 1.3 mg/L/2 hrs.). NOEL = 0.05 mg/L, LEL = 0.115 mg/L (1 day exposed to 0.227 mg/L). (Elevated total leucocyte count and increased SAP in males). Doses tested: 0.05, 0.115, 0.301 mg/L for 2 hrs/day - 21 days.	001966
83-1(b) Feeding- 1 Year Oral Capsule Species: Monkey (Baboon) Ciba-Geigy Corp. Inc. ; 28-Jun-1976	Irgasan tech	00133230 00133231	NOEL = 30 mg/kg/day, LEL = 100 mg/kg/day (failure to eat & diarrhea). Doses tested: 30, 100 and 300 mg/kg/day.	Minimum 003655
83-2(b) Dermal- 18 Month Species: Mouse Industrial Bio-Test Laboratories, Inc. J4915; 01-Jan-1967	Bacteriostat CH 3565 (in acetone) (Irgasan)	00100183	6 month interim report: 0.1 ml of a 0.5% and 1.0 solution in acetone did not produce any systemic or oncogenic effect. Doses tested: 0.5 and 1.0% - Swiss white strain.	001955
83-2(b) Carcinogenicity- 18 Month Species: Mouse Industrial Bio-Test Laboratories, Inc. 622-05278; 25-May-1976	Irgasan	00056976 00094048	IBT - Invalid - Dynamac Corporation Contract No. 68-01-6561 Accepted by EPA: 9/17/82	Invalid 002984

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-2(b) Carcinogenicity- 18 Month Species: Mouse Industrial Bio-Test Laboratories, Inc. J4915; 24-Aug-1965	Bacteriostat CH 3565 in acetone (Irgasan)	00035095 00081390 00107857		Invalid 001956 Invalid 001968 Invalid 002980
83-3(a) Developmental Toxicity Study Species: Rat (Sprague-Dawley (CD)) Bio/dynamics Inc. 91-3665; 16-Apr-1992	Irgacare, Purity: 99.8%	43026606	Irgacare MP was administered to Sprague-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 > or equal to 150 mg/kg/day; the Maternal and Developmental LOEL were not determined.	Supplementary 011304 24-Oct-1994

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(a) Developmental Toxicity Study Species: Rat (Wistar) Unilever Research, Colworth/Welwyn Lab RT/3/84; 01-Dec-1992	Triclosan, Purity: 99.8% [Batch# P405129]	43817502 43817503	The test substance, Triclosan (99.8%), was administered by gavage to pregnant female Colworth Wistar rats (30 rats/treated group and 60 rats in the control group) on days 6-15 of gestation at dose levels of 30, 100, or 300 mg/kg/day, with the day of mating designated as gestation day 0. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 21 of gestation, 25 rats per treated group and 50 control rats were sacrificed and necropsied; uterine weights were recorded. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation. Five rats per treated group and ten control rats were allowed to deliver their litters. Litter weight, pup mortality, and developmental milestones (presence of vibrissae, pinna unfolding, incisor eruption, eyelid opening, and completion of fur growth) were recorded. The pups were killed and necropsied on lactation Day 21, and all pups were processed for skeletal examination. At 300 mg/kg/day, maternal toxicity consisted of transient diarrhea, retarded body weight gain during the period of treatment, and reduced food consumption and increased water consumption from the onset of treatment, throughout the gestation period. Based on these findings: Maternal LOEL = 300 mg/kg/day; Maternal NOEL = 100 mg/kg/day. No evidence of pre- or postnatal developmental toxicity was identified at any dose level under the conditions of this study. Developmental LOEL = Not determined (>300 mg/kg/day); Developmental NOEL > or equal to 300 mg/kg/day This study is classified as ACCEPTABLE and satisfies the 83-3(a) guideline requirement for a developmental toxicity study in rats.	Acceptable 012134 16-Jan-1997
83-3(a) Developmental Toxicity Study Species: Mouse Huntingdon Research Center (UK) 2373/68/251; 26-Aug-1983	GP 41353 (Irgasan)	00034329	Teratogenic NOEL > 100 mg/kg/day0 (HDT), Maternal NOEL = 50 mg/kg/day. Maternal LEL = 100 mg/kg/day (HDT; increased mortality). Fetotoxic NOEL = 50 mg/kg/day. Fetotoxic LEL = 100 mg/kg/day (HDT; weight reduction; asymmetrical sternebrae). Dosing from day 1 to day 16 of gestation at 10, 50 and 100 mg/kg/day.	Minimum 001958 Minimum 003655

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(a) Developmental Toxicity Study Species: Rat (Sprague-Dawley) Bio/dynamics Inc. 91-3665; 16-Apr-1992	Irgacare MP (C-P Sample-No.:38328), Purity: 100% [Lot# 19851206]	43825301	The test substance, Irgacare MP (C-P Sample No. 38328); 100% a.i., was administered by gavage to pregnant female Sprague-Dawley rats on days 6-15 of gestation at dose levels of 1.0, 10.0, 25.0, and 50.0 mg/kg/day. The rats were observed for signs of toxicity; body weight and food consumption values were recorded. On day 20 of gestation, the rats were sacrificed and necropsied; spleen and uterine weights were recorded; spleens were examined histopathologically. The uteri were examined, implantation sites were counted, and the numbers of corpora lutea were determined. The fetuses were removed, weighed, sexed, and examined for external anomalies. They were then processed for visceral and skeletal evaluation. No unequivocal evidence of maternal or developmental toxicity was identified at any dose level under the conditions of this study. Maternal LOEL = Not determined (>150 mg/kg/day); Maternal NOEL > 150 mg/kg/day; Developmental LOEL = Not determined (>150 mg/kg/day); Developmental NOEL > 150 mg/kg/day. This study is classified as SUPPLEMENTARY, not upgradable, and does not satisfy the §83 3(a) guideline requirement for a developmental toxicity study in rats due to the failure of the study to identify a treatment-related effect level for either maternal or developmental toxicity	Supplementary 012134 16-Jan-1997

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(a) Developmental Toxicity Study - Range Finding Species: Rat (Sprague-Dawley) Bio/dynamics Inc. 91-3654; 06-May-1992	Irgacare MP (C-P Sample-No.:38328), Purity: 100% [Lot# 19851206]	43820402	In a range-finding study, Irgacare MP (C-P Sample No. 38828) was administered once daily by gastric intubation on gestation days 6-15 to mated female Sprague-Dawley rats (5/group) at dose levels of 5, 10, 25, 50, or 75 mg/kg/day in a constant volume (5 ml/kg) of 1% carboxymethylcellulose suspension in 20% aqueous glycerin. A concurrent control group received only vehicle. Individual doses were adjusted to the most recent body weight throughout the dosing period. Mortality and clinical observations, body weight data, and food consumption data were recorded throughout the study. Cesarean sections were performed on day 20 of gestation. Gross pathological examinations were performed, and the liver and intact uteri were weighed. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and corpora lutea. Each fetus was weighed, sexed, examined for external abnormalities, and sacrificed. All rats survived to study termination. Clinical observation data did not indicate a response to treatment. In rats receiving 75 mg/kg/day, reductions in mean maternal body weight gain during GD 10-16, as well as for the entire treatment period (GD 6-16), were suggestive of a maternal toxic response. This response, however, was attributed to the weight data for a single animal, all others within the group having weights that were within the range of values for controls. Food consumption data were not affected by treatment. Postmortem examination revealed no treatment-related changes, and absolute and relative (to adjusted gestation Day 20 body weight) liver weight data, were similar between control and treated groups. Mean number of corpora lutea, litter size, number of implantations, pre-implantation loss, and number of resorptions were similar between the control and treatment groups. Mean fetal weights at 75 mg/kg/day were reduced as compared to concurrent and historical controls; no gross external alterations were noted in the fetuses. Based upon the results of this range-finding study, 150 mg/kg/day was selected as a high dose for the subsequent definitive developmental toxicity study in rats. Low- and mid-dose levels chosen were: 15 and 50 mg/kg/day, respectively.	012134 16-Jan-1997

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(b) Developmental Toxicity Study Species: Rabbit (New Zealand White) Huntingdon Research Center (UK) 2403/68/280; 26-Sep-1968	GP 41353 (Irgasan)	00034330	Teratogenic NOEL > 50 mg/kg/day (HDT), Fetotoxic NOEL = 25 mg/kg/day. Fetotoxic LEL = 50 mg/kg/day (HDT; increase in 13 ribbed groups). Levels tested 0, 10, 25, 50 mg/kg/day New Zealand white strain.	Minimum 001958 Minimum 003655
83-3(b) Developmental Toxicity Study Species: Rabbit (New Zealand White) Bio/dynamics Inc. 91-3666; 16-Apr-1992	Irgacare, Purity, 99.8% [Lot No. 19851206C-P 38328]	43026607	In a developmental toxicity study, at least 18 rabbits per dose group of the New Zealand White strain received either 0, 15, 50, or 150 mg/kg/day 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 when compared with 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. 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 damn, (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/day - 7/6 litters, and 150 mg/kg/day - 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. 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.	Guideline 011304 24-Oct-1994

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(b) Developmental Toxicity Study Species: Rabbit (New Zealand White) Bio/dynamics Inc. 91-3666; 16-Apr-1992	Irgacare MP (C-P Sample No.: 38328) (Purity: 100%) [Lot# 19851206]	43820401	The test substance, Irgacare MP (C-P Sample No. 38328); 100% a.i., was administered by gavage to pregnant female New Zealand White rabbits (18/group) on days 6-18 of gestation at dose levels of 15, 50, or 150 mg/kg/day. The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. On day 30 of gestation, the 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 for cardiac abnormalities. 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. Maternal LOEL = 150 mg/kg/day (based upon decreased body weight gain and food consumption during treatment); Maternal NOEL = 50 mg/kg/day No treatment-related developmental toxicity was observed under the conditions of this study. Developmental LOEL = Not determined (>150 mg/kg/day); Developmental NOEL = 150 mg/kg/day This study is classified as ACCEPTABLE and satisfies the 83-3(b) guideline requirement for a developmental toxicity study in rabbits.	Acceptable 012134 16-Jan-1997
83-3(b) Developmental Toxicity Study Species: Rabbit Industrial Bio-Test Laboratories, Inc. J7112; 16-Sep-1969	CH 3565 (Irgasan)	00055002		Invalid 001962 Invalid 001968

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
83-3(b) Developmental Toxicity Study - Range Finding Species: Rabbit (New Zealand White) Bio/dynamics Inc. 91-3655; 06-May-1992	Irgacare MP (C-P Sample No.: 38328) (Purity: 100%) [Lot# 19851206]	43787101	In a range-finding study (C-P Sample No. 38828) was administered once daily by gastric intubation on gestation days 6-18 to mated female New Zealand White rabbits (5/group) at dose levels of 5, 10, 25, 50, or 75 mg/kg/day in a constant volume (2 ml/kg) of 1% carboxymethylcellulose suspension in 20% aqueous glycerin. A concurrent control group received only vehicle. Individual doses were adjusted to the most recent body weight throughout the dosing period. Mortality and clinical observations, body weight data, and food consumption data were recorded throughout the study. Cesarean sections were performed on day 30 of gestation. Gross pathological examinations were performed, and the liver and intact uteri (ovaries attached) were weighed. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and corpora lutea. Each fetus was weighed, examined for external abnormalities, and sacrificed. No treatment-related mortality occurred; the death of one female on GD 9 at 50 mg/kg/day was attributed to a dosing injury. Clinical observation data did not indicate a response to treatment. In rabbits receiving 75 mg/kg/day, there were several intervals during the treatment period when a mean body weight loss and decreased food consumption were evident, and mean weight gain over the treatment period (GD 6-19) was less than control. These findings, although slight, were considered indicative of a treatment-related response. Postmortem examination revealed a greater incidence of red foci/areas in the lungs of does treated at the 50 and 75 mg/kg/day levels, but the toxicological significance of this finding was considered equivocal. Absolute and relative (to adjusted gestation Day 30 body weight) liver weight data, were similar between control and treated groups. Mean number of corpora lutea, litter size, number of implantations, and number of resorptions were similar between the control and treatment groups. Mean fetal weights of treated groups were similar to controls; no gross external alterations were noted in the live fetuses. At 75 mg/kg/day, one late resorption (weighing 16.4 g) with cranial/facial and abdominal closure malformations was identified; however, this finding was considered to be an isolated event and was not attributed to treatment. Based upon the results of this range-finding study, 150 mg/kg/day was selected as a high dose for the subsequent	012134 16-Jan-1997

(continued...)

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Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
(...continued)			definitive developmental toxicity study in rabbits. Low- and mid-dose levels chosen were: 15 and 50 mg/kg/day, respectively.	
83-4 Reproduction Study- 2 Generation Species: Rat Hazleton Lab America 2386-100; 18-Mar-1988	Triclosan 99% Batch # 5202110 (Irgasan)	40623701	Levels tested: 0, 300, 1000, and 3000 ppm. Reproductive NOEL = 1000 ppm. Reproductive LEL = 3000 ppm (decrease viability). Systemic NOEL = 1000 ppm. Systemic LEL = 3000 ppm. Developmental NOEL = 1000 ppm. Developmental LEL = 3000 ppm (decrease body weight ; increased mortality).	Supplementary 006842
83-4 Reproduction/Developmental Toxicity Study Species: Industrial Bio-Test Laboratories, Inc. P7113; 11-Sep-1969	Irgasan	00055002		Invalid 001967
83-4 Reproduction Study - 1 Generation Species: Rat	CH 3565 (Irgasan)		Reproductive NOEL > 100 mg/kg (HDT). Systemic NOEL = 50 mg/kg. Systemic LEL = 100 mg/kg (HDT); body weight depression and increased mortality in males). Fetotoxic NOEL = 100 mg/kg (HDT). Doses tested: 0, 50, 100 mg/kg - Charles River albino strain.	001962
83-5 Feeding/Carcinogenicity- 2 Year Species: Rat (Sprague-Dawley) Ciba-Geigy Corp. Inc. MIN833005; 28-Apr-1986	Fat 80'023 Irgasan DP-300	00161332	Levels tested in Sprague Dawley strain - 0, 300, 1000, and 3000 ppm for 104 weeks and 6000 ppm for 52 weeks. NOEL < 300 ppm (decreased RBC, hemoglobin conc. and hematocrit of males; hepatic necrosis in males). Oncogenicity - negative. At 6000 ppm, decreased body weight in both sexes; decreased Hct in females; decreased in total bilirubin triglycerides, total protein, SGOT and glucose indices; decreased liver weight in M&F; cytoplasmic inclusions and hepatocellular hypertrophy in males. At 3000 ppm, decreased body weight in females; decreased RBC, hemoglobin conc. and hematocrit in males; decreased RBC in females; increased SGPT and SGOT in males and BUN in females; cytoplasmic inclusions and hepatocellular hypertrophy in males; hepatic necrosis in males. At 1000 ppm, decreased RBC, hemoglobin conc. and hematocrit in males; decreased RBC in females; hepatic necrosis in males. Study contained many technical errors, was incomplete and was poorly written.	Supplementary 007098 Minimum 009310
83-5 Feeding/Carcinogenicity- 2 Year Species: Rat Industrial Bio-Test Laboratories, Inc. 622-06047; 01-Dec-1977	Irgasan 99% a.i.	00094046	IBT - Invalid - Dynamac Corporation Accepted by EPA: 3/23/83.	Invalid 002981

EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
84-2 Mutagenic- Ames Species: Huntingdon Research Center (UK) ULR 215/88704; 08-Sep-1988	Triclosan; 2,4,4'-trichloro-2'-diphenyl ether; Irgasan; Purity: 99%	43533301	In two independently conducted Ames assays, S. typhimurium strains TA1535, TA1537, TA98, and TA100 were exposed to triclosan in DMSO at doses of 0.015, 0.050, 0.15, 0.5, or 1.5 ug/plate either in the presence or absence of 3, 10, or 30% S9 from Aroclor 1254-induced rat livers. Triclosan was cytotoxic at 1.5 ug/plate level without S9 and at doses of =>0.5 ug/plate with S9. No mutagenic response was seen at any dose levels with or without S9. This study meets the data requirements for a gene mutation assay (82-2a).	Acceptable 011534 04-May-1995
84-2 Mutagenic- Chromosome Aberration in vivo Species: Rat (Wistar) Ciba-Geigy 218305; 23-Apr-1991	FAT 80'023/Q Purity: 99-100%	43740802	In an in vivo bone marrow cytogenic assay, groups of six male and six female Wistar rats received a single oral gavage administrations of 4000 mg/kg FAT 80'023/Q (99-100%). The test material was delivered to the animals as suspensions prepared in 1% carboxymethyl-cellulose. Animals were sacrificed 6, 24 and 48 hours following compound administration and bone marrow cells from ten animals per group (5 males and 5 females) were harvested and examined for the incidence of structural chromosome aberrations. No signs of overt toxicity or cytotoxicity effects on the target organ were seen in any treatment group. The positive control induced the expected high yield of cells with structural chromosome aberrations. There was also no indication of a clastogenic effect at any sacrifice time.	Acceptable 011744 11-Jan-1996
84-2 Mutagenic- Chromosome Aberration in vitro Species: Hamster (Chinese) Ciba-Geigy 179100; 17-Dec-1990	FAT 80'023/Q Purity: 99-100%	43740801	In an in vitro cytogenic assay, Chinese hamster lung fibroblasts were exposed to FAT 80'023/Q (99-100%) nonactivated doses of 1 ug/mL(7-hour cell harvest), 0.1-3 ug/mL (18-hour harvest), or 3 ug/mL (28-hour harvest) and S9-activated concentrations of 3 ug/mL (7- and 28-hour cell harvests) or 0.1-3 ug/mL (18-hour harvest). The S9 fraction was derived from Aroclor 1254 induced Wistar male rats and FAT 80'023/Q was delivered to the test system in ethanol. No mitotic cells were recovered at any harvest time from cultures treated with > or equal to ug/mL -S9 or > or equal to 10 ug/mL +S(. Findings with the positive controls confirmed the sensitivity of test system to detect clastogenesis. However, nonactivated FAT 80'023/Q at 1 and 3 ug/mL (18-hour harvest) induced a dose-related increase in the yield of cells with abnormal chromosome morphology. The response was significant (p < or equal to 0.001) at the higher concentration. A significant increase (p < or equal to 0.001 was also seen at 3 ug/mL (28-hour harvest). The most frequently observed type of chromosome damage was exchange figures. In the presence of S9 activation, nonsignificant but concentration dependent increases in cells bearing exchange figures were also seen at 1 and 3 ug/mL (18-hour harvest). The data are, therefore, sufficient to conclude that FAT 80'023/Q is active in this test system.	Acceptable 011744 11-Jan-1996

EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
84-2(a) Mutagenic- SPOT Test Species: Mouse Oak Ridge National Laboratory ; 21-Mar-1980	Irgasan DP300	238654	Not a mutagen - However does cause readily apparent toxic effects in embryos and dams receiving I.P. injection (8 mg/kg) or slight toxic effects after dose of 3.2 mg/kg.	Minimum 001965
84-2(a) Mutagenic- Ames Species: Ciba-Geigy Corp. Inc. 78-2511; 01-Mar-1978	Irgasan DP 300	00030398	Not a mutagen in the strains of Salmonella typhimurium (TA 92, TA 98, TA 100, TA 1535, TA 1537) w/ and w/out metabolic activation. Doses tested: 0.01, 0.03, 0.09, 0.77, 0.81, 2.43, 7.29 mg/0.1 mg. Without activation - doses 0.09 mg/0.1 ml and above caused growth inhibition. With activation - only 7.29 mg/0.1 ml caused this inhibition.	Minimum 001965
84-2(a) Mutagenic- SPOT Test Species: Mouse Ciba Geigy Corp. ; 22-Jun-1978	Irgasan DP 300	00030396	Induced about 2.4% color spots of genetic relevance - compared w/a control frequency of 0.1%. Dose tested: 50 mg/kg - this dose had proved maternally toxic in previous exp.	Supplementary 001965
84-2(b) Mutagenic- Chromosomal Species: Mouse Ciba-Geigy Corp. Inc. 78-2903; 01-Dec-1978	Irgasan DP 300	00030403	Not a mutagen (no chromosome aberrations). Doses tested: 189, 378, 756 mg/kg on days 0, 2, 3, 5 and 9.	Minimum 001965
84-2(b) Mutagenic- Chromosomal Species: Mouse Ciba-Geigy Corp. Inc. 78-2904; 23-Feb-1979	Irgasan DP 300	00030404	Not a mutagen (no chromosome aberrations). Doses tested: 189, 378, 756 mg/kg on days 0, 2, 3, 5 and 9.	Minimum 001965
84-2(b) Mutagenic- Chromosomal Species: Hamster (Chinese) Ciba-Geigy Corp. Inc. 78-3105; 01-Jan-1979	Irgasan DP 300	00030402	Not a mutagen (no chromosome aberrations). Test material was administered 3 times a week for 12 weeks. Doses tested: 75, 150, 300, 1600 mg/kg.	Minimum 001965
84-4 Mutagenicity Study Species: University of Stockholm ; 19-Mar-1979	Irgasan	00030405	D. melanogaster - Not a mutagen (did not induce an increase in sex-linked recessive lethals). Dose tested: 1000 ppm in a sucrose solution or corn-oil-ajar.	Minimum 001965
84-4 Mutagenicity Study Species: Ciba-Geigy Corp. Inc. 78-3402; 27-Nov-1978	Irgasan DP 300	00030397	S. cerevisiae - Not a mutagen. Doses tested: 10, 20, 30, 40, 50, 60, 200 mg/L - in MP1 strain.	Minimum 001965

EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
84-4 Mutagenicity Study Species: Ciba-Geigy Corp. Inc. 78-2803; 27-Mar-1978	Irgasan DP 300	00030399	S. typhimurium - Not a mutagen. Strains tested: TA98, TA100, TA1535 and TA1537. Doses tested: 50, 100, 200, 400 mg/kg	Minimum 001965
84-4 Mutagenic- Point Mutation UDS Species: Mouse Ciba-Geigy Corp. Inc. 78-2305; 78-2306; 10-May-1978	Irgasan DP 300	00030400	Not a mutagen [material was tested in vitro and in host mediated (mice) in vivo assay]. Dose tested: 15.8 and 28.9 mg/ml in in vivo assay.	Minimum 001965
84-4 Mutagenicity Study Species: Hamster (Chinese) Ciba-Geigy Corp. Inc. 78-3005; 23-Aug-1978	Irgasan DP 300	00030401	Not a mutagen (no nucleus anomalies) Test material was administered 3 times a week for 12 weeks. Doses tested: 75, 150, 300, 600 mg/kg	Minimum 001965
84-4 Mutagenicity Study Species: Ciba Geigy Corp. ; 22-Jun-1978	Irgasan DP300	00032236	S. cerevisiae - Has definite mutagenic and recombinogenic activity (without metabolic activation) - Does not, though, affect the interallelic recombination system. Dose tested: 0.2 mg/ml.	Supplementary 001965
85-1 Pharmacokinetic- I.V & Intravenous Species: Rat (Wistar)	14C Triclosan	00073390	Volume of distribution = 42%, plasma half life = 8.8 hours, plasma clearance = 77.5 ml/kg/hr. Tissue distribution = plasma, kidney and liver. Rapidly absorbed through the vaginal mucosa. Excreted: 18 and 9% in the feces & urine after i.v. administration Dose tested: i.v. - 5 mg/kg in PEG-400, IV - 5 mg/kg in corn oil. Wistar strain.	Minimum 001965
85-1 Bathing- 90 Day Species: Monkey Hazleton LH 17857; 26-Apr-1979	Triclosan soap (0.1%)	00073387	No compound induced abnormalities noted grossly or upon histologic examination of the tissue, in hematology, or serum chemistry. No effect on body weight also. Dose tested: 5 min. exposure (trunk) for 90 consecutive days to a soap solution containing 0.1% triclosan- Rhesus strain.	Supplementary 001965
85-1 Bathing- 90 Day Species: Monkey (Rhesus) Ciba-Geigy Corp. Inc. ; 04-Jun-1979	Triclosan soap (0.1%)		Blood levels of Irgasan from 0.17 - 0.97 ppm - plateau at 15 days. Found in glucuronide or sulfate form. Urine levels - from 0.3 - 4.8 ppm most present as glucuronide conjugate. Fecal levels - from < 0.1 - 10.5 ppm dropped to trace levels during recovery. Tissue levels detected 1-5 days after treatment period. Highest conc. found in lung, liver, kidney, skin (< 0.1 - 1.9 ppm) - No tissue residues 30 days post treatment. Dose tested 5 min. exp. (trunk) for 90 days to soap solution containing 0.1% Irgasan. Rhesus strain.	Supplementary 001965 Supplementary 001968

EPA Chem. Code 054901 - Irgasan [Triclosan, Chloro-2-(2,4-dichlorophenoxy)phenol]

Citation	Material	MRID No.	Results	Core Grade/ Tox Record No.
85-1 Metabolism Study Species: Rat	Irgasan DP 300		Excreted unchanged in feces and urine (partly conjugated) Is also hydroxylated to 5 different monohydroxy metabolites.	Minimum 001965
85-1 Pharmacokinetic- Oral Species: Monkey (Rhesus) Ciba-Geigy Corp. Inc. ; 19-Oct-1978	Irgasan DP 300		Peak plasma level 3-5 hours and reached non detectable levels 5-7 days later. Conjugated forms exist in blood. Irgasan excreted mainly in urine and feces (83-98%). Feces contained mainly unconjugated Irgasan, while urine contained glucuronide conjugated Irgasan. Rhesus strain tested.	Supplementary 001965
85-1 Metabolism Study Species: Human	14C Bacteriostat 3565		Over 5 day period: 65.4% recovery in urine, 20.6% recovery in feces. Half life = 10 hours. Dose tested: 1 ml.	001962
85-2 Acute Dermal Absorption Species: Monkey (Rhesus) Ciba-Geigy Corp. Inc. ; 05-Jun-1978	Irgasan Soap (0.1%)	00073389	Conjugated Irgasan ranges from 0.25-0.68 ppm in blood samples. Peak at 8-12 hours. Maintained at 24 hours. - 3 day old. Rhesus strain monkeys tested.	Supplementary 001965
85-2 Percutaneous Absorption Study Species: Human	C14-Bacteriostat 3565 (Irgasan)		Applied to forearm in soap solution. About 8.9% absorbed in 24 hrs.	001962
85-2 Metabolism- Dermal Absorption Species: Rat	Irgasan 2% solution in 50% ethanol		0.5 ml of tincture solution (1 mg) applied to shaved back of one rat and 32.2 mg (0.967 mg test substance) to another rat. 0.67% recovered in urine and 22.4% recovered in feces after 48 hrs.	001958
85-2 Metabolism- Dermal Absorption Species: Rabbit	Irgasan 2% solution in 50 % ethanol		0.89, 0.917 and 0.907 mg applied to shaved backs of 3 rabbits respectively. At 48 hrs 1.6% recovered in urine & 21.9% in feces.	001958

DER #1 2-Year Chronic Toxicity/Carcinogenicity Study in Rats MRID # 42027906

SECTION HEAD

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

MAR 23 1989

007098

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Irgasan: Review of Chronic Feeding / Oncogenicity Study in Rats

Caswell No. 186A EPA ID No. 100-502
EPA Record No. 230991

TO: J. Kempter / W. C. Francis, PM (32)
Registration Division (H7505C)

FROM: Whang Phang, Ph.D. 3/20/89
Pharmacologist
Section II
HFAS / Toxicology Branch II / HED (H7509C)

THROUGH: K. Clark Swentzel, Toxicologist K. Clark Swentzel 3/21/89
Acting Section Head
and
Marcia van Gemert, Ph.D. Marcia van Gemert
Acting Branch Chief
HFAS / Toxicology Branch II / HED (H7509C)

The registrant, Ciba-Geigy Corp. has submitted a 2-year feeding/oncogenicity study in rats with Irgasan (Fat 80'023). This study has been reviewed by Dynamac Corp. and approved by Toxicology Branch II. The data evaluation report is attached, and the conclusion is as follows:

1). This study is poorly organized and written. It also contains technical errors in clinical chemistry analyses. In most cases summary data are not prepared.

2). Groups of rats (80/sex/dose) were fed Irgasan at dietary concentrations of 0, 300, 1000, and 3000 ppm for 104 weeks and 6000 ppm for 52 weeks. Under the conditions of the study, the test agent did not show any oncogenic effects.

3). A significant body weight decrease was seen in 6000 ppm females.

4). Consistent and statistically significant decreases in erythrocyte counts were seen in 300, 1000, and 3000 ppm males

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at the measuring periods of weeks 78 and 104. Decreases in hemoglobin concentration and hematocrit in treated males were also present, but they were not consistent and sometimes were not statistically significant. Clotting time in 6000 ppm males was consistently increased.

5). A significant increase in the incidence of accumulation of foamy macrophages in pulmonary alveoli of both treated males and females was seen at 104 week.

6). A significant increase in the incidence of non-neoplastic liver changes such as cytoplasmic inclusions and hepatocellular hypertrophy was seen in 3000 ppm males. An increase in the incidence of hepatic necrosis was seen in 300, 1000, and 3000 ppm males relative to the controls. It seemed to be quite odd that clinical chemistry data did not show consistent changes in SGPT or SGOT levels while the above effects on the liver were found in the treated males. In the absence of the historical control incidence of liver necrosis, the increase in the incidence of liver necrosis in 300, 1000, and 3000 ppm males could not be dismissed.

Based upon the increase in the incidence of liver necrosis, the decrease in the erythrocyte count, and the increase in the incidence of accumulation of foamy macrophages in the pulmonary alveoli, the LOEL is established at 300 ppm which was the lowest concentration tested. At the present a NOEL for chronic toxicity can not be established.

The study is classified as supplementary because it contains many technical errors, is incomplete, and is poorly organized.

(B)

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EPA: 68D80056
DYNAMAC No. 136-A
March 2, 1989

DATA EVALUATION RECORD

IRGASAN

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Puenta (for)

Date: 3-3-89

007098

EPA: 68D80056
DYNAMAC No. 136-A
March 2, 1989

DATA EVALUATION RECORD

IRGASAN

Chronic Toxicity/Oncogenicity Feeding Study in Rats

REVIEWED BY:

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Dynamac Corporation
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Date: March 2, 1989

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(11)

DATA EVALUATION RECORD

GUIDELINE § 83-5

STUDY TYPE: Chronic toxicity/oncogenicity feeding study in rats.

ACCESSION/MRID NUMBER: 263791-263794. 00164332 and 00094047

TEST MATERIAL: Fat 80'023.

SYNONYM(S): Irgasan DP-300; Triclosan.

STUDY NUMBER(S): MIN 833005.

SPONSOR: Dyestuffs and Chemical Division, Ciba-Geigy Corporation, Greensboro, NC.

TESTING FACILITY: Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, NJ.

