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WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of additional data in support of a dermal sensitization study conducted with Dazomet.

EPA Identification Numbers:
DP Barcode: D227120 MRID # 44031801
P.C. Code: 035602 Submission: S507048

TO: Kathleen Depukat / Ron Kendall
Product Manager # 51
Special Review and Reregistration Division (7508W)

FROM: Timothy F. McMahon, Ph.D 10/17/96
Pharmacologist, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

THRU: Jess C. Rowland, M.S. 10/17/96
Acting Section Head, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

and

Yiannakis M: Ioannou, Ph.D.
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

10/17/96

Registrant: BASF Corporation

Action Requested: Review of positive control data submitted to upgrade a dermal sensitization study conducted with Dazomet.

Recommendations: Toxicology Branch II has reviewed the submitted data conducted with dinitrochlorobenzene, a positive control agent used in dermal sensitization studies. The data in the present study satisfy the requirement that the performing laboratory demonstrate the ability to detect substances causing a positive sensitization effect.

The results of this study in conjunction with previously reviewed dermal sensitization data (MRID # 47014505) are sufficient to upgrade the dermal sensitization study to **acceptable**. These two studies satisfy the guideline requirement (OPPTS 870.6200; OPP §81-6) for a dermal sensitization study in guinea pigs. It is also noted that based on these data, Dazomet technical grade is a non-sensitizer in this test system after intradermal and percutaneous induction.

Background

A previously reviewed dermal sensitization study with Dazomet technical grade (MRID # 47014505) was graded core supplementary by Toxicology Branch II, based on the lack of acceptable positive control data (document # 011727; Submission S478165). The registrant has submitted positive control data to upgrade the dermal sensitization study. The executive summary of the submitted data is presented below.

Executive Summary: In a dermal sensitization study (MRID #44031801) with dinitrochlorobenzene (>99% a.i.), female Firbright White Dunkin Hartley guinea pigs were tested using the method of Magnusson and Kligman. Six injections in groups of 2 per animal were administered in the clipped flank area as follows: The test groups received 2 injections of 0.1 ml Freund's adjuvant each without test material with water, in a ratio of 1:1 in the front row; 2 injections of 0.1 ml of test substance (2% concentration) in the middle row; and 2 injections of 0.1ml Freund's adjuvant / water each (1:1) with test substance (2% concentration). The control groups received the same series of injections as the test groups, but without the test material. Skin reactions for both groups were made 24 hours after the beginning of the intradermal phase.

One week following intradermal induction, the percutaneous induction was performed by bringing 2x4 cm strips of filter paper containing approximately 0.3 grams of the test material (in ethanol) into contact with the shoulder region under occlusive conditions for a total duration of exposure of 48 hours. Control groups received identical treatment but without the test material included. Evaluation for skin reactions were made 48 hours post-

application. Challenge phases were performed using approximately 0.15 grams of test substance on strips of filter paper and exposing animals for 24 hours.

Following the challenge phases, animals receiving test article during induction showed increased incidence of reddening and/or edema of the skin compared to control groups. Necrotic changes were also observed in experimental groups vs. control. The data in the present study satisfy the requirement that the performing laboratory demonstrate the ability to detect substances causing a positive sensitization effect.

EPA Reviewer: Timothy F. McMahon, Ph.D. Date: 10/8/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Jess C. Rowland, M.S. Date: 10/10/96
Acting Head, Review Section I (7509C)

Data Evaluation Record

Study Type: Dermal Sensitization - guinea pig
OPPTS 870.6200 (§81-6)

DP Barcode: 227120
P.C.Code: 035602

Submission: S507048
MRID: 44031801

Test Material: Dinitrochlorobenzene, > 99% a.i.

Synonyms: 1-Chloro-2,4-dinitrobenzene

Citation: Dr. Kieczka (1985): Regarding the Study of the Sensitizing Effect of Dinitrochlorobenzene on Guinea Pigs - Maximization Test. BASF Aktiengesellschaft. Project # 30H196/84 W2. MRID # 44031801. Unpublished.

