

2-23-95

011417



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. *Whang Phang 2/16/95*
Pharmacologist
Section III/Tox. Branch II / HED (7509C)

THROUGH: James Rowe, Ph.D. *James N. Rowe 2/17/95*
Section Head
and
Marcia van Gemert, Ph.D. *Marcia van Gemert 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 Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910A.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.

1 of 37

01147

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 Unpublished Study Conducted by Exxon Biomedical Sciences, Inc.; Study No. 139910B.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-day 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**.

2

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-day 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 \approx 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.

011417

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 additional 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

011417

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 thrombo- plastin 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:

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

011417

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.

7

011417

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.

011417

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: The 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 ($p < 0.05$); 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

7

011417

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.

011417

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.

The 90-day 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 ranging study provided a rationale for the dose selection for the 90-day dermal toxicity study, and it was considered as supplementary.

11

Tagasaw Review

Page _____ is not included in this copy.

Pages 12 through 37 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.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