TITLE OF REPORT: Study 1--Fat 80'023. 2 Year Oral administration to Rats; Study 2--Determination of Fat 80'023 in Blood and Tissue Samples Taken during a Two-Year Chronic Oral Toxicity/Oncogenicity Study in Albino Rats (24-Month Final Report).

AUTHOR(S): Study 1--Yau, E. T., and Green, J. D., Study 2--Parkes, D. G.

REPORT ISSUED: Study 1--April 28, 1986; Study 2--April 30, 1986.

CONCLUSIONS: Under the conditions of the chronic toxicity study, Fat 80'023 was not oncogenic when fed to male and female Sprague-Dawley rats at levels of 0, 300, 1000, or 3000 ppm for 104 weeks or 6000 ppm for 52 weeks. There were no overt signs of toxicity or dose-related effects on mortality, clinical observations, ophthalmology, urinalysis, gross pathology, palpable mass observations, or neoplastic histopathology. Body weights were significantly decreased in males and females fed 6000 ppm and females fed 3000 ppm. A compensatory increase in food consumption was exhibited in males only. Erythrocyte counts (RBC), hemoglobin (HGB) concentration, and hematocrit (HCT) of males fed 300, 1000, or 3000 ppm, RBC counts of females fed 1000 or 3000 ppm, and HCT levels of females fed 6000 ppm were found to be decreased. SGOT and SGPT indices were increased in males fed 3000 ppm and BUN levels were increased in dosed females when compared to concurrent controls. Compound-related decreases were found in total bilirubin, triglycerides, total protein, SGOT, and glucose indices. Liver weights of males and females fed 6000 ppm were found to be decreased. Nonneoplastic liver changes (cytoplasmic inclusions and hepatocellular hypertrophy) were exhibited in males fed 3000 and 6000 ppm; hepatic necroses were increased in males fed 300, 1000, or 3000 ppm. Residue levels of the test compound recovered in the blood, kidney, and liver tissues were proportional to the dose levels. The predominant amount of residual Fat 80'023 in the blood and kidney was found as the sulfate conjugate while the unconjugated form was predominant in liver tissue. In general, the blood contained the highest concentration of total residual test compound. Female blood levels of residual Fat 80'023 and residual levels in male livers tended to be increased when compared to residual levels in animals of the opposite sex.

Based on the histopathological incidence of hepatic necrosis, the LOEL is 300 ppm, the lowest dose tested.

Classification: CORE Supplementary. The study contained many technical errors, was incomplete and was poorly written (See Reviewers' Discussion and Interpretation of Study Results.)

A. MATERIALS:

1. Test Compound: Fat 80'023; description: white powder; batch No. 5.2.0211.0; purity: 99%.

2. Test Animals: Species: rat; strain: CrL:COBS CD(SD) BR; age: 37 days at study initiation; weight: males--155.5 to 161.1g, females--127.6 to 129.2 g; source: Charles River Breeding Laboratories, Kingston, N.Y.

B. STUDY DESIGN:

1. Animal Assignment: Following 2 weeks of acclimation and a physical and ocular examination, animals were assigned to the following test groups with a computerized randomization procedure:

Test Group	Dose in Diet (ppm)	Main Study (104 weeks) Males	Main Study (104 weeks) Females	Interim Sacrifice (52 weeks) Males	Interim Sacrifice (52 weeks) Females	Serial Sacrifice[a] (13, 26, and 78 weeks) Males	Serial Sacrifice[a] (13, 26, and 78 weeks) Females
1 Control[b]	0	60	60	20	20	15	15
2 Low (LDT)	300	60	60	10	10	15	15
3 Mid (MDT)	1000	60	60	10	10	15	15
4 High (HDT)	3000	60	60	10	10	15	15
5 Toxic Level	6000	--	--	20	20	--	--

[a]Animals were used for blood and tissue residue determinations (5 animals sacrificed/interval).

[b]Additional groups of 10 animals/sex were used for clinical biochemistry baseline data prior to study initiation.

The study authors indicated that even though the most probable route of exposure of the test material in humans is dermally, Fat 80'023 was administered orally since its metabolic profile is similar by either the oral or dermal route and a sufficient amount of the test material is absorbed following oral administration to cause systemic effects.

2. Diet Preparation: The test compound was mixed weekly with the basal diet at the appropriate test concentrations. The test diet was stored at room temperature. The purity and stability of the test compound and the test diet mixture were determined by the study sponsor. The stability and homogeneity of the test diet mixture were validated by the study laboratory; the concentration of Fat 80'023 in the test diet was determined on study day 1 and monthly thereafter by the study laboratory. The mean body weight and food consumption values were used to calculate the amount of test material needed to maintain the targeted dosage levels on a mg/kg body weight basis.

Results: Homogeneity results of the test diets are presented in Table 1a. The study authors considered the test compound to have been homogeneously distributed in the diet mixtures; dates of homogeneity analyses were not reported. The percent recovery of the low-dose sample was in error for the middle subsample; the corrected range was 98.0 to 131.3%, which is outside the level of acceptability. Other dose mixtures varied only 2 to 8% indicating homogeneity of the subsamples. Stability analyses indicated that the low and high doses of the test diet were stable at room temperature and at 6°C for up to 21 days.

Selected results from the analyses of the diet concentrations at selected months are presented in Table 1b. The mean recovery values of the diets ranged from 94 to 101.7%, 94 to 102.0%, 97 to 103.9%, and 97.4 to 105.2% for 300-, 1000-, 3000- and 6000-ppm diets, respectively. The study authors stated that no test substance was detected in the control feed; however, these control analyses were not presented.

3. Food and Water Consumption: Animals received food (certified Purina Rodent Chow No. 5002) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weights, food consumption, and organ weights were analyzed using Bartlett's test for homogeneity of variance. Based on this outcome, data were analyzed using Dunnett's test or Behren's t test with Cochran's approximation. The analyses of clinical laboratory data were designed to test each parameter for trends existing between treatment groups. The survival distribution was determined using Kaplan-Meier estimates, the Gehan-Wilcoxon test, and the Mantel-Cox logrank test. Microscopic data were analyzed by Fisher's exact test. The method of Peto, Mantel's time-adjusted trend test, Tukey's exact test and the

TABLE 1a. Results of Homogeneity Analyses of Fat 80'023 in Rat Diets

Sample Source	Nominal Concentration (ppm)	Sample Concentration (ppm)	Percent Recovery (%)
Top	300	317	105.7
Middle	300	394	131.3 (98.1)[a]
Bottom	300	294[b]	98.0
Top	1000	934	93.4
Middle	1000	968	96.8
Bottom	1000	980	98.0
Top	3000	2820	94.0
Middle	3000	2977	99.2
Bottom	3000	3086	102.9
Top	6000	5962	99.4
Middle	6000	5953	99.2
Bottom	6000	6061	101.0

[a]Reported by the study authors to be 98.1%; recalculation of the data indicated the percent recovery to be 131.3%.

[b]Possible error in notation.

TABLE 1b. Dietary Analyses of Fat 80'023 in Test Diets as Percent of Targeted Dose in a 2-Year Rat Study

Week	Dietary Level	Target Concentration (ppm)	Target Percent of Targeted Dose (%)[a]
1	300	302	100.6
	1000	961	96.1
	3000	2961	98.7
	6000	5992	99.9
25	300	305	101.7
	1000	1017	101.7
	3000	3068	102.3
	6000	6141	102.4
53	300	291	97.0
	1000	998	99.8
	3000	3087	102.9
	6000	6101	101.7
105	300	299	100.0
	1000	1003	100.3
	3000	3005	100.2
	6000	NA[b]	NA

[a]Range: Low of 94.3% for 300 ppm at week 61 to high of 105.2% for 6000 ppm at week 9.

[b]NA = Not analyzed.

logistic regression method of Dinse and Lagakos were used to evaluate tumor incidence.

5. Quality Assurance: A quality assurance statement was signed and dated April 28, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of moribundity and mortality. Gross signs of toxicity were recorded monthly. Palpable mass observations were conducted monthly for study months 1 to 12 and biweekly thereafter.

Results: Mortality and percent survival were not significantly different between control and dosed rats of either sex (Table 2).

It was reported that there were no abnormal signs suggestive of a compound-related effect. Chromodacryorrhea, foot and tail sores, and alopecia were observed in control and dosed animals in the second year of study. The incidence of pollakiuria and unkempt appearance was slightly increased in animals receiving 3000 ppm.

Palpation of the skin was reported by the study authors to reveal a similar number of masses in dosed and control mice; individual data for females receiving 6000 ppm was not reported.

2. Body Weight: Rats were weighed at study initiation, weekly to week 12, and monthly thereafter.

Results: Table 3 presents mean body weight data at selected intervals. The mean body weights of males receiving 6000 ppm were significantly ($p<0.01$) decreased from study weeks 3 to 52 (reduction of 10.4%), the date of their terminal sacrifice. Males receiving 3000 ppm exhibited significantly ($p<0.01$) reduced body weights from study initiation to study week 6, although these differences were considered to have been due to initial

TABLE 2. Cumulative Mortality and Percent Survival in Rats Fed 80'023 for 104 Weeks

Dose Group (ppm)	Number of Animals		Number of Mortalities (percent mortality) at Week		
	Initial	Termination[a]	52[b]	78[c]	104[a]
			Males		
0	95	22	3 (4.6)	11 (18.3)	38 (63.3)
300	85	18	1 (1.5)	9 (15.0)	42 (70.0)
1000	85	28	0 (0.0)	8 (13.3)	32 (53.3)
3000	85	22	5 (7.6)	11 (18.3)	38 (63.3)
6000	20	19	1 (5.0)	---[d]	---
			Females		
0	95	20	2 (3.1)	11 (18.3)	40 (66.7)
300	85	19	1 (1.5)	21 (35.0)	41 (68.3)
1000	85	21	2 (3.1)	13 (21.6)	39 (65.0)
3000	85	18	3 (4.6)	14 (23.3)	42 (70.0)
6000	20	20	0 (0.0)	---[d]	---

[a]Based on 60 rats/sex/group of the main study.

[b]Percent mortality was based on 65 animals in all groups with the exception of those animals dosed at 6000 ppm; 20 animals/control group were sacrificed at week 52; 10 animals/dose/sex were sacrificed at week 52 for remaining groups.

[c]Percent mortality was based on 60 animals in all groups; 3 to 5 animals/dose/sex were sacrificed at week 78.

[d]Animals were sacrificed at 52 weeks.

TABLE 3. Representative Results of Mean Body Weights of Rats Fed 80'023 for 104 Weeks[a]

Dose Group (ppm)	Mean Body Weight (g ± S.E.) at Week					
	0	3	28	52	76	104
			Males			
0	161.1 ± 1.3	317.1 ± 2.4	644.4 ± 8.1	719.9 ± 11.0	797.8 ± 15.1	725.1 ± 36.2
300	157.3 ± 1.2	316.3 ± 2.4	638.3 ± 7.7	715.0 ± 9.8	785.8 ± 15.1	755.8 ± 34.5
1000	157.5 ± 1.2	309.9 ± 2.8**	624.6 ± 7.7	705.4 ± 8.8	775.4 ± 14.0	711.4 ± 29.1
3000	155.5 ± 1.4**	303.8 ± 2.8**	619.9 ± 10.0	706.9 ± 11.9**	787.3 ± 16.8	763.2 ± 34.5
6000	155.9 ± 2.4	295.8 ± 5.1**	568.3 ± 15.2**	645.1 ± 19.0**	---[b]	---
			Females			
0	128.6 ± 0.9	194.3 ± 1.7	329.4 ± 4.3	396.3 ± 6.8	472.9 ± 15.6	439.6 ± 25.9
300	127.6 ± 1.1	191.9 ± 1.9	328.2 ± 3.8	400.7 ± 6.7	476.1 ± 11.8	414.3 ± 27.0
1000	129.2 ± 1.1	191.4 ± 2.0	328.7 ± 5.1	408.1 ± 8.2	490.7 ± 14.1	442.2 ± 30.2
3000	128.0 ± 0.8	184.9 ± 1.5**	308.2 ± 3.6**	371.7 ± 5.8**	427.1 ± 10.9*	474.3 ± 22.3
6000	128.6 ± 2.0	177.8 ± 3.6**	270.1 ± 4.2**	305.6 ± 7.3**	---	---

[a]Based on rats of the main group.

[b]Animals sacrificed at 52 weeks.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

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differences in body weights between animals of this dose group and concurrent controls; body weight gain between these groups was comparable. Body weights of males receiving 1000 ppm were slightly but nonsignificantly decreased throughout the study. The mean body weights of females receiving 6000 ppm were significantly ($p<0.01$) decreased from study initiation to week 52 (reduction of 22.9%), the date of their terminal sacrifice. Females receiving 3000 ppm exhibited significantly ($p<0.01$, $p<0.05$) reduced body weights from study weeks 2 to 52 (reduction of 6.3%) and week 76 (reduction of 9.3%); body weights of these females were nonsignificantly decreased to study week 96. All other body weights of dosed males and females were similar to concurrent controls.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated at the same intervals as weighings.

Results: Table 4 presents food consumption data at selected intervals. Food consumption of males receiving 3000 and 6000 ppm Fat 80'023 was increased throughout the study when compared to concurrent controls; these increases were significant ($p<0.05$, $p<0.01$) in both dose groups from study weeks 3 to 48 and in rats receiving 3000 ppm from study weeks 56 to 80. Food consumption was slightly increased in this latter dose group from study weeks 80 to 104. Food consumption in females receiving 3000 ppm was slightly increased from study weeks 48 to 104 when compared to concurrent controls. Food consumption of other dosed females was similar to controls throughout the study with the exception of incidental changes at sporadic weekly intervals.

The average test compound intake in all dosed groups was reported by the study authors to decrease by 41 to 68% during study year 1 and 17 to 41% during study year 2 (Table 5); however, the proportionality between the average doses received by different groups remained approximately constant. Mean test compound intake as calculated by the reviewers was 15.3, 52.4, 168.0, and 415.0 mg/kg/day for males receiving 300-, 1000-, 3000-, and 6000-ppm concentrations, respectively. Mean test compound intake for females receiving 300-, 1000-, 3000-, and 6000-ppm concentrations was 20.0, 66.9, 217.4, and 519.3 mg/kg/day, respectively. It should be noted that the mean test compound intake of each dose group was calculated from the data on average daily dose, which was computed weekly from weeks 1 to 12 and monthly thereafter until study termination. In addition, rats dosed with 6000 ppm Fat 80'023 were sacrificed at week 52, which caused an inflated test compound mean intake due to the greater influence of values for young animals.

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TABLE 4. Representative Food Consumption for Rats Fed Fat 80'023 for 104 Weeks

Dose Group (ppm)	Mean Food Consumption (g/day ± S.E.) at Week					
	1	3	6	48	64	104
			Males			
0	23.7 ± 0.2	26.7 ± 0.2	27.0 ± 0.3	28.3 ± 0.3	28.3 ± 0.4	23.0 ± 1.5
300	23.4 ± 0.2	27.0 ± 0.2	27.4 ± 0.3	28.3 ± 0.3	28.9 ± 0.4	23.5 ± 1.0
1000	23.8 ± 0.2	26.5 ± 0.4	27.9 ± 0.3	29.2 ± 0.4	28.8 ± 0.4	24.4 ± 1.3
3000	24.1 ± 0.3	28.0 ± 0.3**	28.8 ± 0.4**	30.2 ± 0.4**	30.1 ± 0.6*	27.0 ± 1.1
6000	23.4 ± 0.5	27.9 ± 0.4*	29.3 ± 0.6**	31.1 ± 0.8**	--[a]	--
			Females			
0	18.9 ± 0.2	19.4 ± 0.2	19.7 ± 0.2	21.3 ± 0.3	22.3 ± 0.4	17.5 ± 1.7
300	18.4 ± 0.2	19.9 ± 0.2	20.0 ± 0.2	21.8 ± 0.5	23.2 ± 0.4	15.4 ± 1.4
1000	18.5 ± 0.2	19.9 ± 0.2	20.4 ± 0.3	22.1 ± 0.3	23.2 ± 0.5	15.6 ± 1.3
3000	18.2 ± 0.2*	19.5 ± 0.2	19.9 ± 0.2	23.3 ± 0.3**	24.1 ± 0.5*	18.2 ± 1.0
6000	16.2 ± 0.3**	19.1 ± 0.5	19.1 ± 0.5	23.0 ± 0.7*	--	--

[a]Animals sacrificed at 52 weeks.

*Significantly different from control values at $p<0.05$ as evaluated by the study authors.

**Significantly different from control values at $p<0.01$ as evaluated by the study authors.

TABLE 5. Calculated Average Intake of Fat 80'023 at Representative Weeks

Dietary Level in Feed (ppm)	Mean Daily Dose (mg/kg/day ± S.D.) at Week				
	1	12	52	80	104
			Males		
300	36.37 ± 2.10	15.51 ± 1.28 (42.6)[a]	11.99 ± 1.30 (33.0)	10.91 ± 1.39 (30.0)	9.31 ± 1.89 (25.6)
1000	129.27 ± 6.74	53.67 ± 2.86 (41.5)	40.49 ± 4.27 (31.3)	36.73 ± 5.99 (28.4)	33.57 ± 9.07 (26.0)
3000	400.19 ± 29.84	171.85 ± 9.10 (42.9)	127.15 ± 13.58 (31.8)	119.81 ± 25.89 (29.9)	107.22 ± 26.53 (26.8)
6000	784.84 ± 55.25	360.54 ± 16.71 (45.9)	246.91 ± 27.59 (31.5)	--[b]	--
			Females		
300	39.87 ± 3.25	21.71 ± 2.21 (54.5)	16.54 ± 2.58 (41.5)	14.24 ± 3.30 (35.7)	10.65 ± 4.01 (26.7)
1000	131.53 ± 9.60	73.70 ± 7.36 (56.0)	55.65 ± 8.07 (42.3)	45.51 ± 10.71 (34.6)	33.98 ± 11.76 (25.8)
3000	394.67 ± 27.72	235.50 ± 25.25 (60.0)	189.91 ± 24.80 (48.1)	151.20 ± 53.12 (38.3)	113.90 ± 25.47 (28.9)
6000	714.74 ± 49.96	453.63 ± 67.78 (63.5)	421.90 ± 69.57 (59.0)	--	--

[a]Number in parentheses is the percent mean daily dose compared to mean daily dose at week 1.

[b]Animals sacrificed at week 52.

4. Ophthalmological: Ophthalmological examinations were performed during the acclimation period and study weeks 52 and 104.

Results: Ophthalmological findings (chromodacryorrhea, corneal stippling, corneal opacity, and lens and retinal abnormalities) were observed in control and dosed males and females and were considered to be normal age- and strain-related changes.

5. Hematology and Clinical Chemistry: Blood was collected following a 12-hour fasting period from the periorbital sinus prior to study initiation from a group of 10 rats/sex and at 13, 26, 52, 78, and 104 weeks from 20 rats/sex/group (assigned as clinical test animals) for hematology analyses. Clinical chemistry analyses were determined from 10 of the 20 rats/sex/group designed as clinical test animals. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)[+]	X	Leukocyte differential count
X	Hemoglobin (HGB)[+]	X	Mean corpuscular HGB (MCH)[c]
X	Leukocyte count (WBC)[+]	X	Mean corpuscular HGB concentration (MCHC)[c]
X	Erythrocyte count (RBC)[+]	X	Mean corpuscular volume (MCV)[c]
X	Platelet count[+]		Coagulation:thromboplastin time (PT)
X	Reticulocyte count (RETIC)[a,b]	X	Clotting time[a]
	Red cell morphology		

Results: Selected hematology results are presented in Tables 6 and 7. Erythrocyte counts (RBC), hemoglobin concentration (HGB), and hematocrit (HCT) were found to be slightly decreased in males fed 300, 1000, or 3000 ppm Fat 80'023 at 78 and 104 weeks (Table 6). These levels differed significantly ($p<0.05$, $p<0.01$) from concurrent controls at several intervals. Erythrocyte parameters of males fed 6000 ppm were similar to controls or only slightly decreased at weeks 13, 26, and 52 with the exception of a slight but significantly ($p<0.05$) decreased RBC count at week 13. Red cell indices (MCV, MCH, MCHC) were concurrently altered with erythrocytic parameters. RETICS were decreased in males but not females fed 6000 ppm. WBC counts were slightly but nonsignificantly increased

[+]Recommended by Subdivision F (October 1982) Guidelines.
[a]Not examined in animals bled prior to study initiation for baseline data.
[b]Evaluated in control and 6000-ppm groups only.
[c]Not consistently reported at all intervals.

TABLE 6. Hematology Parameters (± S.E.) at Selected Intervals in Male Rats Fed Fat 80'023 for 104 Weeks

Parameter/Week	Dietary Level (ppm) 0	300	1000	3000	6000[a]
Hemoglobin (g/dL)					
13	16.07 ± 0.18	16.17 ± 0.12	16.07 ± 0.11	16.06 ± 0.11	15.98 ± 0.11
26	16.16 ± 0.20	15.91 ± 0.15	16.20 ± 0.17	16.53 ± 0.14	16.25 ± 0.20
52	15.13 ± 0.23	15.08 ± 0.19	15.25 ± 0.16	15.50 ± 0.17	15.44 ± 0.16
78	15.27 ± 0.24	14.90 ± 0.14	14.49 ± 0.39	14.32 ± 0.53*	--
104	14.58 ± 0.34	13.45 ± 0.56	13.31 ± 0.53	13.71 ± 0.46	--
Erythrocytes (10^6/cu mm)					
13	8.376 ± 0.09	8.568 ± 0.11	8.347 ± 0.10	8.293 ± 0.09	8.166 ± 0.09*
26	8.374 ± 0.10	8.474 ± 0.11	8.425 ± 0.11	8.483 ± 0.08	8.103 ± 0.11
52	7.824 ± 0.13	7.757 ± 0.10	7.727 ± 0.10	7.847 ± 0.12	7.871 ± 0.07
78	8.039 ± 0.15	7.700 ± 0.11	7.368 ± 0.22*	7.175 ± 0.30**	--
104	7.277 ± 0.21	6.465 ± 0.31*	6.242 ± 0.26*	6.457 ± 0.24*	--
Hematocrit (%)					
13	47.9 ± 0.80	47.5 ± 0.61	46.9 ± 0.43	46.7 ± 0.42	47.2 ± 0.33
26	48.9 ± 0.54	47.7 ± 0.45	47.8 ± 0.51	48.5 ± 0.35	47.5 ± 0.58
52	46.2 ± 0.71	45.6 ± 0.41	45.3 ± 0.46	45.2 ± 0.59	45.1 ± 0.46
78	46.2 ± 0.76	43.9 ± 0.38	42.0 ± 1.05**	42.0 ± 1.40**	--
104	43.9 ± 0.85	40.4 ± 1.64	40.3 ± 1.41	41.1 ± 1.19	--
Clotting Time (Sec)					
13	119.2 ± 8.86	119.6 ± 7.43	130.6 ± 7.64	133.4 ± 7.50	143.6 ± 5.82*
26	114.2 ± 8.59	118.2 ± 6.90	113.1 ± 7.81	144.4 ± 7.99*	150.0 ± 10.08**
52	145.9 ± 17.06	197.5 ± 13.89*	198.8 ± 17.70*	191.0 ± 11.49*	196.9 ± 12.11*
78	152.0 ± 13.11	148.5 ± 8.52	174.5 ± 11.94	151.5 ± 9.90	--
104	112.2 ± 9.71	150.5 ± 11.80	134.3 ± 11.97	173.5 ± 8.21**	--

[a]Sacrificed at week 52.

*Significantly different from controls at $p<0.05$ as evaluated by the study authors.

**Significantly different from controls at $p<0.01$ as evaluated by the study authors.

TABLE 7. Hematology Parameters (± S.E.) at Selected Intervals in Female Rats Fed Fat 80'023 for 104 Weeks

Parameter/Week	Dietary Level (ppm) 0	300	1000	3000	6000[a]
Hemoglobin (g/dL)					
13	16.02 ± 0.11	16.05 ± 0.08	16.12 ± 0.12	16.12 ± 0.13	15.41 ± 0.15**
26	15.89 ± 0.15	15.82 ± 0.17	15.69 ± 0.12	16.20 ± 0.17	15.61 ± 0.14
52	15.10 ± 0.12	14.97 ± 0.22	14.66 ± 0.40	15.43 ± 0.19	15.01 ± 0.16
78	14.89 ± 0.15	14.65 ± 0.18	14.32 ± 0.43	14.85 ± 0.29	--
104	13.47 ± 0.49	13.74 ± 0.43	13.16 ± 0.46	14.51 ± 0.23	--
Erythrocytes (10^6/cu mm)					
13	7.780 ± 0.10	7.815 ± 0.10	7.602 ± 0.12	7.860 ± 0.09	7.698 ± 0.10
26	7.411 ± 0.12	7.381 ± 0.09	7.157 ± 0.14	7.476 ± 0.11	7.426 ± 0.08
52	7.225 ± 0.08	6.953 ± 0.11	6.812 ± 0.24	7.268 ± 0.12	7.234 ± 0.09
78	7.263 ± 0.10	6.997 ± 0.11	6.673 ± 0.20*	6.810 ± 0.18*	--
104	6.001 ± 0.22	6.217 ± 0.16	5.631 ± 0.25	6.523 ± 0.14	--
Hematocrit (%)					
13	46.6 ± 0.72	46.9 ± 0.57	45.9 ± 0.55	46.3 ± 0.44	45.1 ± 0.54*
26	47.4 ± 0.57	46.3 ± 0.47	46.2 ± 0.41	46.3 ± 0.49	45.5 ± 0.44**
52	44.7 ± 0.47	43.4 ± 0.57	42.0 ± 1.07	44.2 ± 0.52	42.4 ± 0.44*
78	43.8 ± 0.46	42.9 ± 0.56	41.6 ± 1.22	43.2 ± 0.70	--
104	39.8 ± 1.30	40.6 ± 1.12	39.0 ± 1.15	42.9 ± 0.68	--
Clotting Time (sec)					
26	94.6 ± 10.01	110.3 ± 8.73	134.5 ± 10.74	115.0 ± 11.65	121.6 ± 12.02
52	128.2 ± 9.54	149.3 ± 12.69	146.8 ± 13.74	156.8 ± 10.84	192.8 ± 10.06
78	140.1 ± 6.50	151.2 ± 9.13	155.7 ± 10.14	141.5 ± 9.46	--
104	144.9 ± 14.93	125.7 ± 9.77	143.3 ± 12.19	147.4 ± 6.15	--

[a]Sacrificed at week 52.

*Significantly different from controls at $p<0.05$ as evaluated by the study authors.

**Significantly different from controls at $p<0.01$ as evaluated by the study authors.

in dosed males at 78 and 104 weeks. Clotting time was sporadically increased in dosed males throughout the study. The study authors reported that red cell morphology appeared altered (increased incidence of polychromasia, hypochromia, poikilocytosis, anisocytosis, and targeting) in males receiving 1000 and 3000 ppm.

RBCs were slightly but significantly ($p < 0.05$) decreased in females fed 1000 and 3000 ppm Fat 80'023 at week 78 and nonsignificantly decreased at week 104; HGB and HCT levels of these animals were similar to concurrent controls throughout the study. Females fed 6000 ppm exhibited slightly but significantly ($p < 0.05$, $p < 0.01$) decreased HCT levels at weeks 13, 26, and 52; HGB and RBC counts of these animals were similar to concurrent controls from study initiation to sacrifice at week 52. Red cell indices (MCV, MCH, MCHC) were concurrently altered with erythrocytic parameters. The reductions in erythrocytic parameters and red cell indices exhibited in dosed males and females were within the range of values for those parameters in historical laboratory controls.[1]

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium*	X	Albumin*
	Chloride*	X	Albumin/globulin ratio
	Magnesium*		Blood creatinine*
	Phosphorus*	X	Blood urea nitrogen*
	Potassium*	X	Cholesterol*
	Sodium*		Globulins
		X	Glucose*
	Enzymes	X	Total bilirubin*
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein*
	Creatinine phosphokinase*	X	Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)*		
X	Serum aspartate aminotransferase (SGOT)*		
X	Gamma glutamyltransferase (GGT)		

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[1]Hazleton Laboratories. 1984. Hematology Reference Ranges --Sprague-Dawley Rats. In: Representative Historical Control Data for Rats and Mice.

*Recommended by Subdivision F (October 1982) Guidelines.

Results: Selected clinical biochemistry results are presented in Tables 8 and 9. Mean SGPT levels were found to be significantly ($p < 0.05$) increased in males fed 3000 ppm at 78 weeks when compared to concurrent controls. There were nonsignificant increases at other intervals. SGOT was also significantly ($p < 0.05$) increased at 78 weeks in males but there were significant decreases at 13 ($p < 0.01$) and 52 ($p < 0.05$) weeks and essentially no change at 104 weeks. Even though these increases were dose related, they were not consistent at other test intervals between sexes. Blood-urea-nitrogen (BUN) levels were slightly but significantly ($p < 0.05$, $p < 0.01$) increased at 13 and 26 weeks in females fed 6000 ppm and in all dosed females at 52 weeks. These values were generally similar to control values at 78 weeks; BUN levels were not increased in dose males.

Significant ($p < 0.05$, $p < 0.01$) decreases were found in total bilirubin, triglycerides, and total protein parameters in dosed males and SGOT, glucose, triglycerides, and total bilirubin in dosed females. Many of these decreases were dose related and consistent over time; generally, these decreased levels recovered prior to study termination. These decreased parameters were considered by the study reviewers to be compound related even though the level of decrease generally remained within the range of values for that parameter in historical laboratory controls.[2] Even though these changes were not biologically significant, they were considered to be the result of toxic effects of the test compound.

6. Urinalysis: Urine was collected from 10 fasted rats/sex/dose at 13, 26, 52, 78, and 104 weeks. The CHECKED (X) parameters were examined:

	Appearance[+]	X	Glucose[+]
	Volume[+]	X	Ketones
X	Specific Gravity[+]	X	Bilirubin[+]
X	pH	X	Blood[+]
	Sediment (microscopic)[+]		Nitrate
X	Protein[+]		Urobilinogen

[+]Recommended by Subdivision F (October 1982) Guidelines.
[2]Hazleton Laboratories. 1984. Clinical Chemistry Reference Ranges--Sprague-Dawley Rats. In: Representative Historical Control Data for Rats and Mice.

