



77-0794-00015

Allied Corporation
Chemical Sector
P.O. Box 1139R
Morristown, NJ 07960-1139

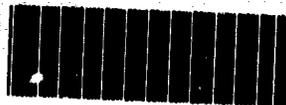


April 15, 1986

INIT 87/26/94

Contains No. 001

Mr. L. Borghi
Senior Scientist
Industrial Chemical Information Section
Dynamac Corporation
11140 Rocksville Pike
Rocksville, MD 20852



84940000145

RECEIVED
OPPT CRIC
94 JUL 26 PM 3 11

Dear Mr. Borghi:

In response to your request of 10/23/85, on behalf of the National Toxicology Program, for information on methyl ethyl ketoxime (MEKO) and alpha methyl styrene (AMS), we are submitting the following:

1. Methyl Ethyl Ketoxime

- a. A 2/14/86 memorandum from G. M. Rusch to R. L. Feder which documents our 1984 submission to EPA of toxicological test data and contains a copy of a sensitization study report which was summarized in that letter and may not have been transmitted to EPA at that time.
- returned* b. A 3/13/86 memorandum from P. O. Milley to R. L. Feder which summarizes the manufacturing, processing, production and importation volume, and consumption by various applications.
- c. A Product Safety Data Sheet.
- d. A 11/18/85 memorandum from D. W. Stidham to E. J. Freeman which summarizes historical data and monitoring results concerning MEKO production at Allied's Hopewell, Virginia plant and contains a copy of a report summarizing area monitoring during the application of alkyd paint containing MEKO and methyl ethyl ketone.
- e. A 3/21/86 memorandum from J. E. Blum to file which summarizes information concerning MEKO air and water emissions at Allied's Hopewell, Virginia plant.

Mr. L. Borghi
Dynamac Corporation
April 15, 1986

- 2 -

Alpha Methyl Styrene

- a. A 12/12/85 memorandum from S. L. Elishewitz to R. L. Feder which provides information concerning manufacturing and processing methods, current production/importation volume data, intermediate and end use information (including consumption by various applications) and environmental data (including fraction released to the environment, concentrations in waste streams and ambient environmental media and degradation/persistence). This memorandum also contains copies of Allied's AMS product specifications, PSDS and other product literature.
- b. A 3/6/86 memorandum from E. J. Freeman to R. L. Feder which summarizes AMS sampling data, both personnel and area samples, from 1976 to 1985.

It is our hope that your program has not been seriously inconvenienced by the delay in sending you this information.

Very truly yours,



R. L. Feder
Director-Product Safety

vs
Att.



Memorandum

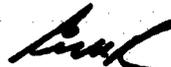
Morris Township, New Jersey

Date: FEBRUARY 14, 1986
To: R.L. FEDER
From: G.M. RUSCH
Subject: DYNAMIC REQUEST FOR INFORMATION ON NEKO
MA-224A

I called Dynamac and discussed their request for information on NEKO with Louis Borghi. When I told him we had submitted most of our toxicology test data to Mr. Robert Jones at EPA during 1984, he requested a copy of the transmittal letters. He was also interested in a copy of the one report we did not submit (a sensitization study) and any information we may have on exposure potential.

To assist you in preparing the response, a copy of the two letters of transmittal for our 1984 submissions to EPA and a copy of the sensitization study report (MA-224-82-2) are attached.

/rb
Attachment


GMR - 3672

*cc: A.F. Ritardi
File

*Transmittal memo only

0005



Allied Corporation
Corporate Health,
Safety and Environmental Sciences
P.O. Box 23329
Morristown, New Jersey 07960

File MA-224A

July 6, 1984

WM. E. RINEHART
JUL 10 1984

Document Control Assistant
TS-793, Room E220
United States Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Attention: Mr. Robert Jones

Re: Methylethylketoxime (MEKO)

Dear Mr. Jones:

A letter was sent to your attention on March 5, 1984 from Joel B. Charm, the Director of Corporate Product Safety, Allied Corporation. Included as part of that communication were summaries of recent tests of the inhalation and dermal toxicities of MEKO to laboratory animals.

