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

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JUL 20 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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. 6/30/88
Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

and

Theodore M. Farber, Ph.D. 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 006842
Section: III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section: III, Tox. Branch (TS-769C) Marcia van Gemert 7/6/88

DATA EVALUATION REPORT

STUDY TYPE: Two-Generation Reproduction-Rats TOX. CHEM. NO.: 186A

MRID NUMBER: 406237-01

TEST MATERIAL: FAT 80'023

SYNONYMS: IRGASAN® DP-300; Triclosan

STUDY NUMBER: HLA No. 2386-100

SPONSOR: CIBA-GEIGY Corporation

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: Two-Generation Reproduction Study in Rats - FAT 80'023

AUTHOR: Sandra L. Morseth, Ph.D.

REPORT ISSUED: March 18, 1988 (QAU release date)

CONCLUSIONS: Administration of FAT 80'023 to rats at levels of 300, 1000, and 3000 ppm in the diet for two generations resulted in reproductive and systemic effects at the high dose only (3000 ppm). Body weights were significantly lower in the high-dose F_1 pups on Days 14 through 21 of lactation and throughout the growth phase. These animals were mated and produced F_2 pups who displayed significantly lower body weights at birth. This body-weight decrement did not persist, however. Body weights of the F_2 pups were comparable among the groups from Day 4 throughout weaning and the postweaning observation period (Days 28-91). The viability index was decreased in the high-dose group in both generations, and the weaning index was slightly lower in the high-dose group compared to control in the F_2 generation. The mean % of pups found dead or missing from Day 0 through Day 4 per litter was increased in the high-dose group in both generations. The % of litters affected was slightly increased in the high-dose group of the F_1 but not the F_2 generation compared to control (74% vs 67%, respectively).

The high-dose produced both reproductive and systemic effects in both generations. The NOEL for both reproductive and systemic effects is 1000 ppm (50 mg/kg); the LEL is 3000 ppm (150 mg/kg).

Before a final assessment of this study can be made, the Registrant should be requested to provide clarification of the procedure used to select the parents of the F_2 generation and to define the terms: adjusted and unadjusted pup body weight.

Classification: Supplementary. This study may be upgraded to Core Minimum upon submission of the requested clarification.

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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 estrous. 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.

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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.

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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.

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	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.

Body 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.

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Weight ups	Group	Day 0 M	Day 0 F	Day 4 M	Day 4 F	Day 4 M	Day 4 F	Day 7 M	Day 7 F	Day 14 M	Day 14 F	Day 21 M	Day 21 F
	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	19/9	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.

er ation	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_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 or 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.

Image Not Relevant

Page _____ is not included in this copy.

Pages 13 through 24 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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