TABLE 8. Selected Clinical Chemistry Data (± S.E.) for Male Rats Fed Fat 80'023 for 104 Weeks

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Parameter/Week	Dietary Level (ppm) 0	300	1000	3000	6000[a]
SGPT (U/L)					
13	27.0 ± 1.23	28.1 ± 2.19	26.5 ± 1.66	26.0 ± 1.26	25.9 ± 1.64
26	34.6 ± 2.05	34.0 ± 1.65	44.2 ± 7.65	38.1 ± 2.82	31.3 ± 1.60
52	34.5 ± 5.78	28.3 ± 1.45	50.5 ± 9.65	53.3 ± 12.40	40.3 ± 6.22
78	36.9 ± 4.04	37.4 ± 3.95	47.4 ± 10.60	68.1 ± 10.57**	--
104	33.5 ± 4.10	46.3 ± 13.37	36.9 ± 6.74	40.3 ± 10.09	--
SGOT (U/L)					
13	112.2 ± 5.04	93.2 ± 6.78*	89.3 ± 7.10**	74.5 ± 2.61**	73.9 ± 3.66**
26	65.8 ± 4.72	86.7 ± 7.03	85.2 ± 8.13	88.1 ± 11.52	63.6 ± 4.20
52	108.3 ± 6.61	96.1 ± 5.90	97.1 ± 7.56	85.1 ± 12.41*	83.6 ± 4.59*
78	102.9 ± 9.23	112.1 ± 11.26	117.3 ± 14.18	150.0 ± 14.04*	--
104	104.0 ± 9.33	118.8 ± 21.53	105.9 ± 8.86	98.4 ± 10.34	--
BUN (mg/dL)					
13	12.7 ± 0.65	13.8 ± 0.39	13.0 ± 0.39	13.0 ± 0.33	14.1 ± 0.59
26	12.6 ± 0.45	14.5 ± 0.56	13.7 ± 0.56	14.3 ± 0.65	14.2 ± 0.51
52	13.4 ± 0.52	14.5 ± 0.48	12.8 ± 0.61	13.4 ± 0.78	11.2 ± 0.44**
78	13.8 ± 0.99	19.1 ± 3.37	17.8 ± 1.48	14.5 ± 0.22	--
104	20.8 ± 3.78	31.0 ± 10.54	23.1 ± 7.89	15.9 ± 1.86	--
Triglycerides (mg/dL)					
13	96.1 ± 7.06	79.5 ± 11.74	151.7 ± 43.82	47.9 ± 4.32	29.3 ± 2.58*
26	114.3 ± 9.81	118.7 ± 14.13	207.1 ± 40.11	81.4 ± 14.81	57.3 ± 10.29*
52	179.0 ± 28.27	276.5 ± 85.14	366.0 ± 111.13	233.0 ± 132.62	78.2 ± 9.15[b]
78	298.3 ± 74.66	419.4 ± 113.44	446.0 ± 155.08	157.9 ± 21.60	--
104	292.6 ± 89.56	217.4 ± 29.24	187.8 ± 39.86	181.9 ± 31.89	--
Total Protein (gm/dL)					
13	6.77 ± 0.11	6.55 ± 0.08	6.74 ± 0.13	6.29 ± 0.08**	6.27 ± 0.09**
26	6.65 ± 0.11	6.59 ± 0.08	7.14 ± 0.11	6.47 ± 0.10	6.49 ± 0.08
52	7.09 ± 0.15	6.94 ± 0.10	7.17 ± 0.12	6.86 ± 0.26	6.79 ± 0.09
78	7.84 ± 0.17	7.78 ± 0.10	7.96 ± 0.18	7.31 ± 0.12*	--
104	7.02 ± 0.23	7.30 ± 0.15	7.09 ± 0.17	6.77 ± 0.21	--
Albumin/Globuin Ratio					
13	1.51 ± 0.05	1.56 ± 0.03	1.53 ± 0.06	1.59 ± 0.04	1.64 ± 0.02*
26	1.69 ± 0.08	1.67 ± 0.05	1.55 ± 0.07	1.73 ± 0.10	1.91 ± 0.08*
52	1.33 ± 0.08	1.17 ± 0.03	1.19 ± 0.06	1.32 ± 0.11	1.51 ± 0.04*
78	1.17 ± 0.06	1.03 ± 0.05	1.03 ± 0.05	1.24 ± 0.06	--
104	1.04 ± 0.06	0.86 ± 0.04	0.94 ± 0.05	1.06 ± 0.09	--
Total Bilirubin (mg/dL)					
13	0.371 ± 0.02	0.289 ± 0.03*	0.249 ± 0.03**	0.214 ± 0.02**	0.177 ± 0.01**
26	0.171 ± 0.02	0.205 ± 0.02	0.216 ± 0.02	0.245 ± 0.02	0.184 ± 0.02
52	0.415 ± 0.04	0.327 ± 0.03	0.375 ± 0.06	0.306 ± 0.06	0.173 ± 0.03**
78	0.318 ± 0.04	0.387 ± 0.06	0.371 ± 0.03	0.322 ± 0.03	--
104	0.390 ± 0.03	0.324 ± 0.03	0.377 ± 0.05	0.311 ± 0.03	--

[a]Sacrificed at week 52.

[b]Reevaluated by the reviewers using Bartlett's test of homogeneity, the Wilcoxon logrank test, and Dunnett's test and found to be significantly different from controls at $p < 0.05$.

*Significantly different from controls at $p<0.05$ as evaluated by the study authors.

**Significantly different from controls at $p<0.01$ as evaluated by the study authors.

TABLE 9. Selected Clinical Chemistry Data (± S.E.) for Female Rats Fed Fat 80'023 for 104 Weeks

Parameter/Week	Dietary Level (ppm) 0	300	1000	3000	6000[a]
SGPT (U/L)					
13	31.4 ± 2.70	30.3 ± 1.28	31.5 ± 2.69	26.9 ± 1.58	26.1 ± 2.67
26	47.4 ± 8.52	33.1 ± 4.47	48.9 ± 9.10	37.9 ± 5.14	18.5 ± 1.19**
52	33.6 ± 3.33	44.2 ± 8.04	40.1 ± 4.49	39.3 ± 5.03	29.0 ± 2.47
78	38.9 ± 3.06	35.8 ± 3.12	40.7 ± 6.14	36.3 ± 3.14	--
104	47.3 ± 9.40	30.1 ± 4.14	33.7 ± 3.35	40.6 ± 4.75	--
Glucose (mg/dL)					
13	104.9 ± 2.60	111.0 ± 4.10	101.6 ± 3.89	87.3 ± 3.19**	89.1 ± 6.51**
26	119.4 ± 6.30	114.8 ± 3.14	124.3 ± 5.22	111.8 ± 4.33	104.5 ± 4.48*
52	105.1 ± 5.25	115.2 ± 8.61	128.2 ± 5.21	117.8 ± 3.09	108.0 ± 6.57
78	123.8 ± 8.58	128.0 ± 4.94	114.4 ± 7.74	111.8 ± 6.74	--
104	101.2 ± 11.06	107.9 ± 11.34	110.5 ± 12.86	127.8 ± 5.32	--
BUN (mg/dL)					
13	16.1 ± 0.82	18.1 ± 0.43	18.1 ± 0.89	18.3 ± 0.70*	19.0 ± 0.83**
26	14.2 ± 0.59	15.2 ± 0.39	17.1 ± 0.98	15.6 ± 0.52	16.2 ± 0.81*
52	10.3 ± 0.34	13.2 ± 0.63**	14.8 ± 0.68**	12.8 ± 0.81**	14.0 ± 0.89**
78	13.1 ± 1.11	13.0 ± 0.67	17.0 ± 2.65	12.6 ± 0.58	--
104	18.1 ± 1.82	20.3 ± 5.17	12.9 ± 0.75	11.2 ± 0.39*	
SGOT (U/L)					
13	87.5 ± 4.68	82.1 ± 4.02	78.1 ± 4.43	78.8 ± 2.99	72.0 ± 3.24**
26	102.6 ± 10.0	74.2 ± 5.37	96.1 ± 11.96	99.5 ± 9.96	72.2 ± 3.48
52	83.0 ± 6.18	83.1 ± 14.30	94.8 ± 7.38	78.2 ± 5.68	81.4 ± 4.29
78	101.5 ± 4.94	97.1 ± 8.12	105.2 ± 15.12	104.5 ± 11.46	--
104	158.9 ± 21.17	129.6 ± 18.92	98.6 ± 10.67**	102.7 ± 6.11**	--
Triglycerides (mg/dL)					
13	81.7 ± 13.84	54.9 ± 6.31*	47.4 ± 4.41**	39.2 ± 5.10**	36.2 ± 2.08**
26	114.8 ± 18.94	109.8 ± 12.62	108.7 ± 20.36	85.1 ± 12.78	44.7 ± 2.43**
52	164.1 ± 33.71	159.2 ± 37.41	169.7 ± 39.08	78.3 ± 15.10	37.5 ± 1.99**
78	288.2 ± 56.05	271.5 ± 53.97	324.9 ± 67.38	176.8 ± 43.95	--
104	210.8 ± 65.46	181.4 ± 37.72	647.4 ± 485.01[b]	213.6 ± 71.71	--
Total Bilirubin (mg/dL)					
13	0.304 ± 0.02	0.212 ± 0.01	0.267 ± 0.02	0.303 ± 0.01	0.191 ± 0.03**
26	0.377 ± 0.02	0.224 ± 0.01**	0.224 ± 0.01**	0.251 ± 0.03**	0.165 ± 0.02**
52	0.318 ± 0.02	0.310 ± 0.03	0.201 ± 0.02**	0.190 ± 0.02**	--
78	0.350 ± 0.04	0.321 ± 0.03	0.358 ± 0.03	0.312 ± 0.02	--
104	0.337 ± 0.03	0.290 ± 0.01	0.287 ± 0.03	0.317 ± 0.02	--

[a]Sacrificed at week 52.

[b]Abnormal group value due to one female (No. 599) with turbid lipemic serum, which may have interferred with assaying method; if this animal is excluded, the group value is 164.4 ± 49.87 mg/dL.

*Significantly different from controls at $p<0.05$ as evaluated by the study authors.

**Significantly different from controls at $p<0.01$ as evaluated by the study authors.

Results: Slight changes were found in the urinary parameters (specific gravity, pH, urinary protein) of males and females receiving 1000, 3000, and 6000 ppm; however, these changes were considered sporadic and were not accompanied by any significant gross or microscopic renal pathology.

7. Residue Analysis: Blood (2 mL minimum) and tissue samples (0.5 g kidney, 3 g liver, 0.5 g spleen, 0.5 g heart, 1 g brain, 1 g skeletal muscle and 1 g retroperitoneal fat) were collected from five designated rats/sex/dose at 13, 26, and 78 (3 to 5 rats/sex/dose) weeks, all interim animals sacrificed at 52 weeks and 50% of the surviving rats at 104 weeks. Samples were sent to the sponsor for residue determinations of free and conjugated Fat 80'023 content. Residue determinations were performed at week 52 only for rats receiving 6000 ppm.

Results: Residue levels of Fat 80'023 found in the blood of dosed animals were proportional to the feeding levels with the largest amount found as the sulfate conjugate (Table 10). Less than 1% existed in the unconjugated form. Females tended to have higher levels of sulfate conjugate at each testing interval; levels remained high until 104 weeks, at which time they were found to decrease. The blood of dosed males exhibited a gradual decrease in sulfate conjugate over the duration of the study. Blood generally contained the highest concentrations of total Fat 80'023 when compared to liver and kidney.

Residue levels of Fat 80'023 found in the kidneys of dosed animals were also proportional to the feeding levels with the largest amount found as the sulfate conjugate (Table 11). Residue levels of the test compound in the kidney appeared to increase over the duration of the study to week 104 when levels appeared slightly decreased. Generally, residue levels were similar in dosed males and females with the exception of slightly increased levels in females fed 3000 ppm at week 104. Unexpected increases were exhibited in conjugated and unconjugated levels of Fat 80'023 in male and female rats dosed with 3000 ppm at 78 weeks; the study authors considered these increases to be a reflection of technical errors and did not regard these values to be an accurate representation of the kidney content of Fat 80'023. The kidney samples of control animals were found to exhibit slight residue levels of the test material at 13, 26, and 52 weeks; these findings were considered to be the result of contamination of the samples following animal sacrifice and were not the result of improper diets.

TABLE 10. Mean Residue Levels of Unconjugated and Conjugated Fat 80'023 in the Blood of Rats Fed the Test Compound for 104 Weeks

Week	Dietary Level (ppm)	Fat 80'023 Content (µg/mL ± SD)							
		Males				Females			
		Unconjugated	Glucuronide Conjugate	Sulfate Conjugate	Total (Acid)	Unconjugated	Glucuronide Conjugate	Sulfate Conjugate	Total (acid)
13[a]	0	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	300	0.91 ± 0.71	5.57 ± 2.41	12.45 ± 2.15	18.93 ± 4.79	0.62 ± 0.36	3.19 ± 1.47	12.79 ± 3.09	16.88 ± 4.31
	1000	0.52 ± 0.22	11.22 ± 4.24	33.74 ± 10.31	45.48 ± 12.50	0.30 ± 0.08	9.04 ± 4.00	41.11 ± 4.81	50.45 ± 5.16
	3000	0.75 ± 0.33	17.10 ± 5.45	78.38 ± 17.09	96.22 ± 20.39	0.69 ± 0.28	20.91 ± 5.68	81.70 ± 22.79	103.50 ± 24.96
	6000[b]	---	---	---	---	---	---	---	--
52[c]	0	0.00 ± 0.00	NA[d]	NA	0.00 ± 0.00	0.00 ± 0.00	NA	NA	0.00 ± 0.00
	300	0.11 ± 0.06	7.15 ± 3.27	6.36 ± 3.11	13.63 ± 5.63	0.06 ± 0.04	5.78 ± 3.25	11.77 ± 5.05	17.60 ± 8.08
	1000	0.21 ± 0.10	12.38 ± 3.68	21.80 ± 7.04	34.39 ± 7.91	0.17 ± 0.08	9.70 ± 4.57	34.27 ± 10.46	44.14 ± 14.21
	3000	0.30 ± 0.07	16.54 ± 4.21	58.39 ± 27.92	75.23 ± 27.79	0.44 ± 0.11	20.52 ± 4.56	85.70 ± 18.14	106.66 ± 20.58
	6000	1.10 ± 0.25	31.68 ± 8.04	101.71 ± 16.87	134.48 ± 20.85	1.58 ± 0.48	25.00 ± 11.01	143.04 ± 37.49	169.62 ± 38.49
78[e]	0	0.00 ± 0.00	NA	NA	0.00 ± 0.00	0.00 ± 0.00	NA	NA	0.00 ± 0.00
	300	0.25 ± 0.19	4.50 ± 1.87	5.13 ± 2.45	9.87 ± 4.04	0.16 ± 0.09	6.10 ± 2.86	14.25 ± 4.32	20.51 ± 7.18
	1000	0.18 ± 0.03	15.96 ± 4.71	18.05 ± 4.72	34.19 ± 9.11	0.29 ± 0.33	16.63 ± 11.26	41.87 ± 10.33	58.79 ± 19.82
	3000	0.45 ± 0.17	20.68 ± 6.84	57.86 ± 25.32	78.99 ± 30.04	0.37 ± 0.10	16.28 ± 3.44	101.50 ± 18.13	118.16 ± 19.56
	6000[b]	---	---	---	---	---	---	---	---
104[f]	0	0.00 ± 0.00	NA	NA	0.01 ± 0.01	0.00 ± 0.01	NA	NA	0.00 ± 0.01
	300	0.05 ± 0.02	3.33 ± 1.33	3.24 ± 1.06	6.61 ± 2.12	0.04 ± 0.03	4.28 ± 4.33	10.56 ± 5.66	16.56 ± 11.27
	1000	0.41 ± 0.31	10.84 ± 3.40	15.84 ± 7.57	27.06 ± 9.85	0.15 ± 0.12	7.70 ± 5.63	18.43 ± 14.70	26.28 ± 19.93
	3000	0.46 ± 0.39	20.31 ± 10.82	33.80 ± 13.95	54.24 ± 16.51	0.32 ± 0.12	18.22 ± 5.85	68.06 ± 22.06	86.60 ± 27.28

[a]Based on five rats/sex/dose.

[b]Determination performed at week 52 only.

[c]Based on 20 animals/sex in control group, 10 animals/sex in 300-, 1000-, and 3000-ppm dose groups, and 19 males and 20 females in 6000-ppm dose group.

[d]NA = not analyzed.

[e]Based on three to five rats/sex/dose.

[f]Based on 9 to 14 males/dose and 8 to 10 females/dose.

TABLE 11. Mean Residue Levels of Unconjugated and Conjugated Fat 80'023 in the Kidneys of Rats Fed the Test Compound for 104 Weeks

Week	Dietary Level (ppm)	FAT 80'023 (µg/g) Males: Unconjugated	Males: Glucuronide Conjugate	Males: Sulfate Conjugate	Males: Total (Acid)	Females: Unconjugated	Females: Glucuronide Conjugate	Females: Sulfate Conjugate	Females: Total (Acid)
13[a]	0[b]	0.05 ± 0.06	0.00 ± 0.00	0.01 ± 0.02	0.06 ± 0.06	0.29 ± 0.17	0.02 ± 0.03	0.05 ± 0.04	0.34 ± 0.16
	300	6.06 ± 2.40	0.81 ± 0.84	4.52 ± 1.30	11.29 ± 2.92	2.52 ± 0.90	1.02 ± 0.86	4.44 ± 2.67	7.97 ± 3.25
	1000	9.86 ± 3.33	2.60 ± 2.52	9.12 ± 2.64	21.58 ± 6.93	5.07 ± 1.16	2.49 ± 1.90	11.31 ± 3.48	18.87 ± 5.27
	3000	17.89 ± 2.01	5.45 ± 5.36	29.56 ± 5.46	52.32 ± 11.11	11.36 ± 2.99	5.95 ± 4.02	33.60 ± 13.20	50.86 ± 14.68
	6000[c]	---	---	---	---	---	---	---	---
52[d]	0	0.01 ± 0.02	NA	NA	0.02 ± 0.03	0.20 ± 1.27	NA	NA	0.01 ± 0.02
	300	2.96 ± 1.38	7.27 ± 2.80	6.20 ± 2.46	16.43 ± 5.03	1.25 ± 0.36	3.62 ± 0.56	6.31 ± 1.67	11.19 ± 2.00
	1000	10.79 ± 3.55	11.20 ± 4.07	13.74 ± 4.23	35.73 ± 8.55	8.07 ± 2.62	10.80 ± 4.86	15.82 ± 6.05	34.69 ± 10.81
	3000	13.74 ± 4.60	26.62 ± 10.79	37.24 ± 10.21	77.60 ± 22.41	5.36 ± 2.02	26.86 ± 8.57	28.35 ± 13.33	60.58 ± 20.34
	6000	16.09 ± 9.12	40.95 ± 16.05	37.71 ± 16.79	93.38 ± 32.18	14.33 ± 6.89	30.48 ± 8.97	76.43 ± 19.97	121.24 ± 25.64
78[e]	0	0.00 ± 0.00	NA	NA	0.01 ± 0.01	0.00 ± 0.00	NA	NA	0.00 ± 0.01
	300	1.53 ± 0.52	3.78 ± 0.69	6.19 ± 4.70	11.49 ± 5.80	1.03 ± 0.51	3.60 ± 1.64	6.65 ± 3.72	11.28 ± 5.64
	1000	5.14 ± 3.34	13.46 ± 1.67	16.38 ± 9.92	34.92 ± 14.09	3.88 ± 1.72	13.70 ± 8.66	21.19 ± 7.18	38.77 ± 16.36
	3000[f]	45.42 ± 8.86	70.00 ± 16.37	125.25 ± 16.23	240.68 ± 12.97	25.29 ± 16.82	63.69 ± 10.07	123.04 ± 20.28	212.02 ± 43.09
	6000[c]	---	---	---	---	---	---	---	---
104[g]	0	0.00 ± 0.01	NA	NA	0.03 ± 0.02	0.01 ± 0.02	NA	NA	0.06 ± 0.07
	300	1.59 ± 0.08	3.79 ± 2.24	6.90 ± 4.76	12.28 ± 7.42	0.97 ± 0.39	3.34 ± 1.68	5.58 ± 3.62	9.89 ± 5.51
	1000	6.09 ± 2.38	9.85 ± 3.32	18.95 ± 8.15	34.89 ± 12.43	4.05 ± 2.85	8.34 ± 5.46	17.87 ± 15.38	30.26 ± 22.82
	3000	13.00 ± 9.02	19.41 ± 8.74	26.15 ± 12.84	58.35 ± 25.55	7.32 ± 4.09	27.81 ± 8.30	51.27 ± 19.15	86.40 ± 25.31
	6000[c]	---	---	---	---	---	---	---	---

[a]Based on five animals/sex/dose.

[b]Residual Fat 80'023 was found in the control samples at 13, 26, and 52 weeks.

[c]Determinations performed at week 52 only.

[d]Based on 20 animals/sex in control group; 10 animals/sex in 300-, 1000-, and 3000-ppm dose groups; and 19 males and 20 females in the 6000-ppm dose group.

[e]Based on three to five animals/sex/dose.

[f]Technical errors involved in resulting high values for males and females at 78 weeks.

[g]Based on 9 to 14 animals/sex/dose.

As with blood and kidney residues, the residue levels of Fat 80'023 found in the livers of dosed males and females were proportional to the dose levels; however, the predominant amount of residual Fat 80'023 was found in the unconjugated form (Table 12). Male livers generally contained higher residual levels; residual levels tended to decline at 104 weeks in males and females. An increased level of the glucuronide conjugate at 52 weeks in males fed 6000 ppm was reported to be a technical error. As with kidney samples, the liver samples of control animals were found to exhibit slight residue levels of the test material at 13, 26, and 52 weeks.

Results of analyses for spleen, heart, brain, skeletal muscle, and retroperitoneal fat were not reported.

8. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Peripheral nerve (sciatic nerve)*
X	Esophagus*	X	Bone marrow (sternum)*	X	Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*		
X	Ileum*				
X	Cecum*				
X	Colon*				
X	Rectum		Urogenital		Glandular
XX	Liver*	XX	Kidneys*	XX	Adrenals*
	Gallbladder*	X	Urinary bladder*		Lacrimal gland*
X	Pancreas*	XX	Testes*	X	Mammary gland*
		X	Epididymides	X	Thyroids*
	Respiratory	X	Prostate	X	Parathyroids*
X	Trachea*		Seminal vesicle		Harderian glands
X	Lung*	XX	Ovaries		
		X	Uterus*		Other
		X	Cervix	X	Bone [vertebra/ femur w/marrow]*
				X	Skeletal muscle*
				X	Skin
				X	All gross lesions and masses

*Recommended by Subdivision F (October 1982) Guidelines.

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TABLE 12. Mean Residue Levels of Unconjugated and Conjugated Fat 80'023 in the Liver of Rats Fed the Test Compound for 104 Weeks

Week	Dietary Level (ppm)	Fat 80'023 Content (µg/mL ± SD)							
		Males				Females			
		Unconjugated	Glucuronide Conjugate	Sulfate Conjugate	Total (Acid)	Unconjugated	Glucuronide Conjugate	Sulfate Conjugate	Total (acid)
13[a]	0[b]	0.05 ± 0.02	0.00 ± 0.01	0.00 ± 0.00	0.05 ± 0.03	0.16 ± 0.13	0.00 ± 0.00	0.01 ± 0.01	0.15 ± 0.12
	300	11.87 ± 2.27	0.68 ± 1.10	0.45 ± 0.53	12.23 ± 2.73	6.56 ± 2.10	0.78 ± 1.37	0.21 ± 0.27	6.54 ± 1.97
	1000	19.14 ± 11.88	0.93 ± 1.21	5.61 ± 5.71	22.96 ± 13.44	18.11 ± 5.88	0.76 ± 1.00	3.87 ± 2.51	21.02 ± 5.72
	3000	69.06 ± 10.39	7.03 ± 5.56	12.89 ± 9.15	87.89 ± 11.38	47.38 ± 8.23	2.03 ± 3.07	11.07 ± 4.59	57.77 ± 13.25
	6000[c]	---	---	---	---	---	---	---	---
52[d]	0	0.02 ± 0.09	NA	NA	0.01 ± 0.05	0.01 ± 0.02	NA	NA	0.01 ± 0.02
	300	9.21 ± 3.36	0.26 ± 0.23	0.41 ± 0.32	9.56 ± 3.51	6.73 ± 2.33	1.28 ± 0.96	1.66 ± 1.11	9.45 ± 3.10
	1000	19.24 ± 3.73	5.05 ± 3.12	1.95 ± 1.52	26.19 ± 6.39	15.36 ± 3.82	0.54 ± 1.23	1.14 ± 1.36	16.71 ± 4.10
	3000	50.12 ± 7.61	10.79 ± 7.17	17.08 ± 9.27	77.99 ± 17.77	41.64 ± 10.59	6.60 ± 7.02	19.45 ± 9.51	67.08 ± 15.63
	6000	76.17 ± 23.40	33.24 ± 63.11[e]	24.69 ± 11.69	120.02 ± 25.82	72.60 ± 13.56	7.83 ± 7.56	36.84 ± 12.28	114.83 ± 20.88
78[f]	0	0.00 ± 0.01	NA	NA	0.02 ± 0.02	0.00 ± 0.00	NA	NA	0.02 ± 0.01
	300	6.82 ± 2.13	0.05 ± 0.10	0.64 ± 0.53	7.15 ± 1.95	6.92 ± 1.68	0.30 ± 0.39	0.90 ± 0.72	7.91 ± 2.54
	1000	12.86 ± 2.99	0.36 ± 0.41	0.83 ± 0.80	13.96 ± 3.68	16.33 ± 5.22	0.86 ± 0.84	2.01 ± 0.92	18.98 ± 5.17
	3000	47.28 ± 8.55	8.09 ± 3.40	6.70 ± 8.96	60.80 ± 21.05	27.55 ± 7.69	8.66 ± 3.43	9.09 ± 5.30	45.24 ± 10.57
	6000	---	---	---	---	---	---	---	---
104[g]	0	0.00 ± 0.01	NA	NA	0.01 ± 0.02	0.01 ± 0.03	NA	NA	0.12 ± 0.30
	300	4.85 ± 1.53	0.20 ± 0.21	0.80 ± 0.41	5.82 ± 1.76	5.19 ± 2.38	0.52 ± 0.46	0.68 ± 0.55	6.37 ± 3.00
	1000	16.65 ± 6.80	1.89 ± 0.95	4.00 ± 2.66	22.51 ± 9.03	7.93 ± 4.59	0.84 ± 0.83	1.37 ± 1.38	9.96 ± 5.78
	3000	31.48 ± 8.97	5.26 ± 2.56	9.50 ± 4.12	46.24 ± 13.20	21.67 ± 9.05	7.28 ± 3.79	8.83 ± 5.25	36.95 ± 15.27

[a] Based on five animals/sex/dose.

[b] Residual Fat 80'023 was found in control samples at 13, 26, and 52 weeks.

[c] Determinations were performed at week 52 only.

[d] Based on 20 animals/sex in control group; 10 animals/sex in 300-, 1000-, 3000-ppm dose group; and 19 males and 20 females in the 6000-ppm dose group.

[e] High variation due to suspected technical error.

[f] Based on three to five animals/sex/dose.

[g] Based on 9 to 14 animals/sex/dose.

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At 52 weeks and terminal sacrifice, all tissues for control and high-dose (6000 ppm at 52 weeks, 3000 ppm at 104 weeks) males and females were examined histologically. The liver, pancreas, and gross lesions were examined histologically for low- and mid-dose rats at 52 weeks; the liver, kidney, lung, and gross lesions were examined for low- and mid-dose rats at 104 weeks. Only the liver and gross lesions were examined histologically for all dosed rats at 13, 26 and 78 weeks. All tissues were examined from animals dying during the study or sacrificed moribund. One female receiving 300 ppm (animal No. 573) died on the day of scheduled sacrifice (day 184); the liver was reported to be the only organ examined histologically for this animal.

Results:

a. Organ Weights: Decreased liver weights of males fed 3000 and 6000 ppm at week 52 were considered a reflection of body weight changes at that study interval since weights appeared similar to concurrent controls in males fed 3000 ppm at 78 and 104 weeks; absolute and relative (organ to body weight ratio) liver weights of females fed 3000 ppm were found to be slightly decreased throughout the study (Table 13). Other changes in organ weights (spleen, adrenals, ovaries) of dosed males and females were considered to be a reflection of body weight changes at those study intervals and were not considered to be of toxicological significance.

b. Gross Pathology: There were no compound-related increases in any gross lesion in dosed animals when compared to concurrent controls.

c. Microscopic Pathology:

1) Nonneoplastic: Table 14 summarizes nonneoplastic findings in the liver, pancreas, kidney, and lung of dosed and control males and females. The incidence of toxic liver changes [cytoplasmic inclusions (ring-shaped or spherical structures) of hepatocytes and centrilobular hepatocytic hypertrophy (enlargement to 1.3 times normal size with flocculent eosinophilic cytoplasm)] in males fed 3000 and 6000 ppm was found to be increased at 13, 52, and 78 weeks when compared to concurrent controls; these increases (incidence of 4/5 for cytoplasmic inclusions and 5/5 for hepatocellular hypertrophy) were found to be significant at 13 weeks for males fed 3000 ppm ($p<0.05$) and 52 weeks for males fed 6000 ppm ($p<0.05$, $p<0.001$).

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TABLE 13. Absolute and Relative Mean Liver Weights of Rats Fat 80'023 for 104 Weeks

Dose Group (ppm)	Mean Liver Weights at Week 26 Absolute (g ± S.E.)	26 Relative (%)	52 Absolute (g ± S.E.)	52 Relative (%)	78 Absolute (g ± S.E.)	78 Relative (%)	104 Absolute (g ± S.E.)	104 Relative (%)
				Males				
0	22.54 ± 1.91	3.497	25.84 ± 1.18	3.448	24.11 ± 2.94	3.163	21.57 ± 0.89	2.948
300	23.54 ± 2.60	3.910	23.87 ± 0.63	3.454	24.44 ± 1.30	2.888	23.13 ± 1.00	3.069
1000	22.29 ± 1.91	3.598	23.44 ± 1.43	3.180	21.95 ± 0.90	3.009	21.75 ± 0.80	3.067
3000	19.90 ± 0.88	3.244	21.54 ± 1.01*	2.899**	25.44 ± 3.74	2.807	21.19 ± 1.03	2.772
6000	---[a]	---	21.26 ± 0.53**	3.304	---	---	---	---
				Females				
0	11.92 ± 0.43	3.281	13.24 ± 0.55	3.301	14.31 ± 1.39	3.817	15.11 ± 0.90	3.439
300	10.47 ± 1.41	3.370	14.06 ± 0.50	3.470	13.99 ± 1.83	3.022	13.86 ± 0.70	3.337
1000	13.17 ± 0.69	3.841	14.66 ± 0.67	3.318	15.87 ± 1.18	3.490	15.10 ± 0.95	3.243
3000	11.20 ± 0.96	3.582	11.86 ± 0.60	3.223	12.61 ± 0.82	3.024	13.92 ± 0.78	2.951
6000	---	---	11.05 ± 0.27**	3.615	---	--	---	---

[a]Relative weights designate organ to body weight ratio.

[b]---indicates liver weights were not measured.

*Significantly different from controls at $p < 0.05$ as evaluated by the study authors.