The complete reports have recently become available and Mr. Charm asked me to forward them for your information. The titles are as follows:

"Acute Inhalation Toxicity Study of MEKO", Report No. MA-224-82-7

"Acute Dermal Toxicity Study of Methylethylketoxime (MEKO)",
Report No. MA-224-82-9

If you should have any questions concerning these reports, please feel free to contact me, (201) 455-4492.

Sincerely

R. J. Buccafusco, Manager
TSCA Compliance

Enclosures (2)
cc: J. B. Charm

bcc: R. L. Feder
H. C. Fogle
W. E. Rinehart
C. E. Sorapure
p.o. milley



Allied Corporation
Corporate Health,
Safety and Environmental Sciences
P.O. Box 23221
Morristown, New Jersey 07960

WM. E. RHEHART
MAR 6 1984

Certified Mail
Return receipt requested

March 5, 1984

Document Control Assistant
TS-793, Room E 220
United States Environmental Protection
Agency
401 M Street, S.W.
Washington, D.C. 20460

Attention: Mr. Robert Jones

Re: Methylethylketoxime (MEKO)

Dear Mr. Jones:

In previous conversations with you, Allied Corporation has indicated its willingness to supply EPA with copies of reports relating to the toxicity of methylethylketoxime (MEKO). The available reports are enclosed.

Studies on the acute dermal and acute inhalation toxicity of MEKO have also been performed and reports of these studies will be submitted when completed in approximately three (3) months. The information, however, on those studies has been included in the summary below. A full scientific critique is to be prepared on the publication by Mirvish ("Carcinogenicity Test of Acetoxime in MRC-Wistar Rats", JNCI, Vol. 69, No. 4, October 1982, Pgs. 961-962) and this will also be submitted to you when complete.

The data on MEKO have been summarized below by our toxicologists.

1. Acute Oral Toxicity (See references 1-5, inclusive)

In mice, the LD₅₀ was reported to be 1.0 g/kg. For rats, 2 separate studies gave results of 2.3 g/kg or 3.7 g/kg, respectively. (By the subcutaneous route, the LD₅₀ in rats was 2.7 g/kg). According to the classification of Hodge and Sterner, the compound would be considered "slightly toxic". The principal toxic effects of MEKO observed in acute studies included: increased respiration; reductions in spontaneous activity, motor coordination and muscle tone, and increased vocalization/irritability. At high dose levels, tremors followed by loss of the righting reflex and complete sedation (narcoleptic effect) were noted. Death generally occurred in the first two days following dosing. Kurita suggests that the acute toxicity is similar to methylethyl ketone.

2. Inhalation Toxicity (See references 1 and 3)

In an old (1958) study, rats were exposed to an essentially unknown (but apparently high) concentration over a period of 2 hours each day for 10 days. All exposed rats went into a stupor or became unconscious. Recovery was complete roughly 1 hour after removal from the chamber.

2. Inhalation Toxicity (Cont.)

The study by Kurita provides even less detail except that exposure seems to have been continuous to a saturated vapor. While death eventually occurred, this may have been due to lack of food and water intake while in a somnolent condition. In a recently completed, but as yet unreported study, Fisher rats were exposed for single 4 hour periods to 0.19, 1.45 or 4.83 mg/l of MEKO vapor. There was no mortality although the high dose group showed evidence of anesthesia after exposure. Production of methemoglobin was noted for the mid and high level. Minor changes in hematology or clinical chemistry noted for all groups are still being evaluated.

3. Repeated Dosing Tests (See references 1 and 7)

Kurita reported on a study in which MEKO was administered subcutaneously at dose levels of 0.1 ml/kg to 0.5 ml/kg daily to rats for four weeks. He observed a narcotic effect soon after dosing. Among the signs of toxicity observed during the study were a depression in growth rate, decrease in erythrocyte number and hemoglobin, secondary leukocytosis, lymphopenia, hypertrophy and congestion of the spleen and atrophy of other lymphatic tissue. A decrease in various serum proteins, diminished activity of erythrocyte and serum cholinesterase and possible impairment of the clotting mechanism were also noted.