Sponsor: BASF Corporation

Executive Summary: In a dermal sensitization study (MRID #44031801) with dinitrochlorobenzene (>99% a.i.), female Firbright White Dunkin Hartley guinea pigs were tested using the method of Magnusson and Kligman. Both intradermal and percutaneous induction was performed, with two challenge phases. Increased incidence of reddening and/or edema was noted after challenge applications in those experimental groups receiving the test substance during induction compared to concurrent controls. This study demonstrates the ability of the performing laboratory to detect substances causing positive dermal sensitization and was submitted in order to upgrade previously reviewed dermal sensitization data (MRID # 47014505). This study in conjunction with the previously reviewed data shows that technical Dazomet is a non-sensitizer using the maximization protocol. The data in the present study together with the previously reviewed data are sufficient to upgrade the dermal sensitization study to **acceptable**. These two studies satisfy the guideline requirement (OPPTS 870.6200; OPP §81-6) for a dermal sensitization study in guinea pigs.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dinitrochlorobenzene, > 99% a.i.
   Batch # 2 432 306 (Merck)

2. Vehicle and Positive Control

   Intradermal Induction: paraffin oil DAB 8 or Freund's adjuvant / distilled water (1:1)

   Percutaneous Induction: paraffin oil DAB 8

   Challenge: Ethanol

3. Test Animals: guinea pig
   Species and Strain: Firbright White, Dunkin Hartley HOE DHPK [EPF-LAC] BO

   Weight at start of treatment: 250-282 grams
   Source: Lippische Versuchstierzucht, Hagemann GmbH & Co. KG, 4925 Extertal 1

   Acclimation period: at least 7 days
   Diet: Kliba 341.4 mm, *ad libitum*
   Water: tap water, *ad libitum;* also, drinking water with approx. 2 g. ascorbic acid per 10 L of water twice a week

   Housing: Makrolon Cage, type IV; 5 animals per cage. Temperature: 20-40 °C; relative humidity 50-70%. Photoperiod: 12 hr light/dark

B. STUDY DESIGN AND METHODS

In a preliminary test, the report stated that a 1% ethanol solution was not found to be an irritant, but was found to have a sensitizing effect. It was also stated that preparations up to 5% in paraffin oil DAB 8 or in Freund's adjuvant / distilled water (1:1) could be injected with a syringe. In a further preliminary experiment (page 24 of the report), it was reported that strips of filter paper (2x2 cm) containing approximately 0.15 grams of test substance were brought into contact with the flank area under occlusive conditions using 2-4 animals. Test substance was applied 3 times every 24 hours over a period of 96 hours. The results of this preliminary experiment were not reported.

The main experiment utilized 10 animals per control group and 20 animals per treatment group. The experimental design is summarized as follows:

Induction Phase

Groups (no animals)	Intradermal induction	Percutaneous induction	First Challenge	Second Challenge
Control 1 (10)	Freund's adjuvant/ water (1:1) or paraffin oil	paraffin oil	ethanol	ethanol
Control 2 (10)	Freund's adjuvant/ water (1:1) or paraffin oil	paraffin oil	ethanol	ethanol
Test Group 1 (20)	2% in Freund's adjuvant/water (1:1) or paraffin oil	10% in paraffin oil	1% in ethanol	1% in ethanol
Test Group 2 (20)	2% in Freund's adjuvant/water (1:1) or paraffin oil	10% in paraffin oil	1% in ethanol	1% in ethanol

During intradermal induction, six injections in groups of 2 per animal were administered in the clipped flank area as follows: The test groups received 2 injections of 0.1 ml Freund's adjuvant each without test material with water, in a ratio of 1:1 in the front row; 2 injections of 0.1 ml of test substance (2% concentration) in the middle row; and 2 injections of 0.1ml Freund's adjuvant / water each (1:1) with test substance (2% concentration).