**Significantly different from controls at $p < 0.01$ as evaluated by the study authors.

TABLE 14. Selected Nonneoplastic Lesion in Rats Fed Fat 80'023 for 104 Weeks

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	300	1000	3000	6000[c]	0	300	1000	3000	6000[c]
Liver[a]	(95)[b]	(85)	(85)	(85)	(20)	(95)	(85)	(85)	(85)	(20)
Cytoplasmic inclusions of hepatocytes	0	0	0	4	4*[d]	0	0	0	0	0
Centrilobular hepatocyte hypertrophy	0	0	0	7	12***[d]	0	0	0	0	0
Cellular alteration	13	17	18	14	0	13	15	9	15	0
Hyperplasia of liver and bile duct	19	13	19	7	0	11	13	11	11	0
Necrosis	1	5	4	4	0	0	0	0	2	0
Telangiectasis	12	21*[e]	24**[e]	16	0	0	2	6	1	0
Congestion	12	18	14	10	0	14	9	8	7	0
Vacuolation	15	3	10	19	9	16	12	9	12	0
Pancreas[f]	(80)			(70)	(20)	(80)			(70)	(20)
Focal atrophy of acinar tissue	19	9[g]	13[g]	18	2	13	4[g]	5[g]	10	5
Hyperplasia of pancreatic islets	3	1[g]	1[g]	4	5	1	0[g]	0[g]	0	0
Kidney[f]	(80)	(60)	(60)	(60)	(20)	(79)	(60)	(60)	(60)	(20)
Microscopic renal calculi	3	5	9	12**[e]	0	19	21	19	9	0
Mineralization	1	3	4	5	0	8	10	20**[e]	10	0

(continued)

TABLE 14. Continued

Organ/Finding	Dose Level (ppm)									
	Males					Females				
	0	300	1000	3000	6000[c]	0	300	1000	3000	6000
Lung[f]	(80)	(60)	(60)	(60)	(20)	(80)	(60)	(60)	(60)	(20)
Accumulation of foamy macrophages (alveoli)	15	29**[e]	22	26*[e]	0	6	16*[e]	19**[e]	14*[e]	0

[a]Includes animals at the 13-, 26-, and 78-week serial sacrifices, at the 52-week interim sacrifice, at terminal sacrifice, and those that died or were sacrificed moribund during the study.

[b]Number in parentheses equals number of tissues examined.

[c]All high-dose animals were sacrificed at 52 weeks.

[d]Significant effect at week 52 as evaluated by the study authors.

[e]Significant effect at week 104 as evaluated by the study authors.

[f]Includes animals at the 52-week interim sacrifice, at the terminal sacrifice, and those that died or were sacrificed moribund during the study.

[g]Finding observed in nonroutine organs; only animals with finding were examined histologically.

*Significantly different from controls at $p < 0.05$ as evaluated by the study authors.

**Significantly different from controls at $p < 0.01$ as evaluated by the study authors.

***Signficantly different from controls at $p < 0.001$ as evaluated by the study authors.

The incidence of hepatic necrosis and telangiectasis was found to be increased in males fed 300, 1000, and 3000 ppm; however, the study authors did not consider these findings to be compound related since the incidence was not dose related. The incidence of renal calculi (unilateral, very small) was significantly ($p<0.01$) increased in males fed 3000 ppm while the incidence of renal mineralization was significantly ($p<0.01$) increased in females fed 1000 ppm at week 104; however, these findings were not considered to be compound related by the study authors since the relative incidence was not significant among rats of the opposite sex.

The accumulation of foamy macrophages in the alveoli of dosed males and females was generally found to be significantly increased ($p<0.05$, $p<0.01$) when compared to concurrent controls; however, the study authors considered this finding to be a normal age-related lesion and not related to compound administration.

2) Neoplastic: Table 15 summarizes the incidence of neoplastic lesions in rats that died, were sacrificed at study termination, or were sacrificed at 52 weeks. The study authors reported that the incidence of neoplastic lesions was similar in dosed and control animals. In addition, the incidence of the neoplastic lesions found was within the range of incidence for historical laboratory controls of this strain[3] with the exception of a slight increase in islet cell adenomas of the pancreas in females fed 3000 ppm. There were no lesions of the pancreas exhibited in females fed 6000 ppm which were sacrificed at 52 weeks.

D. STUDY AUTHORS' CONCLUSIONS:

Dietary administration of Fat 80'023 to male and female rats at concentrations of 0, 300, 1000, or 3000 ppm for 104 weeks and 6000 ppm for 52 weeks resulted in toxic changes in the liver, designated as the primary target organ. This is based

[3]Hazleton, 1984. Neoplasia in Sprague Dawley rats--Untreated Controls. In: Representative Historical Control Data for Rats and Mice.

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TABLE 15. Incidence of Neoplastic Lesions in Rats Fed Fat 80'023 for 104 weeks[a]

Organ/Neoplasm	Dose Level									
	Males					Females				
	0	300	1000	3000	6000	0	300	1000	3000	6000
Adrenal	(79)[b]			(60)	(20)	(80)			(60)	(20)
Cortex--adenoma	1	--[c]	2[d]	2	0	3	4	1	2	0
Pheochromocytoma, benign	6	8	8	7	0	3	1	1	1	0
Liver	(80)	(70)	(70)	(70)	(20)	(80)	(70)	(70)	(70)	(20)
Hepatocarcinoma	1	3	2	3	0	0	0	1	0	0
Hepatocellular adenoma	2	2	1	1	0	4	3	2	2	0
Mammary	(74)			(57)	(20)	(80)			(60)	(20)
Adenocarcinoma	0	--	1	1	0	5	7	11	2	0
Adenoma	0	--	--	0	0	3	3	6	4	0
Pancreas	(80)			(60)	(20)	(80)			(60)	(20)
Islet cell adenoma	2	3	3	4	0	3	1	--	5	0
Parathyroid	(79)			(60)	(20)	(73)			(54)	(20)
Adenoma	0	2	--	1	0	0	--	1	0	0
Pituitary	(77)			(56)	(20)	(80)			(60)	(20)
Pars distalis--adenoma	36	23	31	35	3	52	50	52	50[e]	1
Pars distalis--carcinoma	2	1	1	0	0	2	1	1	3	0
Systemic	(80)			(60)	(20)	(80)			(60)	(20)
Histocytic sarcoma	2	3	1	2	0	1	2	--	2	0
Thyroid	(80)			(60)	(20)	(79)			(60)	(20)
Follicle--adenoma	0	--	1	3	0	2	1	--	1	0

[a] Includes animals at the 52-week interim sacrifice, at the terminal sacrifice, and those that died or were sacrificed moribund during the study.
[b] Number in parentheses equals number of tissues examined.
[c] --Indicates no lesions found in nonroutine organs of animals found dead or sacrificed moribund during the study.
[d] Incidence of lesions found in nonroutine organs of animals found dead or sacrificed moribund during the study.
[e] Three additional adenomas found at week 52; pancreas considered nonroutine organ at this interval.

on slight changes in clinical biochemistry, liver weight data, and nonneoplastic histological evaluation. A dose-related reduction was exhibited in the erythrocytic parameters and indices of all dose groups. However, since the magnitude of these changes was small with the low-dose groups, they were not considered to be of toxicological significance. Clinically significant increases in SGPT and SGOT and decreases in total bilirubin, triglycerides, glucose, total protein, and albumin concentrations occurred at doses $\geq$ 3000 ppm over the initial 52 study weeks. Significant changes in clinical chemistry parameters at lower dose levels ($\leq$ 1000 ppm) were transient and were not considered of toxicologic importance. At 52 weeks, males fed 3000 ppm were found to exhibit decreased mean relative liver weights when compared to concurrent controls.

Nonneoplastic changes were found in the liver (enlarged centrilobular hepatocytes containing hyaline-appearing cytoplasmic "inclusions") of males fed 3000 and 6000 ppm Fat 80'023 at 13 and 52 weeks. Hepatocellular hypertrophy was found in 2/5 males fed 3000 ppm at 78 weeks. These lesions were not found in rats maintained after 78 weeks, suggesting that such compound-induced effects were reversible, repaired through intrinsic mechanisms during continuous exposure to lower relative amounts of the test compound. Tumor incidence was similar in dosed and control animals at 104 weeks. Body weights were significantly decreased in males fed 6000 ppm and females fed 3000 ppm. The percent body weight difference, relative to the control group, was greater for females than for males. A compensatory increase in food consumption was exhibited in males only. Based on body weight data and histological liver findings in males, the maximum tolerated dose and LOEL of dietary Fat 80'023 is 3000 ppm and the NOEL is 1000 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study design was adequate although there were some deficiencies in the conduct of the study and in data reporting. In many areas, the study was poorly reported and written in a confusing manner. Technical and calculation errors were made in homogeneity analyses (Table 1a). The corrected range of source sample recovery at 300 ppm was 98.0 to 131.3%; this is considered to be outside the level of acceptability. Dates and times of testing of homogeneity and stability analyses were not reported. The mean test compound intake over the duration of the study was calculated by the reviewers.

The SGOT level of control males at week 26 varied substantially from the levels reported for these animals at weeks 13 and 52;

the reviewers consider these differences to be the results of technical error. Several clinical chemistry parameters (chloride, phosphorus, potassium, sodium, creatinine phosphokinase) as suggested in EPA Pesticide Assessment Guidelines for combined chronic toxicity/oncogenicity studies, 1982, were not measured. Technical errors in residue analyses of kidney tissue resulted in abnormally high levels of conjugated and unconjugated residues in males and females fed 3000 ppm at 78 weeks (Table 11). Technical errors resulted in abnormally high levels of glucuronide conjugate in the livers of males fed 6000 ppm at 52 weeks (Table 12). Technical errors resulted in residue levels of the test compound in control samples of kidney and liver tissues at 13, 26, and 52 weeks (Tables 11 and 12). Absolute liver weights of males and females fed 6000 ppm were significantly decreased ($p<0.01$) at 52 weeks when compared to concurrent controls. Since liver weights were measured at only 52 weeks it was difficult to determine if these reduced liver weights were an effect of decreased body weights in these animals as reported by the study authors. This explanation, however, appears correct for the reduced liver weights of males and females fed 3000 ppm.

A complete tissue inventory was not included in the study. Histopathological results of neoplastic and nonneoplastic lesions were evaluated and computed by the reviewers. Tissues examined histologically differed for mid-dose rats at each sacrifice interval; the liver was the only tissue consistently examined. In a deviation from the protocol, a complete histopathology was performed on sporadic animals designated for residue analysis at 78 weeks. The results of these complete histopathological examinations in rats fed 300 and 1000 ppm were not reported in summary tables but only as individual data.

The method of statistical analysis utilized by the study authors for clinical biochemistry parameters appears to be incorrect. Our reviewers evaluated triglyceride data for males (Table 8) using Bartlett's test of homogeneity, the Wilcoxon logrank test, and Dunnett's test and found that values reported to be nonsignificant by the study authors were significantly different from controls at $p < 0.05$. The accuracy of the results of many other statistical analyses (e.g., trends) reported by the study authors were questioned by the reviewers but were not recalculated due to time limitations.

The study authors did not consider the incidence of hepatocellular necrosis, renal calculi, and the accumulation of foamy macrophages in the alveoli of male and female rats to be

compound related. Since rats fed 6000 ppm were sacrificed at 52 weeks and the historical incidence of these nonneoplastic findings is unavailable, it is difficult to determine the cause of these findings. However, the reviewers cannot dismiss the increased incidence of liver necrosis in males fed 300-, 1000-, and 3000-ppm Fat 80'023 (5/85, 4/85 and 4/85, respectively, as compared to 1/95 in concurrent controls) when determining the NOEL for the study. Liver enzyme parameters in many of these individual animals were found to be increased.

We agree with the study authors that Fat 80'023 was not oncogenic under the conditions of the study; however, contrary to the study authors who reported the NOEL to be 1000 ppm, we have found the LOEL to be 300 ppm based on the histopathological incidence of hepatic necrosis.

Executive Summary for One-Year Toxicity Study in Baboons (MRID # 00251773)

In a chronic toxicity study, groups of 7 baboons/sex/dose received Irgasan DP 300 orally at doses of 30, 100, and 300 mg/kg/day by capsule for 52 weeks. At 100 and 300 mg/kg/day, the 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 NOEL was determined to be 30 mg/kg/day, and the systemic LOEL was determined to be 100 mg/kg/day, based on clinical signs of toxicity.

003655

DATA EVALUATION REPORT

Compound Irgasan®, DP-300, FAT 80 023/A

Citation

1 Year Oral Toxicity Study in Baboons with compound FAT 80 023/A. J.C. Drake & A. Buxtorf
Geigy Pharmaceuticals, Toxicology Department, Stamford Lodge, Wilmslow, Cheshire. Jun 28, 1976

Reveiwed By

Robert P. Zendzian Ph.D.
Pharmacologist

Core Classification Minimun

Tox Catagory N/A

Conclusion

The subchronic oral toxicity of FAT 80 023/A in the baboon was characterized by effects on the digestive system, vomiting, failure to eat and diarrhoea at 100 and 300mg/kg/day, and depression of red blood cells at 300mg/kg. A NOEL was demonstrated at 30mg/kg/day. This study satisfies the requirement for a nonrodent subchronic study.

Materials

FAT 80 023/A, Irgasan®, DP 300, technical

Fifty-six baboons, 28 male and 28 female, with average body weights of 6.03 kg males and 5.54 kg females. Animals were maintained for at least seven months before the start of the study. All animals were negative for parasites and negative to tuberculin test.

Methods

Animals were assigned to one of four treatment groups of seven males and seven females and dosed with zero, 30, 100 or 300 mg/kg/day FAT 80 023/A orally by capsule for 52 weeks.

Clinical signs and food consumption were recorded daily and body weight weekly. Blood and urine samples were collected before test and on weeks 5, 9, 13, 20, 26, 39 and 52 and during week 56 from the recovery animals. Opthalmic and hearing examinations were carried out before test and on weeks 5, 9, 16, 26, 40 and 52 and during week 56 from the recovery animals

(13)

Hematology

Hemoglobin
Erythrocyte count
Hematocrit
Reticulocyte count
Inclusion bodies
Thrombocyte count
Leucocyte count
Leucocyte differental
Prothrombin time
ESR (sed. rate)
Methhemoglobin

Clinical Chemistry

Sodium
Chloride
Glucose
Urea
SGOT
SGPT
SAP
Total protein
Electrophoresis
Cholinesterase
Cholesterol

Urine analysis

pH
Specific Gravity
Protein
Glucose
Bilirubin
Ketones
Blood
Sediment

Two males and two female from each group were sacrificed at six months, three males and three females from each group were sacrificed at 52 weeks and the remaining animals after a six week recovery period.

Gross necropsy was performed on all animals at sacrifice and the following organs were weighed; adrenals, brain, gonads, heart, kidneys, liver, pituitary and thyroid.

The following tissues were were collected for histopathology:

Adrenals
Brain
Gonads
Kidneys
Lymph nodes, axillary & mesenteric
Muscle
Pituitary
Spleen
Thymus
Aorta
Colon
Gross lesions
Pancreas
Prostate or uterus
Spinal cord
Thyroids
Bone marrow
Eye and optic nerve
Heart
Lungs
Mammary gland
Sciatic Nerve
Small intestine
Urinary bladder
Liver

Body weight and food consumption were analysed by the Profile Analysis Method and laboratory data by the Mann-Whitney U Test.

Results

Compound related signs of digestive tract effects were observed in groups three and four with the majority of effects in group four. Signs included vomiting, failure to eat and diarrhoea. Diarrhoea occured 4-6 hours after dosing or during the night and was the most common toxic sign.

In the male animals, mean growth rate was decreased from week 32 through the end of dosing in groups 3 and 4 but there was no apparent difference between these two groups. In the female animals, mean growth rate was higher then controls from week 25 to the end of the study in groups 2 and 3 and lower than controls from week 25 to the end of the study in group 4.

No pattern of compound related effect on food consumption was observed. Consumption varied from week to week with some isolated depression at the high dose particularly in the males.

Depression of red blood cells at the high dose in both sexes was seen throughout the dosing period. No other changes in hematological perameters were observed.

A decrease in serum potassium and serum alkaline phosphatase was observed at the high dose, in both sexes, throughout the study. Decreased total protein was also observed at this dose but the effect was inconsistantly present. No other effects were observed in clinical chemistry.

No compound related effects were observed in urine analysis.

At necropsy there was some indication of an effect on the lining of the stomach of the high dose animals at each sacrifice. Histopathology was slightly confirmatory of the stomach effect. No other compound related effects were observed in either gross necropsy or histopathology.

SECTION HEAD

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

MEMORANDUM

JUL 20 1988

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Irgasan - Two-Generation Reproduction Study in Rats. Review of Study.

TO: Jeff Kempter
Product Manager (32)
Registration Division (TS-769C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/30/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Marcia van Gemert, Ph.D. M. van Gemert 7/6/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D. W.J. Burn 7/20/88
Chief, Toxicology Branch (TS-769C)

Registrant: CIBA-GEIGY Corporation
Chemical: Irgasan® DP300; FAT 80'023
Project: 8-0759
Caswell No.: 186A
Record No.: 222989

Action Requested: Review of two-generation reproduction study.

Comment: CIBA-GEIGY Corporation has submitted a two-generation reproduction study in rats as fulfillment of reproduction test data requirements.

TB has reviewed this study and the DER is attached. Before final assessment of the study can be made, the Registrant should provide clarification of the procedure used to select the parents of the F_2 generation and define the terms adjusted and unadjusted pup body weight.

The study is classified as Supplementary, pending receipt of clarification. The high-dose produced both systemic and reproductive effects in both generations. The NOEL for both reproductive and systemic effects is 1000 ppm (50 mg/kg) and the LEL is 3000 ppm (HDT - 150 mg/kg), based on body weight effects and viability.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/30/88
Section: III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section: III, Tox. Branch (TS-769C) Marcia Gemert 7/6/88

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - rat- OPPTS 870.3800 (§83-4)

DP BARCODE: SUBMISSION CODE:
P.C. CODE:054901 TOX. CHEM. NO.:186A

TEST MATERIAL (PURITY: FAT 80'023 (Irgasan, Triclosan). Purity: 99%.

SYNONYMS: Irgasan, Triclosan

CITATION: Morseth, S.L. (1988): Two-Generatrion Repoduction Study in Rats - FAT 80'023. Study performed by Hazleton Laboratories America, Inc. For Ciba Specialty Chemicals. Submitted under MRID 40623701. Unpulished.

SPONSOR: Ciba-Geigy Corporation

EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 40623701) FAT 80'023 (Irgasan) was administered in the diet to groups of either 25 or 30 (Fo and F1, respectively)male and female Crl:CD(BR)rats at dose levels of 0, 300, 1000, and 3000ppm (0, 15, 50, and 150 mg/kg/day) for 10 weeks prior to mating and through post-natal day 21 for both generations. In the Fo generation, there were no significant decreases in parental body weight during pre-mating, but a significant increase in mean body weight was observed in 50 mg/kg/day males. Body weight in Fo females during lactation was significantly decreased on post-natal day 7, with a significant negative trend in mean body weight gain for the high dose group of for days 0-7. Increased incidence of liver discoloration in 50 and 150 mg/kg/day parental Fo males was observed. No effects on reproductive performance were observed in the Fo generation. Pups of the Fo generation (F1 pups) showed decreased mean body weight on post-natal days 14 and 21 at the 150 mg/kg/day dose. Increased pup mortality was observed on post-natal days 0-3 in high dose pups. Decreased viability index was also observed at the 150 mg/kg/day dose in F1 pups, as was an increased incidence of dilated renal pelvis. In F1 parental animals, significantly lower group mean body weights were observed during pre-mating at the 150 mg/kg/day dose. Gestational group mean body weight in F1 females was significantly decreased by 12% during the period of gestation, with a significant

negative trend for gestational days 1, 7, 14, and 20. There were no differences in number of pregnant animals, mean gestation duration and mean precoital interval in F1 females. In pups of the F1 parental generation (F2 pups), an increase in number of pups found dead or missing was increased at the 150 mg/kg/day dose. Weaning index was decreased at the high dose in F2 pups, and increased total liter deaths was increased. The Parental Systemic NOEL is 1000ppm (50 mg/kg/day), and the Parental Systemic LOEL is 3000ppm (150 mg/kg/day), based on reduced mean body weights. The Reproductive/Developmental NOEL is 1000ppm (50 mg/kg/day); the Reproductive/Developmental LOEL is 3000ppm (150 mg/kg/day), based on reduced viability of pups and reduced body weights.

This reproductive study in the rat is classified *unacceptable - guideline*. The study is upgradable provided the registrant clarifies the meaning of unadjusted and adjusted body weights and a clarification of the procedure used to select parents of F2 generation.

QUALITY ASSURANCE: A quality assurance statement was provided.

A. MATERIALS:

1. Test compound: FAT 80'023, Description: white powder with lumps, Batch No.: 5202110, Purity: 99%.

2. Test animals: Species: rat, Strain: Crl:CD*(SD)Br, Age: 6 weeks old at start, Weight: 196-230g (males) and 147-172g (females), Source: Charles River Breeding Laboratories, Inc., Newfield, NJ.

3. Statistics: See pages 22-24, appended, for details.

B. STUDY DESIGN

1. Dose:

The test material was incorporated into the basal (Purina Certified Rodent Chow® 5002) at dose levels of 0, 300, 1000, and 3000 ppm, and the appropriate diets and tap water were available ad libitum.

2. Selection of Parents:

F_0 Parents - One hundred male and 100 female rats (after 16-day acclimation period) were assigned to the F_0 test-diet groups by a body-weight-dependent computerized randomization process, which first eliminated those with extreme body weights. There were 25 rats /sex/group.

F_1 Parents - After weaning, one male and one female F_1 pup per litter (when possible) were selected by random card draw from the available litters (up to 2/sex/litter) to form the F_1 parental generation. There were 30 rats/sex/group.

F_2 Parents - To keep open the option of breeding the F_2 animals, potential breeders were chosen on Day 28. Up to 2/sex/group were selected by random card draw from each litter. These animals were not mated.

3. Mating, Delivery, and Subsequent Examination:

One adult (F_0, F_1) male was mated with one adult (F_0, F_1) female from the same dose group after at least 10 weeks of dietary exposure. The F_0 animals were approximately 17 weeks old at the start of breeding. The rats were paired sequentially by ascending number within each group, with one exception. Vaginal examination was performed daily on each female to determine presence of sperm or the presence of a copulatory plug and the stage of eatrous. The day of sperm or plug observation was designated Day 0 of gestation. Females with no evidence of mating and normal estrous cycles, following a maximum 31-day mating period, were placed with proven males of the same dose group for an additional 10 days or until mating was detected. When mating was detected, the sexes were separated. Mated females were placed in nesting boxes on Day 20 of presumed gestation.

Females were allowed to deliver their young; time of birth and the presence of milk in each pup's stomach in Day 0 were recorded. On Days 0, 4, 7, 14, and 21 after birth, the number of live and dead pups of each sex per litter, body weight of each live pup, and clinical observations of live pups were recorded. Dead pups were examined grossly for cervical, thoracic, or abdominal visceral abnormalities and cause of death, and were subsequently discarded. Litters with more than 8 pups on Day 4 were culled to 8. Culling was by random card draw with equal numbers of males and females selected where possible. The culled pups and those not selected for mating were examined for abnormalities as stated above and discarded.

4. Clinical Observations, Body Weight, and Food Consumption

a) Parental - All animals were observed twice daily for mortality and moribundity. Weekly body weights were recorded during treatment and at sacrifice for males and those females not mated, those who failed to produced a litter by Day 26 of presumed gestation, and those who had discontinued lactating. All females were weighed weekly during growth and mating. Confirmed-mated females were weighed on presumed gestation Days 0, 7, 14, and 20, and dams producing litters were weighed on Days 0, 4, 7, 14, and 21 postpartum.

Food consumption of both sexes was measured weekly during the premating period, and on Days 7, 14, and 20 during gestation and Days 4, 7, 14, and 21 of lactation for females who were presumed pregnant or had delivered a litter. After the mating period, weekly food consumption was resumed for males, nonpregnant females or females not confirmed pregnant, and post-lactating females. No food consumption data were collected during mating.

b) F_2 Offspring - Weekly food consumption, body weight, and clinical observations were recorded following weaning of this generation.

5. Sacrifice and Gross Pathology

a) Parental Animals - F_0 males were sacrificed after the F_1 pups were delivered, and the F_0 females were sacrificed after the F_1 pups were weaned. Both sexes were subjected to gross necropsy (not fasted prior to sacrifice). The following tissues were preserved.

vagina	seminal vesicles
uterus	prostate
ovaries	pituitary
testes	liver
epididymides	gross lesion(s)

The uterus of each female not delivering within 26 days of mating was examined for evidence of pregnancy, prior to fixation, and if no evidence was found, proper staining was performed to detect very early implantation scars. The uterus of each female found dead or sacrificed in extremis was examined for implantaion and the ovaries were examined to corpora lutea.

When possible, the extent of development of implantation sites was determined and the gravid uterus was evaluated for resorptions, dead, or normally developing fetuses.

F_1 parental animals were treated in a similar manner, with the exception that the number of implanation sites were recorded at necropsy only for the F_1 females.

b) F_2 Offspring - At approximately 10-13 weeks of age, these animals were sacrificed and subjected of a gross examination of cervical, thoracic, and abdominal viscera of abnormalities.

RESULTS:

Note: All tables and figures referenced are from the final study report and are appended.

1. Diet Analysis

Homogeneity tests indicated that FAT 80'023 was mixed uniformly in the diet with the mean value of greater than 97.7% in all cases. Stability studies showed the test compound to be stable at room temperature for at least 21 days. The concentration of FAT 80'023 in the diet showed the following ranges.

Low	92.2-106.7%
Mid	87.4-102.8%
High	95.8-109.1%

2. Parental Data

a) F_0

1) Mortality - There was only one death reported in the parental animals; one mid-dose male was found dead during Week 13 of the maturation period. This was not noted as related to treatment.

2) Clinical Observations - Only one subcutaneous tissue mass was reported in a control female; no other masses were reported for this generation. The clinical observations noted did not indicate a compound-related effect during any phase of the study.

3) Body Weight and Food Consumption - Mean body weight values were significantly higher in the mid-dose males at Weeks 2 and 8 and in the mid-dose females at Weeks 7-10, compared to controls (Table 3). A significant positive trend was reported for males at Week 2 also. Mid-dose females showed higher total mean body weight gain values compared to control for Weeks 0-10. The mean body weight change for the female controls was 126.6 grams and 142.4 grams for the mid-dose females (112% of control).

Mean food consumption values and mean total food consumption values were comparable among the groups.

During gestation, the mean body weight values were reported to be significantly higher for the mid dose at Day 0 only (Table 7A). The mean body weight change was reported as significantly lower at Days 7-14 for the low group compared to control, with a significant negative trend at Days 14-20 in mean body weight change. Mean food consumption values were significantly lower for the mid-dose group on Days 7-14 and 0-20 compared to control (Figure 6).

During lactation, mean body weight was significantly lower for the high-dose group compared to control only at Day 7 (Table 10). A significant negative trend in the mean body weight gain was reported at Days 0-7 for this group. All other dose levels were comparable to control. Food consumption values were comparable among the groups.

4) Gross Pathology - The only differences reported include an increased incidence of liver findings (mainly discoloration) in 4 mid-dose and 5 high-dose males compared to 2 males in both the control and low-dose groups, changes in the testes (size, texture, or color), which were seen only in treated (2,3, and 3 in the low-, mid-, and high-dose) males, and kidney changes, as follows:

	males				females			
	C	L	M	H	C	L	M	H
kidney, dilated (pelvis)	0	0	1	1	0	1	1	3

There were no other reported differences.

5) Reproductive Performance - The reproduction indices were reported as comparable among the groups. Cycling behavior of the F_0 females was not affected by treatment. The number of pregnant animals was comparable among the groups, as were the mean duration of gestation and the mean precoital interval.

	Fertility Index (%)	Mean Gestation Time	Mean Precoital Interval (days)	Gestation Index (%)
Control -	84	21.7	2.5	100
Low	96	21.8	3.2	100
Mid	96	21.8	2.8	100
High	96	21.4	3.6	100

b) F_1

1) Mortality - One high-dose male was sacrificed during Week 12 for humane reasons (fractured nose, a rough appearance, malocclusion, chromodacryorrhea, and nasal discharge), but this death was not considered related to treatment. One high-dose female was found dead on Day 19 of gestation; no comment was made as to whether the death was related to treatment.

2) Clinical Observations - There was no indication of any toxic effect due to treatment during any phase of the study.

3) Body Weight and Food Consumption - High-dose animals showed significantly lower mean body weights during Weeks 0-12 compared to controls during the growth phase. Significant negative trends were reported in males at the start of the growth phase and at Weeks 5,7,8,10,11, and 12 and in females at start and at Weeks 2-12. Mean body weight changes were comparable at Weeks 1-11 for males compared to controls, but a negative trend in mean body weight change was reported for females for Weeks 0-11.

During gestation, mean body weight for the high-dose females was significantly lower at all intervals and for the low- and mid-dose females on Day 20, with a significant negative trend reported for Days 1,7,14, and 20. Mean body weight change values were significantly lower for all groups at Days 14-20 and 0-20 and for the high-dose at days 0-7, with a significant trend reported at Days 0-7, 14-20, and 0-20 (Table 23A).

On Day 0 of lactation, the body weights of all treated dams were significantly lower than control, but as was the case during gestation, the mid-dose was greater than the low dose. A significant decrease in body weight was seen throughout lactation at all dose levels (except Days 4 and 14 for the mid dose). Mean body weight change was significantly higher in the high-dose dams throughout lactation (Days 0-21) than in controls (Table 26).

During the growth phase and lactation, food consumption was comparable among the groups. During gestation, lower food consumption was reported for the high-dose females compared to control, with significant trends noted for Days 0-7, 14-20, and 0-20.

4) Gross Pathology - The only noteworthy observations were dilated kidney (pelvis), which occurred as follows:

	Males	Females
Control	3	4
Low	1	1
Mid	4	4
High	5	6

Other observations were reported as sporadic and not related to treatment.

5) Reproductive Performance - Cycling behavior was reported as unaffected by treatment in the F_1 females. The number of pregnant animals was comparable among the groups, as were the mean duration of gestation and the mean precoital interval.

	Fertility Index (%)	Mean Gestation Time	Mean Precoital Interval (days)	Gestation Index (%)
Control	100	21.8	4.5	100
Low	86	21.9	3.3	100
Mid	100	21.8	2.9	97
High	90	21.7	3.8	93

3. Offspring Data

a) F_1 Pups

1) Clinical Observations - There were no treatment-related effects reported.