A thirteen week oral gavage toxicity study was performed on MEKO for Allied at Hazleton Laboratories in 1977. MEKO was administered at doses of 25, 75 and 225 mg/kg/day. No mortality, changes in appearance or behavior, or abnormalities in urine values were observed in the rats during the study. Slight to moderately lower mean body weights and food consumption were noted at the high dose level at eight and thirteen weeks in the males. All of the treated groups from both sexes showed dose related decreases in erythrocyte count, hematocrit and hemoglobin values and displayed a moderate to marked reticulocytosis. Heinz bodies, occasional siderocytes, polychromasia, basophilic stippling and Howell-Jolly bodies were generally present in the mid and high dose groups. Blood chemistries revealed an elevation of total bilirubin and erythrocyte cholinesterase in mid dose males and high dose males and females. Alkaline phosphatase levels of high dose males also increased. A slight depression in blood urea nitrogen and plasma cholinesterase levels were noted in the high dose level female group.

Dose related increases in the absolute and/or relative weight of the spleen, liver and kidney were observed in all treatment groups. The spleens and livers appeared large and/or darkened upon necropsy. Histopathological examination revealed extramedullary hematopoiesis and increased amounts of greenish-brown pigment located in macrophages of the spleen and liver. Kidney sections revealed an accumulation of greenish-brown pigment located in macrophages of the spleen and liver.

Kidney sections revealed an accumulation of greenish-brown pigment in the epithelial cells lining the proximal convoluted tubules. These data suggest that MEKO induces a hemolytic anemia in the rat with compensatory erythropoiesis. A NOEL (no observable effect level) was not established but was predicted to be less than 25 mg/kg/day.

4. Eye Irritation (See references 1 and 8)

MEKO has been determined to be corrosive to the eyes of rabbits producing corneal opacity, iritis, conjunctival hyperemia and necrosis, chemosis and discharge. It should be considered a severe eye irritant. A study using only one rabbit indicated that MEKO-induced ocular injury could be prevented by washing the exposed eye with water. Kurita indicated that, in rats, there was only reversible conjunctival irritation.

5. Skin Irritation and Sensitization (See references 1, 9 and 10)

MEKO has been reported to cause mild dermal irritation in the rabbit in two separate studies. The irritation disappeared within 72 hours of exposure in one of the studies.

MEKO produced somewhat equivocal results in a topical sensitization test (Buehler) and positive results in a maximization sensitization test (Morganson-Kligman), both using guinea pigs. There is no evidence, however, of any cases of human sensitization by MEKO in production workers nor any suggestions of such in users by virtue of the lack of customer complaints.

6. Dermal Toxicity (See reference 1)

Kurita dosed rats with MEKO in vaseline daily for 5 weeks and found only dermatological effects. In a recently completed, but as yet unreported study, rabbits were dosed for a single 24 hour period with 0.02, 0.2 or 2.0 ml/kg. Mortality occurred in all animals at 2.0 ml/kg, but there was no mortality at lesser doses. Methemoglobin formation was noted at 0.2 and 2.0 ml/kg and a small increase in reticulocyte levels was seen at 0.2 ml/kg. There were no effects on the blood at 0.02 ml/kg. Nervous system depression (miosis) was the predominant toxic sign for acute doses.

7. Aquatic Toxicity (See reference 11)

A summary of aquatic acute toxicity data available on MEKO is presented in the following table:

<u>Species</u>	<u>Dose</u>	<u>Time of Exposure</u>	<u>Effect</u>
Bluegill sunfish	>116 mg/L	24 h	LC ₅₀
" "	160 mg/L	48 h	LC ₅₀
" "	69 mg/L	72 h	LC ₅₀
" "	48 mg/L	96 h	LC ₅₀
<u>Daphnia magna</u>	1900 mg/L	24 h	EC ₅₀
" "	750 mg/L	48 h	EC ₅₀
" "	280 mg/L	48 h	EC ₀

7. Aquatic Toxicity (Cont.)

These data suggest that MEKO can be considered toxic to both fish and daphnids although relatively more toxic to the former.