The control groups received the same series of injections as the test groups, but without the test material. Skin reactions for both groups were made 24 hours after the beginning of the intradermal phase.

One week following intradermal induction, the percutaneous induction was performed by bringing 2x4 cm strips of filter paper containing approximately 0.3 grams of the test material into contact with the shoulder region under occlusive conditions for a total duration of exposure of 48 hours. Control groups received identical treatment but without the test material included. Evaluation for skin reactions were made 48 hours post-application.

Challenge Phase

Approximately 14 days days after the last induction dose, the first challenge was performed. In this procedure, approximately 0.15g of the test substance contained on filter paper (2x4 cm) was applied to the shoulder region under occlusive conditions. The experimental groups and Control group 1 received this treatment, while Control group 2 received ethanol alone.

For the second challenge, the experimental groups and control groups received the test substance as in the first challenge. In addition, ethanol alone was used as a solvent control in all groups.

Duration of exposure was 24 hours , and skin reactions to challenge were made approximately 24, 48, and 72 hours after application.

According to the report (page 32), observations at 48 hours were considered in determining the sensitization potential of the test material. The report also stated that "primarily the number of sensitized animals will be taken into consideration in the evaluation." There was no other criteria for evaluation of the sensitization potential, in contrast to the previous report (MRID # 47014505), which stated that "significant erythematous reaction (Grade 1 or above) in at least 15% of the animals was considered to be positive."

Compliance

Signed statements of compliance with Good Laboratory Practice and No Data Confidentiality Claims were provided in the report. This study was conducted in accordance with the OECD Principles of Good Laboratory Practice. There was no statement of Quality Assurance provided in this report.

II. **RESULTS AND DISCUSSION**

The design of this study differed from the usual maximization protocol in that a percutaneous induction phase was performed one week after the intradermal induction. This involved exposure of the skin of the shoulder area to approximately 0.3 grams of the test substance for 48 hours. The application was made in the same area as the prior intradermal induction. Readings were made approximately 48 hours after application. Fourteen days after the percutaneous application, non-irritant concentrations of test material (approximately 0.15 grams of test material) were applied to the flank area under occlusive conditions for 24 hours. Readings were performed at 24, 48, and 72 hours after application.

The report stated that in order to "estimate the risk of sensitization in a comprehensive manner," an intradermal application was performed prior to the percutaneous application..."in order to penetrate the skin barrier and this take into account any conceivable increase in absorption through injured areas of the skin." However, intradermal application is the usual method in the maximization test.

First Challenge

Examination of skin for sensitization potential in the control groups after the first challenge showed no significant skin reactions in the first control group. In the second control group, reddening of the skin was observed in three of ten guinea pigs. In the experimental group, reddening and/or edema (graded as slight at 48 hours) was observed in 7 of 19 guinea pigs examined.

Second Challenge

Examination of the skin sites after the second challenge showed very slight reddening in 5 of 9 control group 1 guinea pigs. No skin reactions were observed in the second control group after the second challenge. In the experimental group, reddening and edema as well as necrotic skin changes were observed in 15 of 19 guinea pigs examined. Ethanol itself produced no significant skin reactions in the control or experimental group.

III. **CONCLUSIONS**

The results of this positive control study show evidence of sensitization to dermal application of dinitrochlorobenzene, a chemical used as a positive control agent in dermal sensitization studies. The present study was performed in March of 1985, which was within 6 months of the date of performance of the actual dermal sensitization study. Therefore, the data in the present study satisfy the requirement that the performing laboratory demonstrate the ability to detect substances causing a positive sensitization effect.

The results of this study in conjunction with previously reviewed dermal sensitization data (MRID # 47014505) are sufficient to upgrade the dermal sensitization study to **acceptable**. These two studies satisfy the guideline requirement (OPPTS 870.6200; OPP §81-6) for a dermal sensitization study in guinea pigs. It is also noted that based on these data, Dazomet technical grade is a non-sensitizer in this test system after intradermal and percutaneous induction.

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***HED* Records Reference Center**
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