2) Body Weight, Survival, and Sex Ratios - High-dose pups (viable, both sexes) had slightly lower mean body weights compared to controls (unadjusted and adjusted) on Day 0. The adjusted mean body weight values were significantly lower in this group at Days 14-21. The low- and mid-dose groups were comparable to controls throughout lactation.

ody Weight of Pups		Day 0		Day 4		Day 4		Day 7		Day 14		Day 21	
	Group	M	F	M	F	M	F	M	F	M	F	M	F
F_1	0	6.1	5.8	8.6	8.1	8.6	8.1	13.5	12.7	28.9	27.8	44.8	42.8
	300	6.1	5.7	8.8	8.2	8.9	8.1	14.3	13.1	29.3	27.3	44.6	41.8
	1000	6.2	5.8	9.0	8.4	9.0	8.4	14.1	13.3	30.3	28.7	46.3	43.5
	3000	5.8	5.4	7.8	7.2	7.9	7.2	12.1	11.0	25.5*	23.7*	40.2*	37.7*

*$p \leq 0.05$

Increased mortality occurred in the high-dose group compared to control during the first days of life (Days 0-6). The number of pups found dead or missing/cannibalized is shown below.

	# Pups found dead Interval-Days (% litters affected)			
Group	0-3	4-6	7-13	14-20
Control	15/6	11/5	2/1	0/0
Low	18/10	2/2	2/2	1/1
Mid	11/8	3/2	1/1	0/0
High	47/13	7/7	5/3	0/0

	# Pups missing/presumed cannibalized Interval (Days)			
Group	0-3	4-6	7-13	14-20
Control	9/7	5/4	1/1	0/0
Low	8/5	8/2	2/1	0/0
Mid	3/3	1/1	1/1	0/0
High	12/6	0/0	3/1	0/0

The combined mean % of found dead and missing pups per litter is shown below.

F_1	% litters affected	mean % found dead (or missing) pups/litter
Control	67	11.4± 16.3
Low	57	7.2± 9.0
Mid	52	4.7± 5.2
High	74	18.8± 28.9

The ratio (%) of males to females was comparable among the groups, although at both time intervals the high-dose group showed more males than females.

C	L	M	H
45	46	49	53
51	49	50	57

Other parameters measured are listed below. It is noted that the viability index showed a lower percentage in the high-dose group, which also showed a greater number of litters affected. There were total litter losses in one low-dose and two high-dose dams.

Litter Generation	Dietary Level (ppm)	# Litters Born Live	% Live Pups	Mean # Pups/Litter Day 0	Mean # Dead Day 0 M/F	Viability Index (%)	Weaning Index (%)
F_0 - F_1	0	21	99	14.4	0.1/0.1	90	96
	300	23	97	13.3	0.2/0.2	94	97
	1000	23	99	12.8	0.0/0.1	96	97
	3000	23	99	13.6	0.04/0.1	82	95

3. Gross Necropsy - An increased incidence of kidney findings was reported for the high-dose weanlings (see below).

Kidney*	Control	Low	Mid	High
pelvis(es), dilated	3/2	-	2/2	2/2
dilated	4/3	4/2	7/6	12/8

* #pups affected/#litters

b) F_2 Pups

1) Clinical Observations - There was no indication of any toxic effect related to treatment.

2) Body Weight, Survival, and Sex Ratio - On lactation Day 0, the high-dose pups showed a lower adjusted mean body weight than control and the low- and mid-dose displayed a significantly higher mean body weight on Day 4 (pre- and postcull) compared to controls. Adjusted mean body weight was significantly increased for the mid-dose males and the low- and mid-dose females on Day 7.

›dy Weight f Pups		Day 0		Day 4		Day 4		Day 7		Day 14		Day 21	
	Group	M	F	M	F	M	F	M	F	M	F	M	F
F_2	0	6.4	5.9	7.9	7.4	7.9	7.4	12.0	11.3	26.1	25.2	40.7	39.5
	300	6.3	6.0	9.0*	8.6*	9.0*	8.6*	13.8	13.3*	27.6	26.6	41.0	39.3
	1000	6.1	5.8	8.9*	8.5*	8.9*	8.5*	13.8*	13.2*	28.5	27.0	44.1	41.2
	3000	5.8*	5.5*	8.0	7.5	8.1	7.5	12.5	12.1	25.5	24.0	38.5	36.9

*$p \leq 0.05$

The number of pups found dead/cannabalized during various intervals is shown below.

	# Pups found dead Interval-Days (% litters affected)			
Group	0-3	4-6	7-13	14-21
Control	31/20	14/8	2/2	0/0
Low	11/7	1/1	0/0	1/1
Mid	15/10	7/5	1/1	0/0
High	36/17	19/4	6/3	0/0

	# Pups missing/presumed cannibalized Interval (Days)			
Group	0-3	4-6	7-13	14-21
Control	11/6	8/6	4/4	0/0
Low	3/2	1/1	0/0	0/0
Mid	11/5	3/3	1/1	0/0
High	15/6	4/2	3/3	0/0

The combined mean % of found dead and missing pups per litter is shown below.

F_2	% litters affected	mean % found dead (or missing) pups/litter
Control	72	14.6± 15.6
Low	38	4.9± 7.8
Mid	45	10.1± 18.7
High	72	23.2± 29.2

The percent males on Days 0 and 21 are listed below.

	C	L	M	H
Day 0	54	47	50	53
Day 21	56	50	51	53

Other parameters measured are listed below. There were fewer low- and high-dose litters compared to control and mid-dose, a dose-related decrease (not statistically significant) in the number of pups per litter, the mean pup weight was lowest in the high-dose, and viability and weaning indices were lower (but not significantly) in the high-dose group compared to control. One mid-dose and two high-dose pregnant females failed to deliver pups.

Litter Generation	Dietary Level (ppm)	# Litters Born Live	% Live Pups	Mean # Pups/Litter Day 0	Mean # Dead Day 0 M/F	Viability Index (%)	Weaning Index (%)	Total Litter Deaths
$F_1 - F_2$	0	29	95	12.7	0.1/0.3	87	93	1
	300	24	98	11.9	0.04/0.2	97	99	0
	1000	29	100	11.8	0.03/0.0	90	99	0
	3000	25	93	11.2	0.1.0.4	84	86	4

3. Gross Necropsy - (a) Weanlings: Kidney findings were as follows.

Kidney	Control	Low	Mid	High
pelvis, dilated	4/3	5/4	3/3	2/2

No dose-related changes were reported.

c) F_2-Postweanlings:

1) Clinical Observations - There were no indications of any toxic effect due to treatment.

2) Body Weights and Food Consumption - Postweaning mean body weights (Days 28-91) were comparable among the groups. There was no effect observed on food consumption.

3) Gross Necropsy - The most common findings were in the kidneys, but appeared sporadically and were not dose-related.

Kidneys*	Control	Low	Mid	High
pelvis(es), dilated	9/7	10/7	15/13	8/8
dilated		1/1		

* #pups/#litters affected

DISCUSSION

The effects noted in this study were changes in body weight, which were observed to be decreased mainly in the high-dose group animals, and slightly reduced viability in both the F_1 and F_2 offspring.

The high-dose F_1 pups displayed decreased body weight from Days 14 through 21 and throughout the growth phase. This effect resulted in the delivery of pups of decreased body weight in the next generation (F_2 pups). On Day 20 of gestation, the low- and mid-dose F_1 dams also displayed a significant decrease in body weight compared to control. This apparent decrease at these two lower dose levels may be the result of the large variation in weight seen in the control or due to mathematics rather than a real effect of the compound. The persistent decrease in body weight of the F_1 dams observed at all dose levels during lactation may be attributed to the initial decrease, which was seen on Day 21 of gestation, compounded by the added stress of lactation, and not the result of an effect of the compound on lactation per se.

Additionally, the F_2 high-dose pups had decreased body weights at birth, which may be attributed to the dams' lower body weights. These pups attained comparable body weights to the controls and the other dose groups by Day 4 and maintained comparable weight throughout the remainder of the observation period (Day 91).

In both generations, the viability index was decreased in the high-dose groups compared to control. The weaning index in the F_2 generation high-dose group was slightly lower than control, and total litter deaths in this group were increased above control.

It is concluded that the high-dose (3000 ppm) produced both reproductive and systemic effects in both generations. The NOEL can be set at 1000 ppm for both reproductive and systemic effects; the LEL at 3000 ppm. based on body weight effects and viability.

Note: The Registrant should be requested to provide clarification of the procedure used to select the parents of the F_2 generation. On page 15 of the final report, it is stated:

> "At least one male and one female F_1 pup per litter (when possible) were selected from a preselected pool of 2/sex/litter to serve as the parents to the F_2 generation. These parental F_1 animals... ."

On page 20 of the final report it is stated:

> "After all litters were weaned, one male and one female pup were selected by random card draw from the remaining pups of each litter (up to 2/sex/litter) to form the F_1 parental generation."

The terms: adjusted and unadjusted body weights, need to be defined.

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3-10-1998 HIAR Briefing Package

Page _____ is not included in this copy.

Pages 130 through 141 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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Triclosan (Irgasan) Prenatal Developmental Toxicity in Rats [§83-3(a)]

Reviewed by: Susan L. Makris, M.S. Susan L Makris 7/24/96
Section III, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 7/24/96
Section III, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oral Prenatal Developmental Toxicity in Rats; OPPTS 870.3700 [§83-3(a)]

DP BARCODE: D220590
PC CODE: 054901
SUBMISSION CODE: S496156
CASE NO: 805889
ID NO.: 054901-000100

TEST MATERIAL: Triclosan (99.8%)

SYNONYMS: 2-hydroxy-2',4,4'-trichloro diphenyl ether; Irgasan; 5-chloro-2-(2,4-dichloro phenoxy)phenol

CITATION: Denning, H.J., S. Sliwa, and G.A. Willson (1992) Triclosan: Effects on pregnancy and post-natal development in rats: Volume 1. Environmental Safety Laboratory, Bedford, England. Study No. RT/3/84, December, 1992. MRID Nos. 43817502 and 43817503 (Appendices). Unpublished.

SPONSOR: Unilever Research, Sharnbrook, Bedford MK44 1LQ, England

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

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

No evidence of pre- or postnatal developmental toxicity was identified at any dose level under the conditions of this study. **Developmental LOEL = Not determined (>300 mg/kg/day); Developmental NOEL ≥ 300 mg/kg/day**

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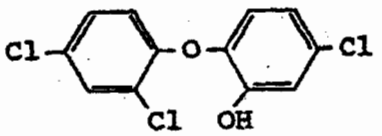
This study is classified as **ACCEPTABLE** and satisfies the §83-3(a) guideline requirement for a developmental toxicity study in rats.

COMPLIANCE: The following signed and dated statements were included in the report:
- Data Confidentiality Claims Statement (none claimed)
- GLP Compliance Certification
- Quality Assurance Statement
- Flagging Statement (negative)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Name: Triclosan; 2-hydroxy-2',4,4'-trichloro diphenyl ether; 5-chloro-2-(2,4-dichlorophenoxy)phenol
   Purity: 99.8%
   Batch No.: P405129
   Description: White powder; melting point = 54.5°C
   CAS No.: 3380-34-5
   Chemical Structure:



2. Vehicle: Material: Corn oil (Mazola)
   ELS Reference No.: S.14,553T$_1$
   Source: Herbert and Herbert, Tavistock St., Bedford, England

3. Test animals: Species: Rat
   Strain: Colworth Wistar
   Source: Not provided
   Age at mating: Approximately 11-16 weeks
   Weight at GD 0: 226-331 g (mean: 285 g)

4. Environment: Housing: Individually
   Temperature: 67-72°F
   Humidity: 50-64%
   Air changes: Not provided
   Light/dark cycle: 12 hours light/12 hours dark
   Food: PCD, ad libitum
   Water: Tap water, ad libitum

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B. PROCEDURES AND STUDY DESIGN

This study was conducted to assess the potential for maternal, developmental, and/or post-natal toxicity of the test substance, Triclosan, when administered by gavage to pregnant Colworth Wistar rats during the period of major fetal organogenesis.

1. In-life dates: Start: May 16, 1991; End: June 18, 1991

2. Group Assignment and Dosage Levels

The female rats were mated 3:1 with male rats; no information on strain, source, or age of the male rats was provided. Each male was allowed a maximum of 5 positive matings. Cage trays were examined each morning for the presence of a copulatory plug; mating was confirmed by the presence of sperm in a vaginal smear. Females were assigned to the following study groups on day 0 of gestation; the five mated females from each male were randomly assigned, one to each treatment group and one to each of the control subgroups, thereby attempting to ensure that there be a representative distribution of any intrinsic male characteristic.

Group	Dose (mg/kg/day)	Concentration (% w/v)	Dose Volume (ml/kg/day)	No. Assigned	
				GD 21 Sacrifice	Natural Parturition
1 (Control)	0[a]	0	6	50	10
2 (Low)	30	0.50	6	25	5
3 (Mid)	100	1.67	6	25	5
4 (High)	300	5.00	6	25	5

a Vehicle control (corn oil).

3. Rationale for Dose Level Selection

In a preliminary study conducted prior to the main study (study number not provided; data not submitted to the Agency), repeated doses of Triclosan were administered by gastric intubation to virgin female Colworth Wistar rats weighing 187-233g and 13-17 weeks of age. Report No. RT/3/84 (MRID 43817502) states that at 400 and 500 mg/kg/day, nephrotoxicity resulted, but no further information was provided on the toxicological profile. Based upon this finding, 300 mg/g/day was selected as the top dose for the pre- and postnatal developmental toxicity study in rats.

4. Dosage Formulation and Analysis

Dosing suspensions were prepared four times during the study, in the following manner: Triclosan was ground to a fine powder with a mortar and pestle, then an

appropriate amount was weighed into a measuring cylinder. Corn oil was added and mixed thoroughly with the test substance, then adjusted to the appropriate volume with additional corn oil. The suspension was homogenized once with a Potter-Eveljhen homogenizer. Suspensions were stored at ambient temperatures. During dosing, the suspensions were continuously stirred on a spin-stirrer.

Prior to the start of the study, the stability of Triclosan in corn oil (2.0 and 5.0% w/v) was demonstrated analytically for a period of 29 days; the method of storage was not stated (report No. RT/3/84, MRID 43817503, page 11). The initial and final batches of the suspensions used for dosing the animals were analyzed for Triclosan concentration; all analytical findings were within ±10% of nominal (report No. RT/3/84, MRID 43817503, page 7). Homogeneity data for dosing suspensions were not presented in the study report.

5. Dosage Administration

The test material was administered once daily to the study animals by gavage on Days 6-15 of gestation. To prevent possible over-distention of the stomach, all rats were denied access to food for 2 hours prior to dosing. Individual dosage volumes were based upon Day 6 body weights and remained constant throughout the dosing period. Control animals received the vehicle (corn oil) in the same manner.

C. Observations

1. Maternal Observations and Evaluations

The study report did not provide a description or schedule of maternal mortality or clinical observations conducted during gestation or lactation. Individual body weights and food and water consumption were recorded daily throughout gestation.

Twenty five rats from each treated group, and fifty control rats were killed on Day 21 of gestation by carbon dioxide asphyxiation. A gross necropsy was performed on each rat; no tissues were saved. The gravid uteri were excised and weighed, corpora lutea of pregnancy were counted, and uterine contents were examined for metrial glands, evidence of abortion, and fetal deaths. The study report does not indicate whether the uteri of apparently nonpregnant females were examined further, e.g. by pressing between glass slides or by staining with ammonium sulfide, for confirmation of pregnancy status.

The study report did not provide a description of sacrifice or postmortem evaluation of the females that were allowed to deliver litters naturally.

2. Fetal Evaluations

Following removal from the uterus, each fetus was examined for external defects, sexed externally, weighed, and measured for crown-rump distance. Individual placental weights were recorded. All fetuses were placed in a warm environment for

20 minutes to enable observations on skin color, movement, and emission of vocal sounds.

The fetuses were killed by immersion in 95% ethanol. After fixation in ethanol, all fetuses were examined internally for visceral anomalies. Approximately two-thirds of the fetuses were fixed in absolute alcohol, macerated in potassium hydroxide, stained with Alizarin Red S, cleared, and examined for skeletal abnormalities. The remaining one-third of the fetuses were decapitated; the heads were preserved in Bouin's fluid for subsequent evaluation of head abnormalities by Wilson's free-hand razor sectioning technique. The remaining body of these fetuses were processed for skeletal evaluation.

3. Postnatal Evaluations

For those rats allowed to deliver naturally (5 rats per treatment group and 10 controls), evaluations of litter weight and mortality were examined from birth to weaning (21 days). According to the study report, litters were also examined for any expression of postpartum effect due to disturbed lactation. The day of age at which the following developmental milestones were observed was recorded for each pup: presence of vibrissae, pinna unfolding, incisor eruption, eyelids opening, and completion of fur growth. The pups were killed on lactation day 21, by carbon dioxide asphyxiation. The internal organs of each pup were examined, and all pups were then processed for skeletal evaluation.

D. Data Analysis

1. Statistical Analyses: Although report No. RT/3/84 did not provide a detailed description of the methods of statistical evaluation, the study data tables indicated that the Student's T-test was used on parametric data (maternal body weight, food consumption, and water consumption; mean uterine and fetal data), and that a "nonparametric equivalent" to Student's T-test was used to evaluate incidences of fetal anomalies. All tests were reported at the 5%, 1%, and 0.1% levels of significance.

2. Historical Data: Historical control data (uterine, fetal, and weanling) for Colworth Wistar rats, from 6 studies conducted at Environmental Safety Laboratory during an unspecified period, were provided (study No. RT/3/84, MRID 43817502, pages 318-324) and are appended as Attachment 1.

II. RESULTS

1. Maternal Mortality and Clinical Observations

During the course of the study, no rats died due to test substance toxicity. One low-dose (30 mg/kg/day) rat was killed due to respiratory distress on gestation day 9 which resulted from an apparent intubation injury. No data from this rat (No. SO 21), through GD 9, were included in the study report.

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Clinical observation data (summarized in Table 1) revealed irritancy of the test substance in 21 high-dose (300 mg/kg/day) rats, as demonstrated by rubbing of the nose and mouth on the cage floor immediately after dosing. In addition, slight diarrhea, usually evident during the first few days of treatment and lasting for 24-48 hours, was observed in 21 rats at this dose level. Individual maternal clinical observation data were not included in the study report.

Table 1. Maternal Clinical Observation Data (incidence a)

Parameter	Dose level (mg/kg/day)			
	0	30	100	300
No. mated	60	30	30	30
No. pregnant	49	27 b	24	29
Moribund sacrifice (pregnant)	0	1	0	0
Diarrhea, slight	0	0	1	21
Irritancy of test substance[c]	0	0	0	21
Hair loss, slight	0	2	2	0
Scab on snout	0	0	0	1
Red urine	1	0	0	1

a Number of rats observed with the finding at least one time during the study.

b Not including the pregnant female that was killed for humane reasons.

c Immediately after dosing, rats rubbed nose and mouth on cage floor.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, page 16.

2. Maternal Body Weight, Food Consumption, and Water Consumption Data

A summary of mean maternal body weight, body weight gain, food consumption, food efficiency, and water consumption data during gestation is presented in Table 2. The body weight data were similar between control and treated groups. A significant decrease in body weight gain was observed in high-dose (300 mg/kg/day) rats during the period of treatment (Days 6-10 and 6-15), although body weight was recovered after cessation of treatment (Days 15-21). Mean food consumption for this group (300 mg/kg/day) was also decreased during the period of treatment (Days 6-10 and 10-15), followed by post-treatment recovery, although there was an additional significant decrease noted for Days 6-21. Food efficiency was significantly decreased for the 300 mg/kg/day group for Days 6-10 of gestation. Mean water consumption data revealed significant increases for all treatment and post-treatment intervals (GD 6-10, 10-15, 15-21, 6-21, and 0-21) at 300 mg/kg/day, with additional significant increases noted at 100 mg/kg/day for GD 10-15, 6-21, and 0-21. Significant increases in mean food and water consumption prior to treatment (GD 0-6) at 100 mg/kg/day were considered to be incidental.

Table 2. Mean Gestation Body Weight, Body Weight Gain, Food Consumption, Food Efficiency, and Water Consumption Data

Parameter	Dose level (mg/kg/day)			
	0	30	100	300
No. pregnant	49	27	24	29
Body weight (g)				
GD 0	199	199	202	202
GD 6	219	219	224	222
GD 10	232	230	235	231
GD 15	252	250	254	250
GD 21	309	304	310	310
Gravid Uterus	67.6	66.6	62.7	67.5
GD 20 Adjusted[a]	242	240	245	242
Body weight gain (g)				
GD 0-6	20	19	21	20
GD 6-10	13	12	12	9***
GD 10-15	20	20	19	18*
GD 15-21	57	54	56	60
GD 6-21	90	86	86	88
GD 0-21	110	105	107	108
Food consumption (g)				
GD 0-6	117	118	127***	118
GD 6-10	74	72	72	63***
GD 10-15	100	98	98	90***
GD 15-21	140	138	143	147*
GD 6-21	314	308	314	300*
GD 0-21	431	425	441	418
Food efficiency				
GD 0-6	0.1671	0.1611	0.1693	0.1685
GD 6-10	0.1760	0.1610	0.1596	0.1496**
GD 10-15	0.2025	0.2067	0.1895	0.2050
GD 15-21	0.4056	0.3928	0.3875	0.4112
GD 6-21	0.2870	0.2792	0.2734	0.2951
GD 0-21	0.2545	0.2457	0.2431	0.2591
Water consumption (g)				
GD 0-6	140	138	148*	141
GD 6-10	91	91	96	120***
GD 10-15	128	127	138*	170***
GD 15-21	192	191	202	226***
GD 6-21	411	410	434*	516***
GD 0-21	550	550	582*	658***

* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
*** Significantly different from control value, $p \leq 0.001$.
a Day 21 body weight minus gravid uterine weight.
Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 17 and 19.

3. Maternal Gross Pathology

No treatment-related lesions were identified at necropsy; postmortem changes occurred sporadically in control and treated groups.

4. Observations Noted at Cesarean Section

The results of the examination of uterine contents at cesarean section are presented in Table 3. The maternal and fetal data were similar between control and treated groups; there were no treatment-related effects on preimplantation or prenatal viability, fetal body weight, litter size, or sex ratio.

Table 3. Summary of Selected Cesarean Section Observations

Parameter	Dose level (mg/kg/day)			
	0	30	100	300
Number assigned to cesarean section	50	25	25	25
Number pregnant	43	23	19	24
Number of maternal deaths	0	1	0	0
Number abortions/premature births	0	0	0	0
Number of litters with live fetuses	43	22	19	24
Mean no. corpora lutea	12.3	11.8	12.4	12.5
Mean no. implantations	11.5	11.1	10.8	11.5
Mean preimplantation loss	0.9	0.7	1.5	1.0
Mean postimplantation loss	1.1	1.1	1.4	1.2
Mean % postimplantation loss	10.02	10.42	11.35	11.27
Mean % late fetal death	0.41	0.73	0.75	1.85
Mean no. live fetuses	10.3	10.0	9.5	10.3
Mean no. live male fetuses	5.6	5.3	4.5*	5.0
Mean no. live female fetuses	4.8	4.6	4.9	5.3
Mean body weights (g)	4.7	4.7	4.6	4.7
Male fetuses	4.9	4.8	4.7	4.8
Female fetuses	4.6	4.6	4.5	4.6
Mean placental weights (g)	0.44	0.45	0.45	0.44
Male fetuses	0.45	0.46	0.46	0.45
Female fetuses	0.43	0.44	0.44	0.42
Mean crown-rump distance (mm)	40.67	40.46	40.67	40.61
Male fetuses	41.11	40.72	40.97	40.88
Female fetuses	40.23	40.21	40.37	40.35

* Significantly different from control value, $p \leq 0.05$.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, page 19.

B. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are summarized in Tables 4 (external and gross visceral defects, anomalies, and variants) and 5 (skeletal defects, anomalies, and variants).

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Table 4. Summary of Selected External and Gross Visceral Major Defects, Anomalies, and Variants in Fetuses

Observation		Dose (mg/kg/day) 0	30	100	300
No. fetuses (litters) examined		444(43)	219(22)	180(19)	247(24)
Total number with any external or visceral defect, anomaly, or variant	Fetus N(%)	101(22.7)	55(25.1)	25(13.9)	34(13.8)*
	Litter N(%)	42(97.7)	20(90.9)	14(73.7)	19(79.2)
Total number with any major external or visceral defect	Fetus N(%)	3(0.68)	3(1.37)	1(0.56)	0
Total number with any external or visceral anomaly	Fetus N(%)	6(1.35)	7(3.20)	1(0.56)	7(2.83)
HEAD - Domed	Fetus N(%)	1(0.23)	1(0.46)	0	2(0.81)
	Litter N(%)	1(2.3)	1(4.5)	0	2(8.3)
BODY - Edema, thoracic [MAJOR DEFECT]	Fetus N(%)	0	1(0.46)	0	0
	Litter N(%)	0	1(4.5)	0	0
MANDIBLE - Brachygnathia [MAJOR DEFECT]	Fetus N(%)	1(0.23)	0	1(0.56)	0
	Litter N(%)	1(2.3)	0	1(5.3)	0
TONGUE - Aglosstomia	Fetus N(%)	1(0.23)	0	1(0.56)	0
	Litter N(%)	1(2.3)	0	1(5.3)	0
TONGUE - Protruding	Fetus N(%)	0	1(0.46)	0	1(0.40)
	Litter N(%)	0	1(4.5)	0	1(4.2)
MOUTH - Small orifice	Fetus N(%)	0	0	1(0.56)	0
	Litter N(%)	0	0	1(5.3)	0
PALATE - Misshapen [MAJOR DEFECT]	Fetus N(%)	0	1(0.46)	0	0
	Litter N(%)	0	1(4.5)	0	0
INNOMINATE ARTERY - Shortened	Fetus N(%)	5(1.13)	5(2.28)	2(1.11)	2(0.81)
	Litter N(%)	5(11.6)	5(22.7)	2(10.5)	2(8.3)
INNOMINATE ARTERY - Missing	Fetus N(%)	3(0.68)	4(1.83)	0	1(0.40)
	Litter N(%)	3(7.0)	2(9.1)	0	1(4.2)
AORTIC ARCH - Displaced	Fetus N(%)	0	2(0.91)	1(0.56)	0
	Litter N(%)	0	2(9.1)	1(5.3)	0
HEART AND GREAT VESSELS - Situs transversus	Fetus N(%)	1(0.23)	1(0.46)	0	2(0.81)
	Litter N(%)	1(2.3)	1(4.5)	0	2(8.3)
BLADDER - Agenesis [MAJOR DEFECT]	Fetus N(%)	1(0.23)	0	0	0
	Litter N(%)	1(2.3)	0	0	0
BLADDER - Rudimentary [MAJOR DEFECT]	Fetus N(%)	0	1(0.46)	0	0
	Litter N(%)	0	1(4.5)	0	0
KIDNEY/URETER - Hydronephrosis and hydroureter	Fetus N(%)	1(0.23)	1(0.46)	0	3(1.21)
	Litter N(%)	1(2.3)	1(4.5)	0	2(8.3)

* Significantly different from control value, $p \leq 0.05$.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 21-22 and 28. Major defects were listed on page 23. Percentages were recalculated by Reviewer.

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Table 5. Summary of Selected Skeletal Major Defects, Anomalies, and Variants in Fetuses

Observation		Dose (mg/kg/day) 0	30	100	300
No. fetuses (litters) examined		312(43)	154(22)	125(19)	172(24)
Total no. with any skeletal defect, anomaly, or variant	Fetus N(%)	185(59.3)	98(63.6)	76(60.8)	110(64.0)
	Litter N(%)	43(100)	22(100)	18(94.7)	24(100)
Total no. with any skeletal defect, anomaly, or variant (excluding extra ribs and sternebrae variations)	Fetus N(%)	128(41.0)	48(31.2)	28(22.4)	46(26.7)
	Litter N(%)	33(76.7)	21(95.5)	14(73.7)	19(79.2)
Total number with any major skeletal defect	Fetus N(%)	2(0.64)	1(0.65)	3(2.40)	1(0.58)
Total number with any skeletal anomaly	Fetus N(%)	2(0.64)	2(1.30)	2(1.60)	0
SKULL - Single nasal orifice [MAJOR DEFECT]	Fetus N(%)	1(0.66)	0	1(0.80)	1(0.58)
	Litter N(%)	1(5.3)	0	1(5.3)	1(4.2)
PALATE - Cleft [MAJOR DEFECT]	Fetus N(%)	0	0	1(0.80)	0
	Litter N(%)	0	0	1(5.3)	0
PALATE - Misshapen [MAJOR DEFECT]	Fetus N(%)	0	1(0.65)	1(0.80)	1(0.58)
	Litter N(%)	0	1(4.5)	1(5.3)	1(4.2)
PALATE - Misaligned	Fetus N(%)	0	0	0	2(1.16)
	Litter N(%)	0	0	0	1(4.2)
MANDIBLE - Fused	Fetus N(%)	1(0.32)	0	1(0.80)	0
	Litter N(%)	1(2.3)	0	1(5.3)	0
MANDIBLE - Brachygnathia [MAJOR DEFECT]	Fetus N(%)	1(0.32)	0	1(0.80)	0
	Litter N(%)	1(2.3)	0	1(5.3)	0
VERTEBRAE - Thoracic, fused arch	Fetus N(%)	0	1(0.65)	1(0.80)	0
	Litter N(%)	0	1(4.5)	1(5.3)	0
VERTEBRAE - Curved spinal column	Fetus N(%)	0	0	1(0.80)	0
	Litter N(%)	0	0	1(5.3)	0
VERTEBRAE - Scoliosis [MAJOR DEFECT]	Fetus N(%)	2(0.64)	0	1(0.80)	0
	Litter N(%)	2(4.7)	0	1(5.3)	0
RIBS - Extra unilateral 7th cervical	Fetus N(%)	4(1.28)	0	5(4.00)	8(4.65)
	Litter N(%)	2(4.7)	0	5(26.3)	3(1.74)
RIBS - Branched	Fetus N(%)	1(0.32)	1(0.65)	1(0.80)	0
	Litter N(%)	1(2.3)	1(4.5)	1(5.3)	0
RIBS - Fused	Fetus N(%)	1(0.32)	1(0.65)	0	0
	Litter N(%)	1(2.3)	1(4.5)	0	0

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 24-25 and 28. Major defects were listed on page 26. Percentages were recalculated by Reviewer.

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The term "defects" appears to be used interchangeably with "malformations" in the study report and refers to changes which are detrimental to the species, cosmetically unacceptable, or incompatible with life. Minor anomalies are described as changes which are unlikely to prove detrimental to the animal but which may not be totally compatible with late fetal development. Variations are considered to be slight differences which occur in a proportion of the animals and do not show any evidence of being detrimental to the stock. Although major defects were identified within the fetal data, no distinction was made between anomalies and variants in the tables of fetal findings.