MEKO was found to biodegrade in an aqueous medium containing a low microbial concentration. Based on a calculated octanol/water partition coefficient of $K_{ow} = 3.98$, it is suggested that MEKO would not accumulate in aquatic organisms.

8. Genetic Toxicity (See references 14 and 15)

MEKO has been evaluated for potential to induce mutation using the Ames Salmonella assay and the sister chromatid exchange (SCE) assay in Chinese hamster (CHO) cells. MEKO failed to induce mutations in the Ames assay. In addition, no increase in SCE was seen following exposure to the compound. This data in combination with the result of a preliminary transformation assay using Syrian hamster cells suggests no presumed oncogenic potential for the compound.

9. Absorption and Distribution (See reference 6)

The similarity of LD₅₀'s in rats dosed by either oral or subcutaneous routes suggests rapid absorption from the gut. In a study utilizing 14 C-labeled MEKO, pregnant female mice were given a single oral dose of this compound on day 14 of gestation and one male was administered MEKO via intratracheal injection. The animals were sacrificed at 0.33, 1.0, 3.0, 9.0 and 24 hours post-dosing. Whole body autoradiography revealed that MEKO was rapidly absorbed from the stomach and lungs. Widespread uptake and distribution of the label over the entire body of the animal were noted. The tissues with the highest concentration of MEKO included bone, bone marrow, liver, gall-bladder, nasal and bronchial epithelium, pancreas, seromucus and salivary glands, spleen, intestinal wall, and thymus. Urine and bile contained significant activity throughout the study. Intestinal activity was minimal. This suggests that MEKO is primarily excreted via the kidneys. Of particular interest is the observation that high concentrations of MEKO were detected in the liver of the fetuses at 24 hours. This concentration was greater than that seen in the liver of the mothers.

10. Metabolism

The metabolism of MEKO has not been specifically studied. By analogy with acetaldehyde oxime, it is expected that the compound would be split to form MEK and hydroxylamine and further handling as such. The toxicology of MEK has been extensively described and is being addressed by the Chemical Manufacturers Association under a voluntary test program with EPA. A review on the toxicology of hydroxylamine has been prepared and is awaiting publication in CRC's Critical Reviews in Toxicology. Of particular interest is the fact that hydroxylamine has been described as possessing possible carcinostatic properties.

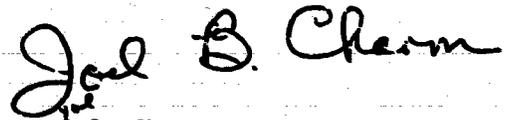
The summary of the data provided in this cover letter can be considered as public information. However, we do request that the full copies of all Allied sponsored

and conducted studies, which are being submitted on a voluntary basis and not pursuant to any rule or regulation, be regarded by EPA as confidential, proprietary, valuable business information, and that, accordingly, EPA use appropriate care in the review, duplication and disposition of these documents.

Methylethylketoxime has been manufactured and used safely for many years. Allied believes the data presented supports the conclusion that MEKO can continue to be manufactured and used safely.

If you have any further questions on this matter, please feel free to contact me.