The litter and fetal incidences of the various findings were comparable between treated and control groups, and generally within the incidence rates observed historically in the performing laboratory (see Attachment 1). The types of malformations and variations observed were varied and did not suggest a response to treatment.

The ossification pattern of small bones is summarized in Table 6. According to the study report, the incidence and distribution of ossification patterns in the sternebrae, cervical centra, caudal vertebrae, forelimbs, and hindlimbs were similar between control and treated groups.

C. Postpartum Phase

1. Litter size and pup survival: Selected litter measurements are presented in Table 7. Litter size was statistically increased ($p \leq 0.05$) in the high-dose group (300 mg/kg/day) at lactation days 7 and 14. This finding was considered incidental. Survival was not notably different between control and treated groups; most deaths occurred postpartum, between Days 0 and 4.

2. Pup Body Weight: Group mean pup body weight data (Table 7) did not reveal any treatment-related differences.

3. Pup Clinical Observations: Developmental milestones were observed, or initiated and completed, at equivalent times for control and treated pups (Table 8).

4. Offspring Postmortem Results: Gross visceral defects, anomalies, and variants that were observed in pups that died or were killed prior to weaning included slight rotation of the heart and stenosis of the innominate artery in one pup at 300 mg/kg/day, supernumerary rudimentary liver lobes in one pup at 100 mg/kg/day, and dextrocardia with transposed lungs in one pup at 30 mg/kg/day (report no. RT/3/84, MRID 43817502, page 32). No skeletal anomalies were identified in these pups.

   External and visceral findings noted at *post mortem* examination for those pups surviving to lactation day 21 are summarized in Table 9a. Skeletal findings for these pups are summarized in Table 9b. Due to the low incidences of these findings, and the lack of a dose-response relationship, they were not attributed to treatment with Triclosan.

Table 6. Incidence of Ossification Patterns of Small Bones[a]

Observation	Dose (mg/kg/day)			
	0	30	100	300
Sternebrae				
6 ossified	51.55	49.23	49.97	48.26
1 unossified	0.80	0.57	2.39	0
2 unossified	0	0	0	0.94
Reduced ossification of 1	41.51	46.15	38.90	46.04
Reduced ossification of 2	1.36	1.22	3.56	0
Asymmetric	10.33	7.22	16.02	12.47
Bipartite	0.55	0.57	0.75	0.42
Cervical vertebrae				
All 7 centra ossified	32.69	34.39	24.50	36.95
1 centrum unossified	43.43	40.27	41.90	44.22
2 centra unossified	10.48	10.81	18.73	9.70
3 centra unossified	10.28	8.66	10.34	3.47
4 centra unossified	2.56	3.41	1.41	3.02
5 centra unossified	0.56	2.46	1.41	2.64
6 centra unossified	0	0	0.66	0
All 7 centra unossified	0	0	1.05	0
Caudal vertebrae				
6 or more ossified vertebrae	93.00	95.18	94.18	94.16
5 ossified vertebrae	7.00	4.82	5.82	5.84
Forelimbs				
Unossified metacarpals	80.00	80.00	80.00	80.00
Unossified phalanges	41.49	41.36	40.70	42.15
Unossified claws	100.00	100.00	100.00	100.00
Hindlimbs				
Unossified metatarsals	99.14	98.90	98.37	99.36
Unossified phalanges	13.12	15.06	13.57	15.48
Unossified claws	100.00	100.00	100.0	100.00
Partially ossified calcaneus	9.73	11.44	15.11	15.08

a Incidence = (no. of fetuses with described pattern of ossification/total no. of fetuses examined) X 100.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 26-27.

Table 7. Selected Litter Measurements

Observation	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
No. mated females assigned to postpartum phase	10	5	5	5
No. pregnant (%)	6(60)	5(100)	4(80)	5(100)
No. of litters	6	5	4	5
Total no. pups born	54	44	42	52
Total no. stillborn pups	0	0	0	0
Total number dead pups: Days 0-21	5	3	6	2
Day 0	1	0	3	0
Days 1-3	4 a	3	3	1
Days 16-21	0	0	0	1 a
Survival indices				
Pup survival: Days 0-4 (%)	49(91)	41(93)	36(86)	51(98)
Pup survival: Days 0-21 (%)	49(91)	41(93)	36(86)	50(96)
Total litter death, Days 0-21	0	0	0	0
Mean litter size				
Day 0	8.8	8.8	9.8	10.4
Day 7	8.2	8.2	9.0	10.2*
Day 14	8.2	8.2	9.0	10.2*
Day 21	8.2	8.2	9.0	10.0
Mean pup weight (g)				
Day 0	5.9	5.7	6.2	5.8
Day 7	14.3	14.4	14.4	14.2
Day 14	28.0	27.7	27.9	25.8
	42.8	42.1	43.4	41.0

* Significantly different from control value, $p \leq 0.05$.

a One pup was killed for humane reasons.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 29 and 30.

Table 8. Mean Developmental Milestones (mean day of observation a)

Observation	Dose (mg/kg/day)			
	0	30	100	300
Vibrissae present	0	0	0	0
Pinnae unfolding[b]	2.5-12.3	2.4-12.8	2.3-12.3	2.2-12.0
Fur growth[b]	4.8-15.8	4.4-15.4	4.0-16.0	4.0-15.8
Incisor eruption	8.8	8.4	8.5	8.6
Eyelids opening[b]	14.2-17.0	14.2-17.0	14.3-17.0	14.2-17.0

a Calculated by reviewer.

b Mean days of initiation and completion of process.

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 239-261.

III. Discussion/Conclusions

A. Investigator's/Reviewer's Conclusions

1. Maternal toxicity

Following gavage administration of the test substance, Triclosan (99.8%), to pregnant Colworth Wistar rats on days 6-15 of gestation at dose levels of 30, 100, or 300 mg/kg/day, maternal toxicity was observed at the 300 mg/kg/day dose level. Findings included transient diarrhea (24-48 hour duration after the initiation of treatment), decreased body weight gain during the treatment period, and a reduction in food consumption and increase in water consumption from the onset of treatment until the end of gestation. Treatment-related effects on dams were not observed at 30 and 100 mg/kg/day.

Maternal LOEL = 300 mg/kg/day (based on transient diarrhea, decreased body weight gain during treatment [GD6-15], and reduced food consumption and increased water consumption from GD6-21); Maternal NOEL = 100 mg/kg/day.

2. Developmental toxicity

For litters that were taken by cesarean section, an assessment of embryonic and fetal development, including litter size, pre- and post-implantation loss, fetal weight and size, placental weight, and sex ratio did not reveal any evidence of treatment-related toxicity. Examination of these fetuses for alterations of external, visceral, and skeletal development revealed no differences between control and treated groups.

For litters delivered naturally, litter weight, pup mortality, and developmental milestones (presence of vibrissae, pinna unfolding, incisor eruption, eyelid opening, and completion of fur growth) were recorded. Survival and development of pups from birth to weaning was comparable between control and treated groups. Evaluation of weanlings at Day 21 revealed no significant increase in the incidence of pups with external, visceral, or skeletal anomalies.

Developmental LOEL = Not determined (> 300 mg/kg/day); Developmental NOEL ≥ 300 mg/kg/day.

Table 9a. Summary of Select External and Visceral Defects, Anomalies, and Variants in Weanling (Day 21) Pups

Observation		Dose (mg/kg/day) 0	30	100	300
No. pups (litters) examined		49(6)	41(5)	36(4)	50(5)
Total no. with any external or visceral defect, anomaly, or variant	Pup N(%)	5(10.2)	11(26.8)	5(13.8)	11(22.0)
	Litter N(%)	4(66.7)	4(80.0)	3(75.0)	5(100)
Eye - Anophthalmia	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Heart - Misshapen	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Heart - Dextrocardia	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Heart - Situs transversus	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Innominate artery - Shortened	Pup N(%)	0	1(2.4)	0	1(2.0)
	Litter N(%)	0	1(20.0)	0	1(20.0)
Innominate artery - Unbranched	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Innominate artery - Agenesis	Pup N(%)	0	1(2.4)	0	1(2.0)
	Litter N(%)	0	1(20.0)	0	1(20.0)
Lungs - Undivided right lobe	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Lungs - Agenesis of post-caval lobe	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Liver - Supernumerary rudimentary lobes	Pup N(%)	1(2.0)	6(14.6)	5(13.8)	5(10.0)
	Litter N(%)	1(16.7)	2(40.0)	3(75.0)	4(80.0)
Liver - Complete division of median lobe	Pup N(%)	0	1(2.4)	0	0
	Litter N(%)	0	1(20.0)	0	0
Gastric transposition	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Kidneys - Transposition	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)

Note: Data were extracted from report No. RT/3/84, MRID 43817502, page 34. Percentages were recalculated by Reviewer.

Table 9b. Summary of Select Skeletal Defects, Anomalies, and Variants in Weanling (Day 21) Pups

Observation		Dose (mg/kg/day) 0	30	100	300
No. pups (litters) examined		49(6)	41(5)	36(4)	50(5)
Total no. with any skeletal defect, anomaly, or variant	Pup N(%)	20(40.8)	12(29.3)	18(50.0)	24(48.0)
	Litter N(%)	6(100)	5(100)	4(100)	5(100)
Total no. with any skeletal defect, anomaly, or variant, excluding extra ribs and sternebrae variants	Pup N(%)	15(30.6)	9(22.0)	17(47.2)	23(46.0)
	Litter N(%)	6(100)	4(80.0)	4(100)	5(100)
Total no. with extra ribs	Pup N(%)	4(10.3)	1(2.4)	3(8.3)	1(2.0)
	Litter N(%)	3(50.0)	1(20.0)	3(75.0)	1(20.0)
Total no. with sternebrae variations	Pup N(%)	1(2.6)	4(9.8)	2(5.6)	3(6.0)
	Litter N(%)	1(16.7)	3(60.0)	2(50.0)	3(60.0)
Eye - Enlarged socket	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Scapula - Shortened and thickened	Pup N(%)	0	0	1(2.8)	0
	Litter N(%)	0	0	1(25.0)	0
Ribs - extra cervical	Pup N(%)	4(10.3)	1(2.4)	2(5.6)	1(2.0)
	Litter N(%)	3(50.0)	1(20.0)	2(50.0)	1(20.0)
Vertebrae, cervical - Missing transverse process on the right 6th	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Vertebrae, cervical - Additional transverse process on the right 5th	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Vertebrae, thoracic - Fused	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Vertebrae, lumbar - Fused	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Vertebrae, lumbar arches - Misshapen	Pup N(%)	0	0	0	2(4.0)
	Litter N(%)	0	0	0	1(20.0)
Vertebral column - Scoliosis	Pup N(%)	0	0	0	1(2.0)
	Litter N(%)	0	0	0	1(20.0)
Humerus and femur - Slightly shortened	Pup N(%)	0	0	1(2.8)	0
	Litter N(%)	0	0	1(25.0)	0

Note: Data were extracted from report No. RT/3/84, MRID 43817502, pages 35-36. Percentages were recalculated by Reviewer.

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B. Study Deficiencies: The following deficiencies in conduct or data analysis were not judged to compromise the validity of the study or the interpretation of results.

1. Homogeneity data for dosing suspensions were not presented in the study report, although adequate purity, stability, and concentration analyses were provided.

2. The female rats were mated 3:1 with male rats, not 1:1 as recommended by guideline §83-3.

3. No information on strain, source, or age of the male rats was provided.

4. The report methods section did not describe the maternal observation schedule for the gestation and lactation periods, although a summary of clinical observations during gestation was provided. The individual maternal clinical observation data, upon which the summary was based, were not included in the study report. The study methods did not indicate if clinical observations were performed on pups during lactation, and no data were provided for this parameter.

5. The study report did not provide a description of sacrifice or postmortem evaluation of the females that were allowed to deliver litters naturally.

6. A detailed description of the methods of statistical evaluation was not included in the study report. Footnotes on the summary tables were the only clue to what statistical treatments were applied to the data.

7. No data were included in the study report for one low-dose rat (No. SO 21) that was killed on gestation day 9 due to respiratory distress which resulted from an apparent intubation injury.

8. Fetal/weanling and litter percentages presented in the summary tables of external, visceral, and skeletal findings, all apparently generated by the same computer system, contained numerous inconsistencies. The reason for these inconsistencies was not clear, since the statistical treatment of data was not adequately described in the report. In order to eliminate some of the confusion such inconsistencies could generate, all percentages for individual findings in fetuses and weanlings were recalculated by the Reviewer for presentation in the DER (Tables 4, 5, 9a and 9b). Percentage incidences of small bone ossification, however, were not recalculated since the observations were not recorded in a manner that would enable verification. Whether or not the percentages were accurately calculated, a review of the individual fetal and weanling incidence data in the study report (MRID 43817503) did not indicate the need for concern regarding the incidence or distribution of these observations.

9. Although major defects were identified within the fetal data, no distinction was made between anomalies and variants in the tables of fetal findings.

10. The study report did not indicate the period of time over which the studies that provided historical control data were conducted.

ONE-LINER

Study Type: Oral Prenatal Developmental Toxicity in Rabbits; OPPTS 870.3700 (§83-3)

Test Material: Irgacare MP (C-P Sample No.: 38328) (100%)

Synonyms: Irgasan; 5-chloro-2-(2,4-dichlorophenoxy)phenol; Triclosan; 2-hydroxy-2',4,4'-trichloro diphenyl ether

EPA MRID No.: **43820401** and 43787101 (range-finding)

Testing Facility: Bio/dynamics, Inc., East Millstone, NJ

Study No.: **91-3666** and 91-3655 (range-finding)

Report Issued: **April 16, 1992** and May 6, 1992 (range-finding)

Executive Summary: The test substance, Irgacare MP (C-P Sample No. 38328); 100% a.i., was administered by gavage to pregnant female New Zealand White rabbits (18/group) on days 6-18 of gestation at dose levels of 15, 50, or 150 mg/kg/day. The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. On day 30 of gestation, the 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 for cardiac abnormalities.

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. **Maternal LOEL = 150 mg/kg/day (based upon decreased body weight gain and food consumption during treatment); Maternal NOEL = 50 mg/kg/day**

No treatment-related developmental toxicity was observed under the conditions of this study. **Developmental LOEL = Not determined (>150 mg/kg/day); Developmental NOEL = 150 mg/kg/day**

This study is classified as **ACCEPTABLE** and satisfies the §83-3(b) guideline requirement for a developmental toxicity study in rabbits.

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DER #5 Developmental Toxicity in Rabbits MRID # 43787101

Irgasan Prenatal Developmental Toxicity in Rabbits [§83-3(b)]

Reviewed by: Susan L. Makris, M.S. 7/17/96
Section III, Toxicology Branch II (7509C)
Secondary reviewer: James N. Rowe, Ph.D. 7/17/96
Section III, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oral Prenatal Developmental Toxicity in Rabbits; OPPTS 870.3700 [§83-3(b)]

DP BARCODE: D220524
PC CODE: 054901
SUBMISSION CODE: S496150
CASE NO: 805889
ID NO.: 054901-000100

TEST MATERIAL: Irgacare MP (C-P Sample No.: 38328) (100%)

SYNONYMS: Irgasan;5-chloro-2-(2,4-dichlorophenoxy)phenol;Triclosan;2-hydroxy-2',4,4'-trichloro diphenyl ether

CITATIONS: Schroeder, Raymond E. (1992) A segment II teratology study in rabbits with Irgacare MP (C-P Sample No. 38328). Bio/dynamics, Inc., East Millstone, NJ. Study No. 91-3666, April 16, 1992. MRID No. 43820401. Unpublished.

Schroeder, Raymond E. (1992) A range-finding study to evaluate the toxicity of Irgacare MP (C-P sample No. 38828) in the pregnant rabbit. Bio/dynamics, Inc., East Millstone, NJ. Study No. 91-3655, May 6, 1992. MRID No. 43787101. Unpublished.

SPONSOR: Colgate-Palmolive Company, Piscataway, NJ; C-P study Nos. 91-006 and 91-014 (R-F)

EXECUTIVE SUMMARY: The test substance, Irgacare MP (C-P Sample No. 38328); 100% a.i., was administered by gavage to pregnant female New Zealand White rabbits (18/group) on days 6-18 of gestation at dose levels of 15, 50, or 150 mg/kg/day. The rabbits were observed for signs of toxicity; body weight and food consumption values were recorded. On day 30 of gestation, the 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 for cardiac abnormalities.

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. **Maternal LOEL = 150 mg/kg/day (based upon decreased body weight gain and food consumption during treatment); Maternal NOEL = 50 mg/kg/day**

No treatment-related developmental toxicity was observed under the conditions of this study. **Developmental LOEL = Not determined (>150 mg/kg/day); Developmental NOEL = 150 mg/kg/day**

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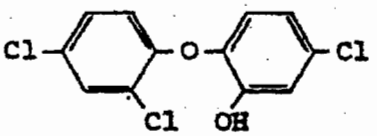
This study is classified as **ACCEPTABLE** and satisfies the §83-3(b) guideline requirement for a developmental toxicity study in rabbits.

COMPLIANCE: The following signed and dated statements were included in the report:
- Data Confidentiality Claims Statement (none claimed)
- GLP Compliance Certification
- Quality Assurance Statement
- Flagging Statement (negative)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Name: Irgacare MP (C-P Sample No. 38328); Irgasan; 5-chloro-2-(2,4-dichlorophenoxy)phenol
   Purity: 100%
   Lot No.: 19851206
   Description: Dosing suspensions; clear colorless liquid; density: 1.051 g/ml
   CAS No.: 3380-34-5
   Chemical Structure:



2. Vehicle: Formulation: 1% (w/w) carboxymethylcellulose in a 20% aqueous glycerine suspension
   Lot Nos.: Not provided

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Source: Hazleton Research Products, Inc., Denver, PA
   Age at mating: Approximately 5-5.25 months
   Weight at GD 0: 3028-4190 g

4. Environment: Housing: Individually in stainless steel suspended cages
   Temperature: 59-76°F
   Humidity: 33-90%
   Air changes: Not provided
   Light/dark cycle: 12 hours light/12 hours dark
   Food: Purina Certified High Fiber Rabbit Chow #5325, ad libitum
   Water: Tap water, ad libitum

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B. PROCEDURES AND STUDY DESIGN

This study was conducted to assess the potential for maternal and/or developmental toxicity of the test substance, Irgacare MP (C-P Sample no. 38328), when administered by gavage to pregnant New Zealand White rabbits during the period of major fetal organogenesis.

1. In-life dates: Start: June 15, 1991; End: July 25, 1991

2. Group Assignment and Dosage Levels

Following a 40-day period of acclimation, the female rabbits were mated naturally with male rabbits (proven breeders, used solely for mating purposes, approximately 2-4 months of age) of the same strain and source. Each female was mated with two different males in the same day; mating was confirmed by visual verification of copulation. Females were assigned to the following study groups on Day 0 of gestation in a nonrandom manner which provided an equal distribution of mated females among groups and equalized, as best as possible, the gestation Day 0 mean group body weights.

Group	Dose (mg/kg/day)	Percent Concentration (w/w)	Actual Concentration [b] (mg/ml)	Dose Volume (ml/kg/day)	No. per Group
1 (Control)	0[a]	0	0	3.80	18
2 (Low)	15	0.375	3.95	3.80	19
3 (Mid)	50	1.250	13.1	3.82	18
4 (High)	150	3.750	39.2	3.83	19

a Vehicle control (1% w/w carboxymethylcellulose in a 20% aqueous glycerin suspension).
b Actual concentration = concentration (mg/g) x density of suspensions (g/ml)

3. Rationale for Dose Level Selection

In a range-finding study (Bio/dynamics study No. 91-3655; C-P study No. 91-014; MRID No. 43787101), Irgacare MP (C-P Sample No. 38828) was administered once daily by gastric intubation on gestation days 6-18 to mated female New Zealand White rabbits (5/group) at dose levels of 5, 10, 25, 50, or 75 mg/kg/day in a constant volume (2 ml/kg) of 1% carboxymethylcellulose suspension in 20% aqueous glycerin. A concurrent control group received only vehicle. Individual doses were adjusted to the most recent body weight throughout the dosing period. Mortality and clinical observations, body weight data, and food consumption data were recorded throughout the study. Cesarean sections were performed on day 30 of gestation. Gross pathological examinations were performed, and the liver and intact uteri (ovaries attached) were weighed. The uteri and ovaries were examined for the number and distribution of implantation sites, early and late resorptions, live and dead fetuses, and

corpora lutea. Each fetus was weighed, examined for external abnormalities, and sacrificed.

No treatment-related mortality occurred; the death of one female on GD 9 at 50 mg/kg/day was attributed to a dosing injury. Clinical observation data did not indicate a response to treatment. In rabbits receiving 75 mg/kg/day, there were several intervals during the treatment period when a mean body weight loss and decreased food consumption were evident, and mean weight gain over the treatment period (GD 6-19) was less than control. These findings, although slight, were considered indicative of a treatment-related response. Postmortem examination revealed a greater incidence of red foci/areas in the lungs of does treated at the 50 and 75 mg/kg/day levels, but the toxicological significance of this finding was considered equivocal. Absolute and relative (to adjusted gestation Day 30 body weight) liver weight data, were similar between control and treated groups.

Mean number of corpora lutea, litter size, number of implantations, and number of resorptions were similar between the control and treatment groups. Mean fetal weights of treated groups were similar to controls; no gross external alterations were noted in the live fetuses. At 75 mg/kg/day, one late resorption (weighing 16.4 g) with cranial/facial and abdominal closure malformations was identified; however, this finding was considered to be an isolated event and was not attributed to treatment.

Based upon the results of this range-finding study, 150 mg/kg/day was selected as a high dose for the subsequent definitive developmental toxicity study in rabbits. Low- and mid-dose levels chosen were: 15 and 50 mg/kg/day, respectively.

4. Dosage Formulation and Analysis

Previously-formulated suspensions of Irgacare MP (C-P Sample No. 38328) in 1% w/w carboxymethylcellulose in a-20% aqueous glycerine suspension were received by the performing laboratory from the study sponsor. Four batches of dosing suspensions were mixed during the study. The dosing suspensions were stored refrigerated when not being used, were resuspended prior to being dispensed for dosing, and were continuously stirred during the dosing procedure.

Analytical chemistry data were provided in a separate memo submitted to the Agency by the Registrant. Prior to the start of the study, the test substance was mixed in an aqueous solution of 1.0% carboxymethyl cellulose and 20% glycerin at concentrations of 0.10% and 3.70% w/w to approximate the low and high-dose concentrations on the study. These suspensions were maintained at room temperature for one year; they were analyzed at 2 weeks, 4 weeks, and one year. The analysis at 4 weeks showed a 9-10% degradation of the 3.70% sample, but this finding was considered to be due to improper sampling or inadequate mixing, since no evidence of degradation was observed at 2 weeks or 1 year. Additional evidence in support of this explanation is that analyses of the dosing formulations during the inlife phase of the study demonstrated no appreciable reduction in concentration.

Samples from the top and bottom of the dosing suspensions were analyzed to determine concentration and homogeneity. There was, with the exception of only a few incidences, minimal variance between the two values obtained, thus validating the adequacy of the mixing procedure. Actual concentration values were found to be slightly below nominal, but were generally within 10% of target, with only one low (0.375%) and two mid-dose (1.25%) samples that were found to be within 12-17% of target. These data confirmed that the study animals received adequate doses of test substance.

5. Dosage Administration

The test material was administered once daily to the study animals by gavage on Days 6-18 of gestation. Individual dosage volumes were adjusted throughout the dosing period, based upon the most recent body weight value. Control animals received the vehicle (1% w/w carboxymethylcellulose in a 20% aqueous glycerine suspension) in the same manner.

C. Observations

1. Maternal Observations and Evaluations

The rabbits were observed for mortality/moribundity and for signs of pharmacologic or toxicologic effects twice daily. Clinical observations were recorded on gestation days 0, 6-19 (daily, one hour after dosing), 24, and 30. Individual body weights were recorded on gestation Days 0, 6, 8, 10, 12, 14, 19, 24, and 30. Food consumption was measured on the following gestation days: 1, 3, 5-19 (daily), 24, and 29.

Surviving does were sacrificed on Day 30 of gestation by intravenous injection of sodium pentobarbital via the marginal ear vein. A gross necropsy was performed on each rabbit, with examination of the external surface, all orifices, the cranial cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities and their viscera, and the cervical tissues and organs. Liver and intact gravid uterine weights (ovaries attached) were recorded. Corpora lutea of pregnancy were counted, and uterine contents were examined for pregnancy status, number and distribution of implantation sites, early and late resorptions, and live and dead fetuses. The uteri of does that died during the study were examined for the presence of implants to determine pregnancy status. If no uterine implants were observed in does that died prematurely or were killed at study termination, the uterus was stained with ammonium sulfide for confirmation of pregnancy status. No tissues were saved.

2. Fetal Evaluations

Following removal from the uterus, each fetus was examined for external variations or malformations, weighed, and sexed externally. Following sacrifice by an intraperitoneal injection of sodium pentobarbital, fetuses were evaluated for visceral variations and malformations, using a microdissection procedure similar to that described by Staples (1974), and fetal sex was confirmed by internal inspection of the

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gonads. The fetuses were eviscerated and skinned. The eyes were examined at the time of skinning, and the internal structure of the brain was evaluated by a transverse cut through the cerebral hemispheres. All fetuses were then fixed, macerated, stained with alizarin red S (modified method of Crary, 1962), cleared, and examined for skeletal alterations.

D. Data Analysis

1. Statistical Analyses: Methods of statistical evaluation were described in report No. 91-3666, pages 45-50. Mean body weight, body weight change, food consumption, organ weight, uterine count, and fetal weight data were analyzed in the following manner: first, Bartlett's test was performed. If variances were equal, parametric procedures were used; this consisted of standard one-way ANOVA, using the F distribution to assess significance and Dunnett's test to determine significance from control. If variances were unequal, nonparametric procedures were used: Kruskal-Wallis test was used to determine the equality of means, and a ranked sum test (Dunn) was used to determine which treatments differed from control. A statistical test for trend was also performed. For parametric data, standard regression techniques with a test for trend and lack of fit were used, and for nonparametric data, Jonckheere's test for monotonic trend was used.

   Incidence data (litters with resorption sites, mortality rates, pregnancy rates, and fetal variations or malformations) were performed using contingency tables. A chi-square analysis was first performed, then each treatment group was compared to the control using a 2x2 Fisher Exact Test, corrected via the Bonferroni inequality. Thirdly, Armitage's test for linear trend was performed. The unit of measure was not indicated (i.e., the litter versus the fetus). All tests were reported at the 5% and 1% level of significance.

2. Historical Data: Historical control data (maternal and fetal) for 28 studies conducted at Bio/dynamics, Inc. during 1984-1989 were provided (study No. 91-3666, pages 322-334) and are appended as Attachment 1.

II. RESULTS

A. Maternal Toxicity

1. *Maternal Mortality and Clinical Signs:*

During the study, intubation injuries were the primary cause of mortality. The deaths or early sacrifice of one control, two Group 2, and one Group 4 rabbits were attributed to dosing injury. Only one additional death was reported, in a Group 2 female that died on gestation Day 19; the cause of death for this animal could not be determined.

A slight increase in the number of does with staining of the anogenital area during the

treatment interval (GD 6-18) was observed in all treated groups but not in controls. The number of rabbits that exhibited this finding during treatment, but not as a pretreatment condition, was: 2/19 in Group 2, 3/18 in Group 3, and 1/19 in Group 4. In spite of the fact that AG staining did not occur in the control rabbits, the lack of dose response within the treated groups suggests that the AG staining was not attributable to treatment. All other reported clinical findings were observed at similar incidences between control and treated groups.

2. Maternal Body Weight and Food Consumption Data

A summary of mean maternal body weight, body weight change and food consumption values during gestation is presented in Table 1.

Table 1. Mean (±S.D.) Gestation Body Weight, Body Weight Change, and Food Consumption Data (g)

Parameter	Dose level (mg/kg/day)			
	0	15	50	150
No. pregnant	16	15	15	16
Body weight				
GD 0	3466±303	3451±323	3365±178	3357±154
GD 6	3662±324	3637±366	3537±185	3540±203
GD 19	3866±367	3790±317	3752±255	3615±221
GD 30	4086±312	3999±291	3989±289	3910±219
Gravid Uterus	628±100	573±147	570±107	555±112
GD 30 Adjusted[a]	3458±333	3426±307	3419±243	3354±210
Body weight change				
GD 0-6	196±111	186±73	173±51	183±81
GD 6-8	2±60	-26±86	5±39	-68±52 **
GD 6-19	219±84	171±95	215±106	75±159**
GD 19-30	220±135	209±95	237±107	295±155
GD 0-30 Adjusted[b]	-189±149	-193±161	-119±129	-186±147
Food consumption				
GD 6-7	63±12	60±17	59±14	49±14*
GD 7-8	63±11	56±18	61±8	46±19**
GD 15-16	58±16	56±11	63±8	39±23*
GD 24-25	49±7	50±10	54±7	58±12*

* Significantly different from control value, $p \leq 0.05$.
** Significantly different from control value, $p \leq 0.01$.
a Day 30 body weight minus gravid uterine weight.
b Day 0-30 body weight change minus gravid uterine weight.
Note: Data were extracted from report No. 91-3666, pages 53, 58, 64, and 70-71.

Significant treatment-related decreases in mean body weight change values were noted during the period of treatment for the high-dose group (150 mg/kg/day) on gestation

days 6-8, 12-14 (not shown), and 6-19. However, when corrected for gravid uterine weight, which was reduced, although not significantly, at the 150 mg/kg/day level, the day 0-30 mean body weight gain value was not significantly different from control. The decreases in mean body weight gain during test substance administration were attributed to maternal toxicity. Maternal recovery was also observed as increased mean body weight gain during the post-treatment period (GD 19-30). These findings were consistent with the gestation food consumption data which were significantly decreased during periods of treatment (Days 6-9 and 12-16) and increased post-treatment (Days 24-25).

3. Maternal Gross Pathology and Organ Weight Data

No treatment-related lesions were identified at necropsy. Absolute liver weight values and liver-to-body weight ratios were similar among control and treated groups (report No. 91-3666, page 100).

4. Observations Noted at Cesarean Section

The results of the examination of uterine contents at cesarean section are presented in Table 2. The maternal and fetal data were similar between control and treated groups; there were no effects on preimplantation or prenatal viability, fetal body weight, litter size, or sex ratio. A significant trend in the mean number of resorptions and mean number of resorptions per implantation did not result in a significant difference in resorption incidence in any treated group compared to control, and was not judged to be treatment-related.

Table 2. Summary of Selected Cesarean Section Observations

Parameter	Dose level (mg/kg/day)			
	0	15	50	150
Number mated	18	19	18	19
Number pregnant (%)	17 (94.4)	16 (88.9)	15 (83.3)	16 (88.9)
Number of maternal deaths	1	3	0	1
Number abortions/premature births	0	0	0	0
Number of litters with live fetuses	16	15	15	16
Mean no. corpora lutea	9.7±1.6	9.2±2.1	9.4±1.4	9.6±1.2
Mean no. implantations	9.2±1.6	8.5±2.7	9.1±1.3	8.7±1.6
Mean preimplantation loss	0.049±0.078	0.087±0.167	0.034±0.060	0.093±0.114
No. viable fetuses	143	126	125	123
No. dead fetuses	0	0	4	1
Mean no. fetuses/doe (live + dead)	8.9±1.7	8.4±2.6	8.6±1.5	7.8±1.9
Mean no. male fetuses	4.3±1.6	4.2±1.6	3.6±2.1	4.3±1.7
Mean no. female fetuses	4.6±1.7	4.2±2.4	4.5±2.0	3.4±1.6
Mean no. resorptions *	0.3±0.8	0.1±0.3	0.5±0.6	0.9±1.5
Mean no. resorptions/implantation **	0.027±0.079	0.006±0.022	0.052±0.075	0.102±0.169
No. litters with resorptions (%)	2 (12.5)	1 (6.7)	6 (40.0)	7 (43.8)
Mean live fetal body weights (g)	49.74±3.47	49.30±4.61	47.50±3.05	50.44±4.88
Male fetuses	50.25±3.77	50.54±5.14	47.97±3.53	51.29±5.13
Female fetuses	49.20±3.66	48.23±4.68	47.22±3.77	49.47±5.70
Ratio of males/females	0.9	1.0	0.8	1.3

* Significant ordered response to dosage (Jonckheere's Statistic), $p \leq 0.05$.
** Significant ordered response to dosage (Jonckheere's Statistic), $p \leq 0.01$.
Note: Data were extracted from report No. 91-3666, page 80.

B. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are summarized in Tables 3 (malformations) and 4 (visceral and skeletal variations). The litter and fetal incidences of the various malformations and variations observed were comparable between treated and control groups, and generally within the incidence rates observed historically in the performing laboratory. The types of malformations observed (Table 3) were varied and did not suggest a response to treatment.

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Table 3. Summary of Malformations

Observation		Dose (mg/kg/day) 0	15	50	150
EXTERNAL					
No. fetuses examined		143(16)	126(15)	129(15)	124(16)
Total external malformations	Fetus N(%)	2(1.4)	0	1(0.8)	1(0.8)
	Litter N(%)	2(12.5)	0	1(6.7)	1(6.3)
TONGUE - Protruding	Fetus N(%)	0	0	1(0.8)	1(0.8)
	Litter N(%)	0	0	1(6.7)	1(6.3)
EXTERNAL NARES - Absent	Fetus N(%)	0	0	1(0.8)	0
	Litter N(%)	0	0	1(6.7)	0
SNOUT - Shortened, asymmetric	Fetus N(%)	0	0	1(0.8)	0
	Litter N(%)	0	0	1(6.7)	0
CRANIUM - Domed	Fetus N(%)	1(0.7)	0	0	0
	Litter N(%)	1(6.3)	0	0	0
FOREPAWS - Ectrodactyly: (digit 1 agenesis, bilateral)	Fetus N(%)	1(0.7)	0	0	0
	Litter N(%)	1(6.3)	0	0	0
FORELIMBS - Shortened	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
HINDLIMBS - Abnormal flexure	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
VISCERAL					
No. fetuses examined		143(16)	126(15)	125(15)	124(16)
Total visceral malformations	Fetus N(%)	1(0.7)	1(0.8)	2(1.6)	1(0.8)
	Litter N(%)	1(6.3)	1(6.7)	1(6.7)	1(6.3)
BRAIN - Lateral ventricles distended	Fetus N(%)	1(0.7)	0	1(0.8)	0
	Litter N(%)	1(6.3)	0	1(6.7)	0
AORTIC ARCH - Defect	Fetus N(%)	0	0	2(1.6)	0
	Litter N(%)	0	0	1(6.7)	0
HEART - Small atrium(a)	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
HEART - Interventricular septal defect	Fetus N(%)	0	0	2(1.6)	0
	Litter N(%)	0	0	1(6.7)	0
KIDNEYS - Small	Fetus N(%)	0	1(0.8)	0	0
	Litter N(%)	0	1(6.7)	0	0

Note: Data were extracted from report No. 91-3666, pages 190 and 206.

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Table 3. Summary of Malformations - continued

Observation		Dose (mg/kg/day) 0	15	50	150
SKELETAL (selected)					
No. fetuses examined		143(16)	126(15)	129(15)	124(16)
Total skeletal malformations	Fetus N(%)	12(8.4)	4(3.2)	11(8.5)	6(4.8)
	Litter N(%)	9(56.3)	3(20.0)	7(46.7)	5(31.3)
HYOID ARCH(ES) - Angulated	Fetus N(%)	8(5.6)	3(2.4)	9(7.0)	6(4.8)
	Litter N(%)	6(37.5)	3(20.0)	6(40.0)	5(31.3)
MANDIBLE - Short, misshapen	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
HEAD - Multiple cranial malformations	Fetus N(%)	0	0	1(0.8)	0
	Litter N(%)	0	0	1(6.7)	0
RIBS - Wavy	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
RIBS - Fused	Fetus N(%)	0	0	1(0.8)	0
	Litter N(%)	0	0	1(6.7)	0
FOREPAW - Absence of digital ossifications	Fetus N(%)	1(0.7)	0	0	0
	Litter N(%)	1(6.3)	0	0	0
THORACIC GIRDLE - Scapula/ scapular spine/clavicle bent	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
FORELIMBS - Radius, ulna, and and humerus bent	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
HINDLIMB - Tibia, fibula, and femur bent	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)

Note: Data were extracted from report No. 91-3666, pages 225-228.

No external variations were observed. Visceral variations (Table 4) included only a dark red iris in one high-dose fetus, a small gall bladder in one mid-dose fetus, and additional subclavian arteries (arteries branching off the aortic arch at the level of the left subclavian) in fetuses of all treatment groups, at a similar fetal and litter incidence as control. The overall incidence of skeletal variations was comparable among treated and control groups (Table 4). The majority of the skeletal variations observed appeared to represent reversible delays in ossification. Reductions in ossification were noted for the hyoid, various cranial and facial bones, sternebrae, and metacarpals or phalanges; the incidences are not included in the summary table because they are minimal and not related to dose. The incidence of 13th ribs (rudimentary, unilateral, short, or floating) was comparable among groups, and few vertebral findings were reported.

Table 4. Summary of Selected Visceral and Skeletal Variations

Observation		Dose (mg/kg/day) 0	15	50	150
VISCERAL					
No. fetuses examined		143(16)	126(15)	129(15)	124(16)
Total visceral variations	Fetus N(%)	5(3.5)	6(4.8)	5(3.9)	13(10.5)
	Litter N(%)	4(25.0)	5(33.3)	4(26.7)	6(37.5)
IRIS - Dark red	Fetus N(%)	0	0	0	1(0.8)
	Litter N(%)	0	0	0	1(6.3)
MAJOR VESSELS - Presence of additional subclavian arter(ies)	Fetus N(%)	5(3.5)	6(4.8)	4(3.1)	12(9.7)
	Litter N(%)	4(25.0)	5(33.3)	4(26.7)	5(31.3)
GALL BLADDER - Small	Fetus N(%)	0	0	1(0.8)	0
	Litter N(%)	0	0	1(6.7)	0
SKELETAL					
No. fetuses examined		143(16)	126(15)	129(15)	124(16)
Total skeletal variations	Fetus N(%)	114(79.7)	96(76.2)	97(75.2)	91(73.4)
	Litter N(%)	16(100)	15(100)	15(100)	16(100)
PHALANGES - Forelimb, mid-phalanges incompletely ossified	Fetus N(%)	12(8.4)	11(8.7)	14(10.9)	18(14.5)
	Litter N(%)	6(37.5)	5(33.3)	9(60.0)	9(56.3)

Note: Data were extracted from report No. 91-3666, pages 207 and 229-234.

III. Deviations

The temperature and humidity of the animal room were occasionally higher than desired ranges:

Temperature: Desired = 60-70°F; actual = 59-76°F

Humidity: Desired = 40-60%; actual = 33-90%

These deviations were not considered to compromise the integrity of the study, because the high temperature and humidity values were attained infrequently and since the animals did not appear to suffered any ill effects from these conditions.

IV. Discussion/Conclusions

Following oral administration of the test substance, Irgacare MP (C-P Sample No.: 38328), to pregnant rabbits on days 6-18 of gestation, 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.

Maternal LOEL = 150 mg/kg/day
(based upon decreased body weight gain and food consumption during treatment)

(12)

3-10-1998 HIAR Briefing Package

Page _____ is not included in this copy.

Pages 175 through 187 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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DER #6 Developmental Toxicity in Rabbits MRID # 43022607

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

011304

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

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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.

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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.

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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)

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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*	-
Interventricular Septal Defect	-	-	2/1*	-
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

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

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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

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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.

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DER #7 Subcronic Dermal Toxcity in Rats MRID # 43328001

C11417

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

FEB 23 1995

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Irgasan: Review of a 14-day dermal dose rangefinding study and a 90-day dermal toxicity study in rats

Caswell No. 168A **DP Barcode.** D206560
MRID No. 432519-01: 14-day dermal dose rangefinding study
433280-01: 90-day dermal toxicity study
Chemical No. 054901

TO: Bonnie Adler/Kathryn Davis, PM Team 52
Special Review and Registration Division (7508W)

FROM: Whang Phang, Ph.D. 2/16/95
Pharmacologist
Section III/Tox. Branch II / HED (7509C)

THROUGH: James Rowe, Ph.D. 2/17/95
Section Head
and
Marcia van Gemert, Ph.D. 2/21/95
Branch Chief
Tox. Branch II/HED (7509C)

The registrant, Ciba-Geigy, submitted a 14-day dermal dose rangefinding study and a 90-day dermal toxicity in rats. The 14-day dose rangefinding study was submitted earlier, and it was decided to reviewed the two studies together because the 14-day dermal dose rangefinding study provided information for selecting the doses employed in the 90-day study. These two studies have been reviewed, and the evaluations for them are presented in a single Data Evaluation Report (DER) which is attached. The citation and the conclusion of each study are presented below:

Citation:

Trimmer, G.W. (1993) 14-Day dermal rangefinding study in the rat with Irgasan DP300 (MRID-92-399). Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910A. Sept 3, 1993. Submitted to EPA by Ciba Geigy Corp.; EPA MRID No. 432519-01.

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Trimmer, G.W. (1994) 90-Day subchronic dermal toxicity study in the rat with satellite group with Irgasan DP300 (MRID-92-399). Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910B. July 14, 1994. Submitted to EPA by Ciba Geigy Corp.; EPA MRID No. 433280-01.

Conclusion: **14-day dose rangefinding study:** Groups of rats (2/sex/group) received Irgasan in propylene glycol (PPG) at dose levels of 0, 10, 25, 50, 100, and 200 mg/kg or in Drakeol at dose levels of 0, 25, and 200 mg/kg. The test animals were exposed to the test article for 6 hrs. Skin irritation was seen in test animals which received 25 mg/kg or above, and that in 100 and 200 mg/kg groups was more marked. No dermal irritation was seen in the 10 mg/kg group. There was no systemic toxicity in any test groups. Based on these results doses of 10, 40, and 80 mg/kg were selected for the 90-day dermal toxicity study.

90-day dermal toxicity study: Groups of rats (10/sex/group) received Irgasan in PPG by dermal application at dose levels of 10, 40, and 80 mg/kg for 6 hrs/day for 90 days. Dermal irritation at the application site was found in all dose groups, and the severity of the dermal irritation was dose-related. However, the dermal irritation was reversible after a certain recovery period.

An increase in the incidence of occult blood in the urine of 80 mg/kg males and females was found. No additional systemic toxicity was seen. Under the conditions of this study, the LEL for the systemic toxicity was 80 mg/kg; NOEL, 40 mg/kg.

The **90-dermal toxicity study** meets the data requirements for a subchronic dermal toxicity study (82-3), and this study is classified as **minimum.** The **14-day dermal dose rangefinding study** provided a rationale for the dose selection for the 90-day dermal toxicity study, and it was considered as **supplementary.**

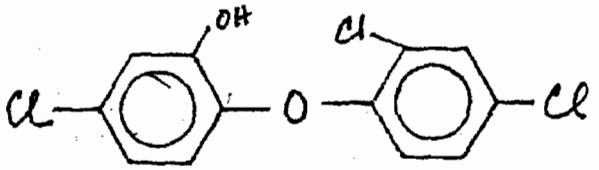
Reviewer: Whang Phang, Ph.D.
Tox. Branch II (7509C) Whang Phang 2/16/95

Secondary Reviewer: James Rowe, Ph.D.
Tox. Branch II (7509C) James N. Rowe 2/17/95

DATA EVALUATION REPORT

Study Type: 14-day dermal dose rangefinding study in rats and 90-day dermal toxicity study in rats

Chemical: 2,4,4'-trichloro-2'-hydroxy-diphenyl ether; Irgasan® DP 300; FAT80'023/Q



Caswell No. 168A **DP Barcode.** D206560
MRID No. 432519-01: 14-day dermal dose rangefinding study
433280-01: 90-day dermal toxicity study
Chemical No. 054901

Sponsor: Ciba Geigy Ltd.

Testing Facility: Exxon Biomedical Sciences Inc.
Toxicology Laboratory
Mettlers Rd.; CN2350
East Millstone, NJ 08875-2350

Citation: Trimmer, G.W. (1993) 14-Day dermal rangefinding study in the rat with Irgasan DP300 (MRID-92-399). Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910A. Sept 3, 1993. Submitted to EPA by Ciba Geigy Corp.; EPA MRID No. 432519-01.

Trimmer, G.W. (1994) 90-Day subchronic dermal toxicity study in the rat with satellite group with Irgasan DP300 (MRID-92-399). Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910B. July 14, 1994. Submitted to EPA by Ciba Geigy Corp.; EPA MRID No. 433280-01.

Conclusion: **14-day dose rangefinding study:** Groups of rats (2/sex/group) received Irgasan in propylene glycol (PPG) at dose levels of 0, 10, 25, 50, 100, and 200 mg/kg or in Drakeol at dose levels of 0, 25, and 200 mg/kg. The test animals were exposed to the test article for 6 hrs. Skin irritation was seen in test animals which received 25 mg/kg or above, and that in 100 and 200 mg/kg groups was more marked. No dermal

irritation was seen in the 10 mg/kg group. There was no systemic toxicity in any test groups. Based on these results doses of 10, 40, and 80 mg/kg were selected for the 90-day dermal toxicity study.

90-day dermal toxicity study: Groups of rats (10/sex/group) received Irgasan in PPG by dermal application at dose levels of 10, 40, and 80 mg/kg for 6 hrs/day for 90 days. Dermal irritation at the application site was found in all dose groups, and the severity of the dermal irritation was dose-related. However, the dermal irritation was reversible after a certain recovery period.

An increase in the incidence of occult blood in the urine of 80 mg/kg males and females was found. No additional systemic toxicity was seen. Under the conditions of this study, the LEL for the systemic toxicity was 80 mg/kg; NOEL, 40 mg/kg.

The **90-dermal toxicity study** meets the data requirements for a subchronic dermal toxicity study (82-3), and this study is classified as **minimum**. The **14-day dermal dose rangefinding study** provided a rationale for the dose selection for the 90-day dermal toxicity study, and it was considered as **supplementary.**

Methods and Materials

Test Article: Irgasan DP300; off white powder with batch No. 5.2.0211.0 which is the same for both 14-day and 90-day dermal toxicity studies. The purity of the test material was 99.7%.

Propylene glycol (PPG) was explored as a vehicle in the 14-day dermal dose rangefinding study, and it was selected as a vehicle for the 90-day dermal toxicity study. PPG was obtained from J.T. Baker Company, Phillipsburg, NJ. The Batch No. was C40638.

Drakeol 19 was also explored as a possible vehicle in the 14-day dermal dose rangefinding study. This chemical was obtained from Penreco, Karns City, PA. The Lot No. was 71-90.

Test Animals: Approximately 7-8 weeks old, Crl:CDBR (VAF/Plus) rats were obtained from Charles River Laboratories, Inc., Kingston, NY. These rats weighed ≈246-287 gm for males and 195-242 gm for females. They were acclimated to the laboratory environment for 11 days and were fed Purina Certified Rat Chow (mash), ad libitum.

The same strain of rats were used for both the 14-day dermal dose rangefinding and the 90-day dermal toxicity studies.

Study Design: The day before the initiation of the study, the dorsal surface (from the shoulder to the lumbar region) of each test animal was clipped (≈10% of the body surface). The skin was left intact. Subsequently, the animals were clipped each week or as needed.

14-Day Dermal Dose Rangefinding Study: For this study, the test chemical was mixed in the PPG or Drakeol at the concentrations presented in Table 1A. Table 1A also showed that 5 Groups of rats (2/sex/dose) received the test chemical in PPG at concentrations ranging from 0.5 to 10% (w/v) or (10 to 200 mg/kg). Two groups received the test chemical in Drakeol at concentrations of 1.25 and 10 %(w/v) or (25 and 200 mg/kg). A PPG and a Drakeol control group were also included in the study. The test solution was prepared weekly and stirred for 20 to 30 minutes prior to dermal application. The test material was applied to the shaved back of each test animal under a porous gauze dressing which was then secured to the animal with non-irritating tape. The applied volume was 2.0 ml/kg bwt. To avoid evaporation and ingestion by the test animal, the application site was wrapped with COBAN. The test animals were treated for at least 6 hours/day. After approximately 6 hrs, the application site was rinsed with purified water and dried with a paper towel.

The animals were observed for clinical signs and viability twice daily. The dermal response on the application sites were evaluated on days 0, 1, 3, 7, and 10, and scored according to the Draize Method of Scoring (Appendix A).

Body weights were measured prior to the initiation of the study and on days 0, 3, 7, and 14. Food consumption was measured weekly during the study.

All test animals were sacrificed on day 14, and gross examination was conducted on all animals sacrificed or those that died spontaneously.

90-Day Dermal Toxicity Study: For the subchronic dermal toxicity study, the test material was dissolved in PPG. The dose levels, concentrations, and the number of test animals per group are presented in Table 1B. In this study, 10 rats/sex/dose received Irgasan at doses of 0, 10, 40, and 80 mg/kg with dermal application. A satellite group or recovery group, which received the test material at 80 mg/kg was also included. For this group, the treated was terminated at 90 days and observed for an additonal 28 days. The dose levels and the vehicle for administration were selected based on the results of the 14-Day Dermal Dose Rangefinding Study (see

Results). The entire procedures for test material application and removal for this study were similar to those just described for the 14-Day Dermal Dose Rangefinding Study.

The test animals were observed daily during weekdays and once daily during weekends for clinical signs and viability. The dermal response on the application sites were evaluated on days 0, 1, and 4 and twice weekly thereafter according to the Draize Method of Scoring (Appendix A).

Body weights were measured prior to the initiation of the study, on day 0, and weekly thereafter. Food consumption was also measured weekly during the treatment period.

Prior to the beginning of the study and on the week prior to the termination of the study, ophthalmological examination was performed on all test animals.

At the termination of the study, blood samples were collected from each test animal which was fasted overnight prior to blood collection. The following hematological and clinical chemistry parameters were analyzed from the samples collected:

Hematological parameters	
leukocyte counts (total and differential)	erythrocyte counts
hemoglobin	hematocrit
Mean corp. volume	mean corp. hemoglobin
concentration	platelet counts
prothrombin time	activated partial thromboplastin time

Serum Clinical chemistry parameters	
sodium	potassium
chloride	calcium
inorganic phosphorus	alkaline phosphatase
total bilirubin	cholesterol
aspartate aminotransferase (AST)	alanine aminotransferase (ALT)
creatine phosphokinase (CPK)	urea nitrogen
creatinine	total protein
albumin	glucose
triglycerides	CO_2

Urinalysis: The urine samples were collected during the fasting period prior to study termination. The following parameters were analyzed:

semi-quantitative parameters	quantitative parameters
Color & appearance	volume
microscopic elements	specific gravity
Ph	protein
ketones	glucose

occult blood
bilirubin
urobilinogen

Gross examination: All test animals received a postmortem gross examination. Representative tissue samples were collected from each animal and fixed in 10% neutral-buffered formalin where appropriate. The following organs were weighed:

liver	testes
brain	ovaries
kidneys	

Histology: The following tissues were processed, stained, (hematoxylin and eosin) and examined microscopically:

adrenals	lymph nodes (mesenteric)
aorta	lungs with bronchi
sternum with marrows	mammary glands
brain	pancreas
eye	pituitary
esophagus	stomach
duodenum	urinary bladder
jejunum	ileum
cecum	colon
rectum	prostate & seminal vesicles
salivary glands	sciatic nerve
ovaries	testis with epididymis
skin	spinal cord
spleen	uterus (corpus, cervix)
thymus	thyroid and parathyroid
heart	trachea
kidneys	liver

The methods for statistical analyses for the results of this study are presented in Appendix B.

Signed statements of quality assurance, GLP, and no claims of confidentiality are included in both reports.

RESULTS

14-Day Dermal Dose Rangefinding study

No compound-related clinical signs were observed in any of the treated rats. One Group 4 (50 mg/kg) female died on day 11, and another Group 6 (200 mg/kg) died on day 10. These two deaths were not apparently related to treatment.

The body weights of Groups 5 and 6 male rats were slightly decreased, but the drop was not significantly different from that of the control. A slight decrease was also seen in Group 9 males. In females, the body weights of compound-treated animals were comparable to those of the controls (Table 2).

In general, food consumption data were comparable between the treated and the control rats.

Dermal examinations showed that with repeated dermal applications of the test material in PPG, an increase in the incidence of erythema was seen in 25, 50, 100, and 200 mg/kg groups. An increase in the incidence of erythema was also seen in 25 and 200 mg/kg groups with Drakeol as a vehicle (Table 3). The increase in the incidence of edema was smaller than that of erythema, and it was seen in 200 mg/kg group with Drakeol as vehicle and in 100 mg/kg group with PPG as vehicle. No incidence of erythema or edema was seen in the controls or the 10 mg/kg groups with PPG as vehicle. The incidence of skin irritation was more prominent in 100 and 200 mg/kg groups (Table 3).

Supplemental dermal observation data indicated that an increase in the incidence of eschar, desquamation, and pinpoint eschar was reported in test animals which received Irgasan in PPG at dose levels of 25 mg/kg or above or in Drakeol of 200 mg/kg groups (Table 4).

The gross examination data did not show a significant increase in any compound-related effects (Table 5).

The test material was soluble in PPG, but it formed a suspension in Drakeol. For the 90-day dermal toxicity study, PPG was selected as the vehicle, and doses of 10, 40, and 80 mg/kg were chosen.

90-Day Dermal Toxicity Study

1. Clinical observations: Treatment-related clinical signs were not observed.

2. Mortality: There were five deaths during the study from five different groups including the control (1 death/group). No compound related death was found.

3. Dermal toxicity: An increase in the incidence of erythema at the application site was found in all treatment groups of male and female rats including the satellite group (Tables 6 & 7). The increase was more marked in the 40 and 80 mg/kg groups. An increase in the incidence of edema was mostly seen in the 40 and 80 mg/kg groups at various periods of the study.

One rat in the 10 mg/kg group was reported to have edema towards the end of the study (Table 6).

The supplemental dermal observations also indicated that an increase in the incidence of desquamation, eschar, and exfoliation of the skin in 10 mg/kg females and 40 and 80 mg/kg males and females (Table 8). In addition, in 40 and 80 mg/kg male and female rats, an increase in the incidence of atonia of the application site was also reported. The vehicle control (PPG) did not induce skin irritation (Table 8).

In the satellite group, an increase in the incidence of desquamation, eschar, exfoliation, and atonia was also seen. Approximately 20 days after cessation of the treatment, the skin toxicity recovered in almost all of animals of the satellite group (Table 9).

4. Body weights and food consumption: The body weights of the treated and the control animals were comparable (Table 10). Irgasan did not affect food consumption in the treated animals.

5. Hematology: Tht levels of hemoglobin and hematocrit were significantly decreased ($p<0.05$) in the 80 mg/kg males (Table 1). There were other sporadic changes in certain hematological parameters in some treated groups, but the changes were slight and not dose-related (Table 11).

6. Clinical chemistry: There were slight and sporadic changes in some clinical chemistry parameters, and some changes even showed a statistical difference from the control. Most of the changes were not dose-related and could not be considered as compound related effects. However, Irgasan significantly depressed triglyceride levels in 80 mg/kg males ($p\leq0.05$); a decrease in this level was also found in 40 mg/kg males. The effects of Irgasan on the triglyceride level in treated male rats also showed a dose-related effect (Table 12). A slight decrease in cholesterol level was seen in 80 mg/kg males and females. When the decrease of triglyceride and cholesterol were considered together, the reduction in the triglyceride level appeared to be a compound-related effect in 80 mg/kg male rats. However Irgasan did not affect any serum chemistry levels in the satellite group (Table 13).

7. Ophthalmological examination: Compound-related eye effects were not found in any test groups.

8. Urinalysis: The individual animal data indicated an increase in the incidence of occult blood in the urine in 80 mg/kg males of regular and the satellite groups (regular 80 mg/kg, 2/9; satellite 80 mg/kg, 3/9; Control, 0/10). In addition 1 female rat in the 80 mg/kg satellite group also had

occult blood in the urine. The report did not summarize the incidence of occult blood. This set of data should be tabulated and explored more fully in terms of whether or not it was a compound-related effect. No other compound-related effects were seen in any other groups of the treated animals.

9. Gross examination: Besides the dermal irritation findings in the application sites, no additional treatment-related gross finding was reported.

10. Organ weights: Organ weights among various test groups were comparable (Table 14).

11. Histopathology: Compound-related histological changes were not seen in other organs or tissues examined except the skin application sites, where inflammation, hyperplasia, exudate, necrosis, and hyperkeratosis in Irgasan treated animals. The severity of these findings in the application sites showed a dose-related effect. The incidence of necrosis and inflammation was dramatically decreased in the satellite group (Table 15).

Discussion

In a **14-day dose rangefinding study** groups of rats (2/sex/group) received Irgasan in PPG at dose levels of 0, 10, 25, 50, 100, and 200 mg/kg or in Drakeol at dose levels of 0, 25, and 200 mg/kg. The test animals were exposed to the test article for 6 hrs. Skin erythema was seen in test animals which received 25 mg/kg or above, and that in 100 and 200 mg/kg groups was more marked. During gross examination, increased incidence of eschar and desquamation was also found in animals which received dose levels of 25 mg/kg or above. There was no systemic toxicity. No dermal irritation was seen in the 10 mg/kg group. Based on these results doses of 10, 40, and 80 mg/kg were selected for the 90-day dermal toxicity study. **Considering the dermal toxicity induced by this chemical at 100 and 200 mg/kg, the doses selected for the 90-day were adequate for studying the subchronic dermal toxicity of this chemical.**

For the **90-day dermal toxicity study**, groups of rats (10/sex/group) received Irgasan in PPG by dermal application at dose levels of 10, 40, and 80 mg/kg for 6 hrs/day for 90 days. Dermal irritation at the application sites characterized by erythema, eschar, desquamation, and exfoliation at the application site was found in all dose groups. The severity of dermal irritation also showed a dose-related effect. Dermal irritation was reversible at approximately 20 days after termination of treatment.

There were decreases in the levels of hematocrit, hemoglobin, and triglyceride in 80 mg/kg males, but these decreases were not enough to affect the general health of this group of test animals. In addition, the reduction in the above levels was probably related to the severe skin irritation. An increase in the incidence of occult blood in the urine of 80 mg/kg males in the regular group and in males and females of the satellite group was also found. There was no histological finding in the urinary system to elucidate from where the blood might have originated. However, the finding of occult blood in urine was rare. It should be considered as a treatment-related effect. Therefore, under the conditions of this study, the LEL for the systemic toxicity was 80 mg/kg; NOEL, 40 mg/kg.

- no biochem. treatment effect on the red cell

The **90-dermal toxicity study** meets the data requirements for a subchronic dermal toxicity study (82-3), and this study is classified as **minimum.** The **14-day dermal dose rangefinding study** provided a rationale for the dose selection for the 90-day dermal toxicity study, and it was considered as **supplementary.**

3-10-1998 HIAR Briefing Package

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DER #8 Subcronic Oral Toxcity in Dogs MRID # 001968

Executive Summary for 90-Day Toxcity Study in Dogs (HED document # 001968)

In a subchronic oral toxicity study in dogs (HED document # 001968) Irgasan DP-300 [Triclosan] was administered in gelatin capsules to grops of 4 male and 4 female beagle dogs at doses of 0, 12.5, 25, 50, and 100 mg/kg seven days per week for 13 weks. An additional test group of 2 male and 2 female dogs were given the test material at 50 mg/kg/day for 13 weks and allowed a four wek recovery period prior to termination. Body weight, clinical observations, hematology, clinical chemistry, urinalysis, and gross and microscopic histopathology were investigated in all dogs under study. Body weight gain in females at 12.5 mg/kg/day was significantly lower in relation to untreated controls, but body weight decrements were not observed at higher doses in either sex. One male dog at 100 mg/kg/day was sacrificed in extremis after 26 days on test, while another male dog at 100 mg/kg/day was found dead on day 23. One female dog at 50 mg/kg/day was sacrificed in extremis after 57 ays on test. Increased serum alkaline phosphatase activity was observed at 50 and 100 mg/kg/day in male and female dogs, while those dogs in the recovery group showed that elevation of alkalion phosphatase was a reversible effect. Histopathologic examination of tissues from those dogs sacrificed or that died showed hepatoxicity resulting in obstructive jaundice. Changes in liver histopathology were observed in dogs receiving test material at the 25, 50, and 100 mg/kg/day dose levels which consisted of focal acidophilic to granular degeneration of cytoplasm of a few individual or small groups of hepatocytes. Based on the results of this study, the systemic NOEL is 12.5 mg/kg/day, and the Systemic LOEL is 25 mg/kg/day, based on histopathologic changes in the liver of treated dogs.

Registration: 9687-RR

Product: Irgasan DP-300

Subject: 90 day subacute oral toxicity study with Irgasan DP-300 in Beagle Dogs.

Conclusion: On the basis of this study NEL = 12.5 mg/kg

Test Organization:

Group	No. of Animals Male	Female	Dose Level (mg/kg/day)
Control	4	4	None
T-I	4	4	12.5
T-II	4	4	25
T-III	4	4	50
T-IV	4	4	100
T-V*	2	2	50

*Allowed a four week recovery period (off test) after termination of the 90-day investigation.

Dosage administered orally via gelatin capsule seven days per week.

Parameters: The following determinations were conducted upon each dog from the untreated control group and five test groups just prior to the inception of the study and after 42 and 85 days of testing.

significance - will be covered by computation for standard deviation or primary hypothesis tested?