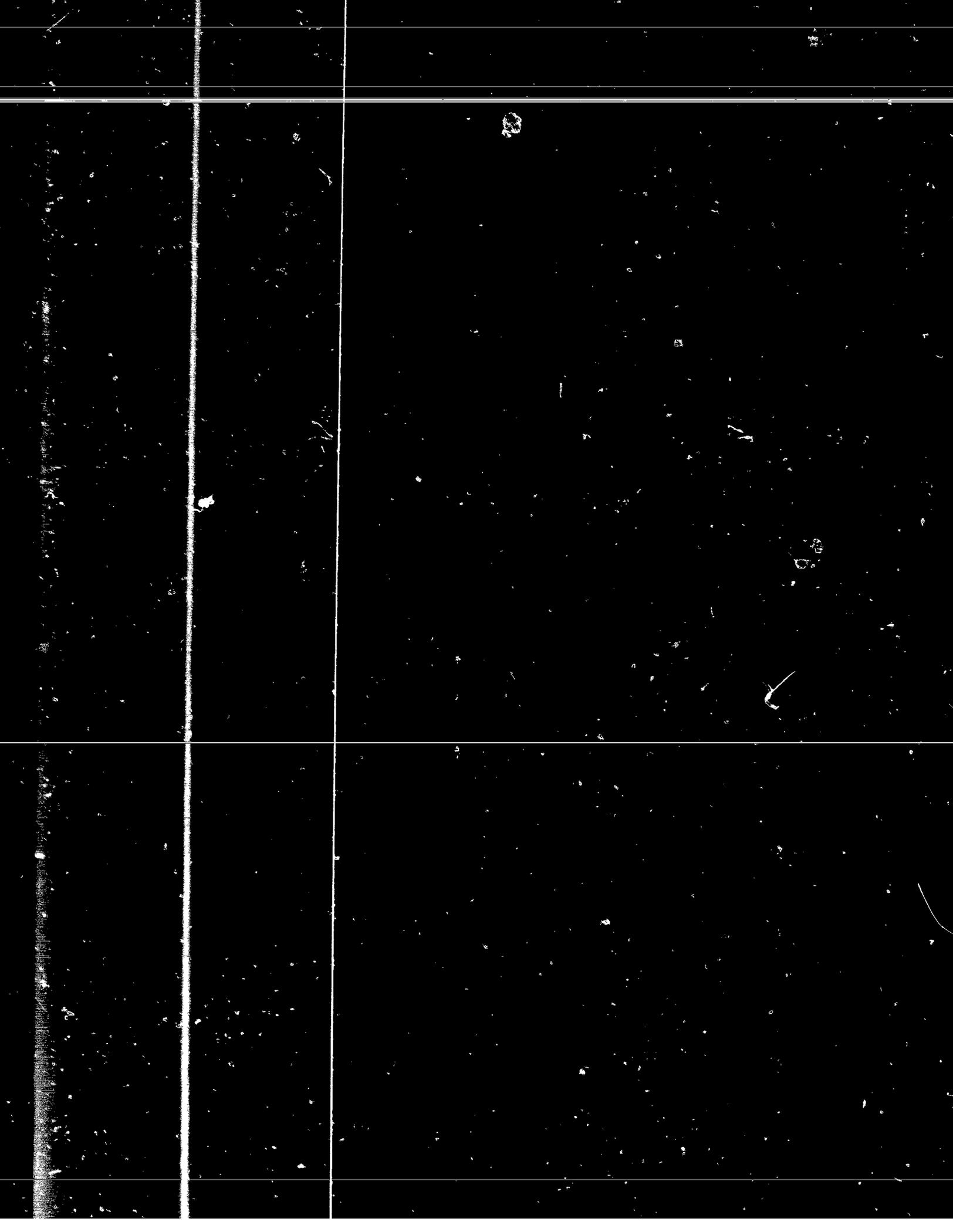
Sincerely yours,



Joel B. Charn
Director, Corporate Product Safety

JBC/gbl
attachments

cc: Dr. C. S. Aaron
Dr. W. E. Rinehart



**REPORTS SUBMITTED
TO EPA RE METHYLETHYLKETOXIME (MEKO)
WITH LETTER DATED MARCH 5, 1984**

Reference No.

1. Experimental Studies on Methyleneketoxime Toxicity by Hideo Kurita, Dept. of Hygiene, Nagoya University School of Medicine Nagoya J. med. Sci. 29: 393-418, 1967.
- ✓ 2. Methyl Ethyl Ketoxime (B16) - Pure Oral LD₅₀ Results in Sprague Dawley Rats, Project No. MA-65-78-2, Sample No. 34972-5 (Memo to Dr. S. Zakhari from R. J. Tivey dated 8/14/78).
3. AOB-Ethyl Methyl Ketoxime SU-23; BC 72137, Final Report (Report to National Animal Health Div., Buffalo 5, NY from Syracuse University Institute, Syracuse, NY dated 2/13/58).
4. 1980 Registry of Toxic Effects of Chemical Substances, Volume One, Y-358956.
5. Methyl Ethyl Ketoxime (B13) - Impure Oral LD₅₀ Results in Sprague Dawley Rats, Project No. MA-65-78-1, Sample No. 34972-5 (Memo to S. Zakhari from R. J. Tivey dated 8/14/78).
6. Specialty Oximes, MA-13A, Transmittal of Pharmakon Research Foundation, Inc. Reports on Autoradiographic Studies of Acetaldehyde Oxime (AAO) MA-13-77-62 and Methyl Ethyl Ketoxime (MEKO) MA-13-77-61, CMA Report MA-13-77-28 (from G. M. Rusch dated 8/17/81).
7. 13-Week Toxicity Study in Rats, Methyl Ethyl Ketoxime (AOB) Final Report (submitted to Allied Chemical Company, Morristown, NJ from Hazleton Laboratories America, Inc., 1/24/77).
- ✓ 8. Test For Eye Irritants, Methyl Ethyl Ketoxime, Code: B-16, Project No. MA-65-78-3, Sample No. 34972-5, Notebook Reference #35013, pp. 17-18 (Memo to Dr. D. M. Aviado from B. J. Dunn dated 9/8/78).
9. Primary Dermal Irritation Test, Methyl Ethyl Ketoxime, Code: B-16, Project No. MA-65-78-4, Sample No. 34972-5, Notebook Reference #8008, pp. 26-30 (Memo to Dr. D. M. Aviado from B. J. Dunn dated 9/14/78).
- ✓ 10. Guinea Pig Skin Hypersensitization Test, Methyl Ethyl Ketoxime, Code: B-16, Project No. MA-65-78-5, Sample No. 34972-5, Notebook Reference #8010, pp. 94-100 (Memo to Dr. D. M. Aviado from B. J. Dunn dated 9/18/78).

REPORTS SUBMITTED
TO EPA RE METHYLETHYLKETOXIME (MEKO)
WITH LETTER DATED MARCH 5, 1984

(Continued)

Reference No.

11. Environmental Screening, Studies of Methyl Ethyl Ketoxime (MEKO) Project No. MA-65-78, Report No. MA-65-78-6, Sample No. 34972-5, 34993-14 (JTC Environmental Consultants, Inc., Report #379 dated 2/6/79).
14. Transmittal of Report Methyl Ethyl Ketoxime (MEKO), MA-224B, Evaluation of Methyl Ethyl Ketoxime in the Ames Salmonella Mutagenicity Assay, Report No. MA-224-82-3, dated 10/26/83.
15. Evaluation of Methyl Ethyl Ketoxime (MEKO) in the Sister Chromatid Exchange (SCE) Test: In Vitro Results in Chinese Hamster Ovary (CHO) Cells, Report No. MA-224-82-6, dated 11/11/83.

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MEMORANDUM

NA-RR-83-484

9/9/83

TO: B. F. Himmelsbach

FROM: B. J. Dunn
G. M. Rusch

SUBJECT: TRANSMITTAL OF REPORT
MEKO/Specialty Rubber Application
NA-2248
Guinea Pig Maximization Test of MEKO
Department of Toxicology Sample No. 8211-74A
NA-224-82-2

Attached is Department of Toxicology Report No. NA-224-82-60 entitled "Dermal Sensitization Study: Guinea Pig Maximization Test of Allied Corporation Department of Toxicology Sample No. 8211-74A". Methylenechloroform (MEKO), Sample No. 8211-74A, was assessed for its potential to produce dermal sensitization in female Hartley strain guinea pigs. The test procedure was validated concurrently with a standard positive control material, dinitrochlorobenzene, using animals from the same shipment.