Hematologic Studies

total leukocyte count
erythrocyte count
hemoglobin
hematocrit
differential leukocyte count

Blood Chemistry Studies

blood urea nitrogen
serum alkaline phosphatase
SGOT
serum glucose
serum bilirubin
SGPT

Urine Analyses

albumin
pH
erythrocytes
glucose
specific gravity
crystals
bilirubin
leukocytes

At the conclusion of 90 days of testing, the dogs from the untreated control group and Test Groups I, II, III and IV were sacrificed by electric shock. All major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland, pituitary gland.

The following tissues and organs excised from these animals were examined histologically.

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lungs
Lymph Nodes (cervical, mesenteric)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (Submaxillary)
Small Intestin
Spinal Cord
Spleen
Stomach
Trachea
Thyroid Gland
Uterus
Urinary Bladder
Muscle (skeletal)

Animals in Test Group V were taken off test after 90 days and allowed a four-week recovery period, after which time they were sacrificed and examined according to procedures described above.

Results: The mean overall body weight gain for females receiving 12.5 mg/kg Irgasan DP-300 was significantly lower than that for untreated control females. However, mean gains seen at higher dose levels showed no significant deviation from untreated controls.

One male receiving 100 mg/kg died after 23 days on test; another 100 mg/kg male was sacrificed in extremis after 26 days. One female receiving 50 mg/kg was sacrificed in extremis after 57 days - Each of the three animals that died or was sacrificed during the study displayed weight loss, anorexia, lethargy and symptoms of jaundice (a distinct yellow cast to ocular and oral mucous membranes) three to five days prior to death. No abnormal reactions were noted among any of the other test animals during the study.

Increases in serum alkaline phosphatase activity were noted among animals receiving 100 mg/kg Irgasan DP-300 similar elevations were noted in the two groups (T-III, T-V) dosed with 50 mg/kg. Serum alkaline phosphatase activities among dogs at lower levels (25 or 12.5 mg/kg) were comparable to the untreated control. Animals in test group V (50 mg/kg) were allowed a four-week withdrawal period (off test) after which time formerly elevated serum alkaline phosphatase activities returned to normal.

With respect to the other blood chemistry studies, data revealed no significant abnormalities at any of the levels tested.

Histopathologic examination of tissues from the three animals that died or were sacrificed in extremis during the study, revealed that death was attributed to hepatotoxicity which resulted in abstructive jaundice. No table lesions in other major organs (kidneys, spleen) were also seen and are considered to be related to the systemic effects of the hepatotoxicity and inanition.

001968

Histopathologic examination of tissues derived from animals surviving the 90-day test period revealed treatment related morphologic changes in the livers of most animals at the 25, 50, 100 mg/kg dose levels. Changes consisted of focal acidophilic to granular degeneration of the cytoplasm of a few individual hepatocytes or small numbers of adjacent hepatocytes. Histopathologic examination of tissues from the animals dosed with 12.5 mg/kg and the four 50 mg/kg dogs allowed a four-week recovery period (off test) revealed no abnormalities attributable to the ingestion of the test material.

Blood Levels: 200 mg/kg Dog - single dose oral
Blood Drawn 0, 1, 2, 4, 8, 24, 48, 72 hours
Blood Level Max. 0.322 ppm (free) at 4 hours - 72 hours .023 ppm

100 mg/kg - Single dose (oral) Max. conc. 50 ppm total
0.50 ppm Free - Reached 7 hours

Compound forms B-glucuronide or sulfate fairly rapidly.

Excretion complete in approx. 5 days.

Excretion major route feces to a lesser extent urine. Smaller animals relative more excretion takes place in the urine.

Tissue distribution after 50 mg/day for 6 days

Brain = 0.17 ppm
Liver = 12.6 ppm
Kidneys = 6.37 ppm
Muscle = 0.80 ppm
Fat = 2.06 ppm

90 day Beagle study
Blood Levels after 90 days:

Dose Levels	Free	Total
12.5 mg/kg/day	.111 ppm	36.1 ppm
25.0 mg/kg/day	.218 ppm	67.4 ppm
50.0 mg/kg/day	.350 ppm	87.7 ppm
100.0 mg/kg/day	.489 ppm	107.4 ppm

DER #9 Subcronic Oral Toxcity in mice MRID # 43022605

011304

DATA EVALUATION REPORT

IRGACARE

Study Type: Subchronic Oral Toxicity in Mice

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer: Pia Lindstrom — Date: 4/19/94
Pia Lindstrom, D.P.H.

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

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

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

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[a]

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

[a]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. MRA 483-287, Table 3A.

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

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*

[a]Data extracted from Study No. MMA 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.)[a]

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

[a]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	Globulins
	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 electrophoresis
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)	16.0 ± 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*	33.9 ± 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 buffered 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)	2.61 ± 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. MMA 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 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. MRA 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]

Observation	Dose level (mg/kg/day) 0	25	75	200	350	750	900
Spleen							
Extramedullary hematopoiesis, increased	7/15 (1.4)	---[d]	8/10 (1.4)	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	4/15 (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. MRL-[illegible], Tables 14 A and 14 B and page 51
[b]Includes animals found dead and [illegible] sacrifice
[c]Within parentheses, mean severity grade (based on number of organs in which change was observed)
[d]Not examined

E. STUDY DEFICIENCIES

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.

EPA Reviewer: Timothy F. McMahon, Ph.D. Date: 1/7/98
Senior Scientist, RASSB/AD (7510W)
EPA Secondary Reviewer: James O. Pennings, Ph.D. Date 1/7/98
Pharmacologist, HED/TOX I

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity (feeding) - mouse (Non-guideline)

DP Barcode: 240164 Submission: S532102
P.C. Code: 054901 MRID: 44389707

TEST MATERIAL: FAT 80023 (TRICLOSAN); purity: > 99%

CITATION: Thevenaz, Dr. phil. II ph. (1987): Final Report: FAT 80023: 28-Day Toxicity Study in Mice (Administration in Feed) with Special Reference to Histopathology. Ciba-Geigy Ltd., Basle, Switzerland. Laboratory Project # 864005. MRID # 44389707. Unpublished.

SPONSOR: CIBA-GEIGY LTD.

EXECUTIVE SUMMARY: In a twenty-eight day toxicity study, a total of 40 mice (MAGf [SPF], 5/sex/dose) received technical triclosan admixed into pelleted feed at dose levels of 0, 50, and 1000 ppm (6.48 and 135.59 mg/kg/day in males, 8.25 and 168.78 mg/kg/day in females) for 4 weeks. Five males and 5 females were given the high dose and allowed to "recover" (feeding of non-treated feed) for 2 weeks. There were no reported effects on mortality, body weight, or food consumption. Hematological effects were observed at the high dose (135.59 mg/kg/day in males; 168.78 mg/kg/day in females), and included significant decreases in erythrocytes, hemoglobin, and hematocrit in males, and a significant decrease in hemoglobin in females. Both sexes showed significant increases in thrombocytes at this dose, the effects of which were not fully reversible after two weeks recovery. Clinical chemistry alterations (significant increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase; significant decrease in glubulin fraction) were observed at the high dose in male and female mice. Elevated serum enzyme activities were evident after the two week recovery period. Absolute weight of the liver and liver/body weight ratio were significantly increased at the high dose in male and female mice. Histopathological examination of the liver showed an increased incidence of liver cell necrosis (as single cells or small cell groups), hemosiderosis of Kupffer cells in the vicinity, cytoplasmic vacuoles in hepatocytes, and liver cell hypertrophy. The presence of necrosis was still evident (2/5 males and 3/5 females) after the recovery period.
Based on the biochemical and morphological effects of Irgasan treatment on the liver of male and female mice, a systemic LOEL of 135.59 mg/kg/day for males and 168.78 mg/kg/day is assigned. The

systemic NOEL is considered to be 6.48 mg/kg/day in males, and 8.25 mg/kg/day in females. This study is classified as **acceptable** and provides relevant toxicologic data on the effects of Irgasan treatment to the liver of male and female mice. This study was not conducted to fulfill a specific guideline requirement, but provides useful data for the risk assessment of Irgasan.

COMPLIANCE: Signed and dated statements of GLP, Quality Assurance, and Data Confidentiality were **not** provided. This study was conducted according to the OECD guideline No. 407, "repeated dose oral toxicity - rodent: 28-day or 14-day study."

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: FAT 80023 (Triclosan)
   Description: faintly yellowish crystalline powder.
   Lot/Batch No. P.4-11-210
   Purity: > 99%
   Stability: there was no stability analysis performed in this study.

2. Vehicle: pelleted, certified standard diet Nafag 890 Tox.

3. Test Animals: Species: Albino mouse
   Strain: Tif: MAGf (SPF), hybrids of NIH x MIG
   Age: 5-6 weeks old at delivery
   Weight: males, 28-32 g; females, 23-27 g at first weighing session.
   Source: Animal Production, Ciba-Geigy Ltd., Switzerland
   Housing: Individually in macrolon cages type 2 with standardized granulated soft wood bedding.
   Diet: Pelleted, certified, standard diet Nafag No.890 Tox, ad libitum.
   Water: Tap water, quality according to the specifications of the "Schweizerisches Lebensmittelbuch"

Environmental Conditions: Air-conditioned room maintained at a temperature of 22±2 °C, relative humidity of 55±10%; 12 hour light/dark cycle, and 16-20 air changes per hour.

Acclimation period: 11 days.

B STUDY DESIGN:

1. In-life dates: start: 11/7/86 end: 12/5/86

2. Animal assignment: Animals were distributed according to the Table below.

TABLE I: STUDY DESIGN

Animal No.	GROUP 1 CONTROL	GROUP 2 50 PPM	GROUP 3 1000 PPM
Males I	1-5	6-10	11-15
Males II	---	---	16-20
Females I	21-25	26-30	31-35
Females II	---	---	36-40

I - 5 mice/sex/dose for toxicity evaluation

II - 5 mice/sex for evaluation of reversibility of toxicity after 14 days recovery.

3. Diet Preparation and Analysis:

FAT 80023 was weighed on a calibrated Mettler balance. Pulverized feed was then homogenously mixed with the appropriate concentrations of the test article. Approximately 25% water was added before pelleting, to ensure necessary pellet quality. Pellets were subsequently air dried. There was no specific statement as to the analysis of pellets for test article concentration and homogeneity. Intake of test article was, however, reported on pages 24-25 of the report.

4. Statistics: In this study, non-parametric methods were applied. Both pair-wise and trend tests were applied. Specific tests were not listed. The report noted (page 22) that the recovery period was conducted with only one group without the use of corresponding controls. Evaluation of this group was done by comparing values collected at the end of the recovery period with those obtained in the control animals of Group I at the end of the treatment period.

C. METHODS:

1. Observations:

Daily examinations were performed for observation of clinical toxicity. All animals were checked twice daily (once a.m. and once p.m.) and once on weekends (a.m.) for observation of mortality.

2. Body weight: Individual body weight was recorded weekly.

3. Food consumption and compound intake:

Individual food consumption was recorded weekly. Food efficiency was calculated according to the following formula:

$$\frac{\text{food consumption (g) between 2 measurements}}{\text{body weight at midweek}} \times 1000$$

4. Ophthalmoscopic examination:

There was no ophthalmoscopic examination performed in this study.

5. Blood collection: Sampling for hematology and clinical chemistry was done between the hours of 7:00 and 9:00 a.m. to minimize biological variability due to circadian rhythms. Food was withheld for approximately 18 hours prior to blood collection. Blood was collected from the orbital sinus in all cases, using microhematocrit glass capillary tubes. For hematology measurements, blood was collected into individual vials containing EDTA, while for clinical chemistry, blood was collected into individual vials containing heparin.

a. Hematology

The following parameters were measured:

X	Erythrocyte count (RBC) Hemoglobin (HB) Hematocrit (HCT) Mean corpusc. vol. (MCV) Mean corpusc. HB (MCH)	X	Mean corpusc. HB conc. (MCHC) Leukocyte count (WBC) Leukocyte differential Thrombocyte count

b. Clinical chemistry

X	ELECTROLYTES Calcium Phosphate (inorganic) ENZYMES aspartate aminotransferase (AST) alanine aminotransferase (ALT) alkaline phosphatase (Alk. -P)	X	OTHER Glucose Urea Creatinine Total protein Albumin Globulins A/G Ratio

6. Urinalysis

Urine was collected from individual animals fasted overnight. The following parameters were measured:

X	Urine volume Specific gravity pH Sediment Protein Glucose	X	Ketones Bilirubin Blood Urobilinogen

7. Sacrifice and Pathology

At the end of treatment (group I) or recovery (group II), all surviving animals were exsanguinated under ether anesthesia and subjected to detailed necropsy. The checked (X) tissues were collected and preserved in 10% neutral formalin for histopathological examination, and the (XX) organs were weighed:

X	DIGESTIVE	X	CARDIOVASCULAR	X	NEUROLOGIC
	esophagus		aorta	XX	brain
	stomach		heart		spinal cord
	small int.	XX	spleen		eye
	large int.		thymus		optic nerve
	salivary gland		lymph nodes		pituitary
XX	liver		bone marrow		
	gall bladder		trachea		
	pancreas				GLANDULAR
	tongue		UROGENITAL		adrenal
	RESPIRATORY	XX			lacrimal
	lung		kidney		thyroid
			bladder		parathyroid
			seminal vesicle		mammary
			testis		OTHER
			epididymis		skin
			vagina		skeletal muscle
			prostate		orbital gland
			uterus		lacrimal gland
			ovary		

The report noted the following (page 20): "Additional paraffin sections of all livers were stained by the PAS method, and in some animals liver sections were also stained with Chromotrope Anilinblue (CAB), Prussian Blue for detection of iron, and Kossa for the demonstration of calcium. Frozen sections of formalin fixed livers from all animals were stained with Sudan for fat."

"Kidney sections from all animals were additionally stained with PAS and Gomoeri methods."

In addition to histological examination of tissues through staining, small pieces of liver and kidney were prepared used for electron microscopic examinations.

II. **RESULTS**

A. Observations:

1. Toxicity - According to the report, clinical observations in individual animals are part of the raw data. The report stated that there were no signs of systemic toxicity observed during the

study.

2. Mortality - There were no deaths during the conduct of this study.

B. Body weight and weight gain: Mean body weight and weight gain in animals used in this study is summarized in the following tables:

TABLE Ia
BODY WEIGHTS IN **MALE** MICE ADMINISTERED DIETARY IRGASAN DP300 FOR 4 WEEKS[a]

Dose (ppm)	Week -1	Week 1	Week 2	Week 3	Week 4	**Weight gain**
0	30.4	30.4	33.4	35.2	35.6	5.2
50.0	29.6	29.6	32.4	32.6	33.4	3.8
1000.0	29.9	29.9	33.7	34.8	34.7	4.8

TABLE Ib
BODY WEIGHTS IN **FEMALE** MICE ADMINISTERED DIETARY IRGASAN DP300 FOR 4 WEEKS[a]

Dose (ppm)	Week -1	Week 1	Week 2	Week 3	Week 4	**Weight gain**
0	25.2	25.2	26.0	26.6	27.7	2.5
50.0	25.0	24.4	26.4	26.6	28.0	3.0
1000.0	24.6	24.7	27.0	27.0	28.0	3.3

[a]data obtained from pages 32-34 of the report.

As shown above, there were no significant effects on body weight or body weight gain in male or female mice in this study.

C. Food consumption and compound intake

Mean food consumption in male and female mice is shown in the following tables:

1. Food consumption

TABLE IIa
FOOD CONSUMPTION IN **MALE** MICE ADMINISTERED DIETARY IRGASAN DP300 FOR 4 WEEKS[a]

Dose (ppm)	Week -1	Week 1	Week 2	Week 3	Week 4	
0	44.4	38.8	49.4	42.4	44.6	
50.0	42.0	35.8	45.0	35.8	40.4	
1000.0	44.3	37.3*	48.8	41.4	44.9	

TABLE IIb
FOOD CONSUMPTION IN **FEMALE** MICE ADMINISTERED DIETARY IRGASAN DP300 FOR 4 WEEKS[a]

Dose (ppm)	Week -1	Week 1	Week 2	Week 3	Week 4	
0	40.3	34.2	44.0	40.6	43.9	
50.0	38.8	33.4	45.4	42.0	44.1	
1000.0	38.3	36.2	46.5	42.0	47.7	

[a]data taken from pages 41-44 of the report. Data are expressed as grams/animal/day food consumption.

As noted, the only statistically different value for food consumption occurred in male mice during week 1 of treatment at the 1000 ppm dose level, where a significant decrease in food consumption was observed relative to control. However, this change was small in comparison to the control value, and there were no subsequent changes in food consumption in male mice at this dose, nor were there any significant changes in food consumption in female mice during the study at any dose level tested. Thus, the difference in food consumption in male mice at this time point is not considered related to treatment.

2. Compound consumption

Nominal concentrations of test article in the diet and mean test article intake were presented in the report and are summarized in the following tables:

TABLE 3 INTAKE OF IRGASAN DP300 (MG/KG/DAY) IN MALE AND FEMALE MICE[a]				
Week of study	Male Mice 50 ppm	Male Mice 1000 ppm	Female mice 50 ppm	Female mice 1000 ppm
1	6.39	130.63	7.24	153.47
2	7.34	151.63	9.09	180.34
3	5.80	124.57	8.35	162.89
4	6.39	135.54	8.32	178.45
Mean	6.48	135.59	8.25	168.78

[a]data taken from pages 23-24 of the report.

The report noted that the percentage of actual amount of test substance was 74.0% for male and mice at the 50 ppm dose, and 73.3% of nominal for male and female mice at the 1000 ppm dose, respectively. Thus, actual intake of test chemical in mg/kg/day was reported as shown above. Female mice received a slightly higher average dose than male mice in this study.

D. Ophthalmoscopic examination

As noted previously, there was no ophthalmoscopic examination performed in this study.

E. Blood Work:

1. Hematology

Hematological effects of Irgasan administration on male mice is summarized in the following table:

TABLE 4a Hematological Effects of Irgasan Administration in Male Albino Mice[a]				
Parameter	0 mg/kg/day	6.48 mg/kg/day	135.59 mg/kg/day	Recovery group
erythro-cytes (T/L)	10.3	10.7	9.5*	10.3
hemoglobin (mmol/L)	10.0	10.2	9.0*	9.8
hematocrit	0.46	0.47	0.43*	0.47

thrombo-cytes (g/L)	1400	1406	1676	1551
MCV (FL)	45	45	45	46
MCH (FMOL)	0.97	0.95	0.95	0.95
MCHC (mmol/L)	21.7	21.5	21.1	20.8
leukocytes (g/L)	2.1	1.8	2.5	2.4

[a]data obtained from pages 56 and 58 of the report.

TABLE 4b
Hematological Effects of Irgasan Administration in Female Albino Mice[a]

Parameter	0 mg/kg/day	8.25 mg/kg/day	168.78 mg/kg/day	Recovery group
erythro-cytes (T/L)	10.2	10.8	9.8	10.4
hemoglobin (mmol/L)	10.2	10.4	9.5*	10.1
hematocrit	0.47	0.49	0.45	0.48
thrombo-cytes (g/L)	1155	1260	1436*	1372
MCV (FL)	46	45	46	47
MCH (FMOL)	1.00	0.97	0.97*	0.97
MCHC (mmol/L)	21.9	21.3	21.1*	21.0
leukocytes (g/L)	2.8	2.7	3.2	2.4

[a]data obtained from pages 60 and 62 of the report.

As shown from the data in the above tables, the high dose of the test chemical (135.59 mg/kg/day in males, 168.78 mg/kg/day in females) caused depression of erythrocyte count, hemoglobin, and hematocrit in male and female mice. Decreases in these parameters ranged from 7-10% in males, and from 4-7% in female mice. The decreases were statistically significant for male mice for all three parameters, while in female mice, the decreases, while apparent for all three parameters, were statistically significant for only hemoglobin. A statistically significant trend for the

decrease in erythrocytes, hemoglobin, and hematocrit was also identified in male mice, while a statistically significant trend was identified for the decrease in hemoglobin for female mice. Corresponding to the decreases in erythrocytes, hemoglobin, and hematocrit was an increase in thrombocytes in both sexes at the high dose of test chemical (increase of 19% in males, 24% in females). Statistically significant trends were also indentified for the increase in thrombocytes in both sexes. 90 The results of the hemotologic examinations in this study are consistent with published literature (J. Toxicol. Enviorn. Health 8 (1-2): 215-224) suggesting the potent hemolytic activity of hydroxy chlorodiphenyl ethers.

It is also noted from the hematology data that the recovery group showed reversal of the effects of Irgasan administration in large part, with the possible exception of thrombocytes, which appeared elevated after the 2 week recovery period.

2. Clinical Chemistry

Clinical chemistry results are summarized in the following tables:

TABLE 5a
Effects of Irgasan Administration on Blood Chemistry in Male Albino Mice[a]

Parameter	0 mg/kg/day	6.48 mg/kg/day	135.59 mg/kg/day	Recovery group
Alk. Phos. (U/L)	226.7	253.9	701.6*	225.0
alanine aminotrans-ferase (U/L)	28.8	45.9	89.9*	40.1
aspartate aminotrans-ferase (U/L)	76.2	93.2	107.5*	109.6
urea (mmol/L)	9.7	9.7	12.7*	11.9
glucose (mmol/L)	9.7	8.1	10.3	9.6
total globulin (g/L)	22.9	20.8	19.5*	22.1
albumin	35.4	35.7	35.3	34.9
A/G ratio	1.55	1.72	1.82*	1.58

[a]data obtained from pages 56-59 of the report.

TABLE 5b
Effects of Irgasan Administration on Blood Chemistry in Female Albino Mice[a]

Parameter	0 mg/kg/day	8.25 mg/kg/day	168.78 mg/kg/day	Recovery group
Alk. Phos. (U/L)	292.9	273.6	428.0*	239.7
alanine aminotrans-ferase (U/L)	32.6	33.0	100.7*	45.4
aspartate aminotrans-ferase (U/L)	137.5	162.1	249.9*	127.3
urea (mmol/L)	7.7	8.1	10.6*	10.5
creatinine (µmol/L)	29	33	35*	34
glucose (mmol/L)	6.9	8.4	9.2	10.3
total globulin (g/L)	18.5	18.0	16.6*	19.7
albumin	37.7	37.1	35.6*	36.1
A/G ratio	2.04	2.06	2.15	1.85

[a]data taken from pages 60-63 of the report.

The data above show that, at the high dose of test chemical, elevation of serum enzymes associated with liver function (alkaline phosphatase, alanine and aspartate aminotransferase) were significantly elevated, a reflection of possible damage to liver cells. Elevations were also observed in both sexes in serum urea and serum creatinine. The elevation in serum urea could possibly be based on increased production of nitrogenous waste, increased protein metabolism, or impairment of excretion of this waste product. The elevation in glucose, while not significant, was apparent also at the high dose of the test chemical. Serum albumin and globulin were decreased significantly in female mice at the high dose level, while only serum globulin was decreased in male mice at the high dose level.
As it is known from other longer term studies that the liver is a target organ of Irgasan toxicity, the changes observed in mice in this study are not surprising. It is noted that in male mice, the activities of alanine and aspartate aminotransferase did not return completely to normal, and in fact appeared elevated after

the two week recovery period. In females, activity of aspartate aminotransferase returned to normal after the recovery period, but activity of alanine aminotransferase was still somewhat elevated.

F. Urinalysis

Urinalysis data were presented on pages 56-59 for male mice, and on pages 60-63 for female mice. Although the report noted that urine was collected from individual mice in this study, the number of mice actually reported under urinalysis data was variable, without explanation as to why. For male mice, 2, 1, and 5 mice were reported with urine data in the 0, 6.48, and 135.59 mg/kg/day dose groups, while in female mice, 2, 4, and 9 mice were reported with urine data in the corresponding groups. The variability in sample size (and the small sample sizes in some cases) might preclude determination of a significant effect. Examination of urine data for individual mice (pages 142-144 for males; pages 155-157 for females) showed no definitive effect on the parameters measured (protein, glucose, ketones, bilirubin, blood, or urobilinogen as stated in methods section) but as stated, the small and variable sample size would not lead to definitive conclusions unless the effect were very pronounced.

G. Sacrifice and Pathology

1. Organ weight

As expected, a statistically significant increase in absolute and relative liver weight was observed for male and female mice at the high dose of test chemical. In male mice, absolute liver weight was increased 80% over control, while in female mice, absolute liver weight was increased 70% over control at this dose. Relative liver weight in male mice (liver/body) was increased 76% over control, while relative liver weight in female mice (liver/body) was increased 65% over control. Liver to brain weight ratio was also affected in both sexes, with an increase in this ratio of 87% in males, and 71% in females at the high dose. Organ weight data are shown below (Table 6):

TABLE 6: ORGAN WEIGHTS IN MICE GIVEN DIETARY IRGASAN[a]

	Dose (mg/kg/day)					
	Males			**Females**		
Organ	0.0	6.48	135.59	0.0	8.25	168.78
body	29.96	27.54	30.52	21.84	21.92	22.24
brain	0.504	0.500	0.482	0.521	0.502	0.513
brain/b.w.	1.686	1.817	1.579	2.391	2.292	2.318
liver	1.678	1.532	3.019*	1.297	1.325	2.193*
liver/b.w.	5.608	5.567	9.885*	5.946	6.049	9.866*
liv./brain	333.85	308.24	626.53*	249.80	263.95	429.02*

kidneys kid/b.w. kid/brain	0.568 1.903 113.00	0.500 1.813 99.89	0.500* 1.641 104.07	0.394 1.806 75.89	0.379 1.731 75.34	0.375 1.696 73.08
spleen spleen/b.w. spln/brain	0.069 0.232 13.80	0.069 0.249 13.68	0.083 0.272 17.15	0.074 0.341 14.25	0.066 0.301 13.15	0.062 0.279 12.14

[a]data taken from pages 65-68 of the report.

2. Gross Pathology

Data on the gross examination of mice in this study were presented in summary form on pages 71-72 of the report, and as individual animal data on pages 180-201 of the report. The summary tables on pages 71-72 showed no data indicating any gross abnormalities in male or female mice. Examination of individual animal data also showed no reported gross abnormalities.

3. Microscopic pathology

Summary of microscopic pathology observed in mice in this study was presented on pages 73-78 of the report, and was presented also as individual animal data on pages 180-201 of the report. A summary of the findings is presented in the following table (Table 7):

TABLE 7: MICROSCOPIC FINDINGS IN IRGASAN TREATED MICE[a]

Microscopic Observations	Dose (mg/kg/day) Males			Females		
	0.0	6.48	135.59	0.0	8.25	168.78
Liver -inflammatory cell infiltration	0/5	1/5	0/5	1/5	0/5	1/5
- calcification	0/5	0/5	3/5	0/5	0/5	1/5
- fatty change	0/5	0/5	4/5	0/5	0/5	1/5
- kupffer cell hemosiderosis	0/5	0/5	3/5	0/5	0/5	1/5
- necrosis	0/5	0/5	5/5	0/5	0/5	5/5
- vacuolization	0/5	0/5	5/5	0/5	0/5	0/5
- hypertrophy	0/5	0/5	5/5	0/5	0/5	5/5
Kidney cortex - inflammatory cell infiltration	0/5	1/5	0/5	1/5	0/5	2/5

polymorphonuclear infiltration	0/5	0/5	0/5	0/5	0/5	2/5

[a]data from pages 73-78 of the report.

In addition to the above dose groups, microscopic examination of those mice in the recovery group (high dose followed by 2 weeks recovery) showed the following:

	Recovery Group	
	Males	Females
Liver		
inflammatory cell infiltration	1/5	1/5
portal tract hemosiderosis	0/5	1/5
kupffer cell hemosiderosis	0/5	2/5
hepatocyte necrosis	2/5	3/5
Kidney		
tubular proliferation	0/5	1/5
basophilic proliferation	0/5	1/5

According to the report (page 69), those mice treated at the high dose of Irgasan showed "marked hypertrophy accompanied by focal liver cell necrosis" in parenchymal cells, especially from centrilobular regions. In the report, necrotic liver cell foci were also noted which presented as "either single cell necrosis or necrosis of small cell groups." These areas, the report noted, were slightly more numerous in subcapsular regions of the liver. In 3 of 5 males at the high dose, it was further noted that the subcapsular foci contained calcifications which were bordered by macrophages. Fat content in perilobular regions of the liver parenchyma was slightly increased in 4 of 5 male mice and markedly increased in 1 of 5 female mice at the high dose. In the recovery group, inflammatory liver cell changes as well as necrosis were still present in some of the mice at the high dose after the 2 week recovery period. The report also noted the presence of numerous cytoplasmic granules in all males from the recovery group which stained pink by HE and blue by CAB stain. The vacuoles occurred most frequently in midzonal regions of the liver lobules.

The report stated that there were no changes in renal parenchyma in treated male or female mice in this study. Minor, nonspecific lymphocytic inflammatory cell infiltrates were observed in the interstitium of the renal pelvis. However, the report did not address the inflammation observed in the renal cortex as reported above.

Electron microscopic analysis of selected tissues showed pronounced hypertrophy of smooth endoplasmic reticulum and slight elevation of peroxisome number in hepatocytes of high dose male mice. In females, the number and size of peroxisomes was increased while the smooth endoplasmic reticulum was only slightly hypertrophied. There were no pathological alterations reported from electron microscopic examination of kidneys from high dose male mice.

The changes observed in liver of both male and female mice in the high dose treatment group as well as in the recovery group are considered treatment-related.

III. DISCUSSION

The present study examined systemic and organ toxicity of Irgasan DP300 to male and female albino mice after administration in the diet for 28 days at dose levels of 0, 50, and 1000 ppm (0, 6.48, and 135.59 mg/kg/day in male mice; 0, 8.25, and 168.78 mg/kg/day in female mice). Recovery from systemic effects was also assessed in a group of 5 male and 5 female mice administered the high dose of test chemical for 28 days and then control diet for 2 weeks prior to assessment of systemic toxicity.

There was no reported effect of Irgasan administration on mortality, body weight, or food consumption at any dose level tested. Hematological effects of Irgasan administration were observed in male and female mice treated with the high dose of test chemical (135.59 mg/kg/day in males; 168.78 mg/kg/day in females), and included depression of erythrocyte count, hemoglobin, and hematocrit in male and female mice. Accompanying this change was an increase in thrombocytes in both sexes (equivalent to platelets in humans). This indicates a hemolytic type of anemia at the high dose of the test chemical, and is consistent with published literature (J. Toxicol. Enviorn. Health 8 (1-2): 215-224) suggesting the potent hemolytic activity of hydroxy chlorodiphenyl ethers. The recovery group of mice showed reversal of the depression of red cells, hemoglobin, and hematocrit, while thrombocytes still appeared elevated after the 2 week recovery period.
Clinical chemistry alterations were also apparent at the high dose of test chemical in male and female mice, and included increased activity of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. These increases were in the range of approximately 2-3 fold over control, and were statistically significant by pair-wise comparison as well as by trend analysis.