MEKO produced dermal sensitization reactions in at least eight of ten guinea pigs, classifying it as a strong dermal sensitizer. Dermal contact with MEKO should be avoided, as the results of this test suggest that it may represent a potential sensitization risk for humans.

B.J.D.
BJD - 6077

G.M.R.
GMR - 3672

/ae

cc: B. A. Burns*
E. W. Callahan
R. L. Feder*
P. L. Foreman*
S. C. Gad
J. A. Hathaway
T. M. Hellman
D. Levine
P. O. Milley
W. E. Rinehart
A. C. Smith - Archives
Information Services
File

H. Fleig, BASF AG, ZNT-2470, 6700 Ludwigshafen,
Federal Republic of Germany (mailed 6/21/85)

*Transmittal memorandum only

0 0 1 4



FOOD & DRUG RESEARCH LABORATORIES, INC.
P.O. BOX 107, RT. 17C, WARREN, NY 14882-0107 (607) 565-8131

DERMAL SENSITIZATION STUDY:
GUINEA PIG MAXIMIZATION TEST
OF ALLIED CORPORATION DEPARTMENT OF TOXICOLOGY
SAMPLE No. 8211-74A

TOX Computer #MA-224-82-60
9/6/83

FDRL Study No.: 1705

Submitted to: Allied Corporation
Morristown, NJ 07960

Date: August 25, 1983

Joseph C. Siglin
Joseph C. Siglin, B.A.
Associate Toxicologist
Study Director

Peter J. Becci
Peter J. Becci, Ph.D.
President

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any members of its staff, may be used in connection with the advertising or sale of any product or services without written authorization.

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SUMMARY

Allied Corporation, Department of Toxicology sample no. 8211-74A (MEKO) was evaluated for its potential to produce dermal sensitization in 10 guinea pigs using the maximization test technique. In accordance with the classification system of Kligman (1966), the sensitizing potential of MEKO was rated as strong-extreme.

The positive control material used for this study (1-chloro-2, 4-dinitrobenzene) produced dermal sensitization in a separate group of 10 animals indicating that the test system could detect potential dermal allergens.

INTRODUCTION

The purpose of this study was to evaluate Allied Corporation, Department of Toxicology sample no. 8211-74A for its potential to produce dermal sensitization using the guinea pig maximization test. This study was conducted in accordance with the regulations for Good Laboratory Practice as described by the FDA (21 CFR Part 58) and FDRL Standard Operating Procedures. All original data and pertinent records have been retained in the FDRL archives and are available upon request.

Initial range finding studies were conducted from June 28, 1983 to July 4, 1983. The main study was initiated on July 7, 1983 and completed on July 31, 1983.

MATERIALS AND METHODS

The study protocol is given in Appendix I.

Sample Identification

The test article was received at FDRL on June 23, 1983 and was identified as follows:

<u>Sponsor's I.D.</u>	<u>FDRL I.D.</u>
Sample No. 30424	83-0494
DOT No. 8211-74A	
MEKO (AOB)	

The positive control material (1-chloro-2, 4-dinitrobenzene) was purchased from Fisher Scientific, Rochester, NY and was received at FDRL on March 31, 1982. This material was identified as follows:

FDRL Study No. 7705

Chemical I.D.

1-chloro-2, 4-dinitro-
benzene (DNCB)
Lot # D9D

FDRL I.D.

82-0217

Animals and Animal Care

All animal housing and care conformed to the standards established in "Guide for the Care and Use of Laboratory Animals" DHEW Publication No. (NIH) 78-23.

Thirty-eight female Hartley derived albino guinea pigs were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. The animals were individually housed in wire mesh bottom cages in an environment-controlled room and had access to food (NIH Animal Feed A, certified feed, Zeigler Brothers, Gardners, PA) and fresh tap water ad libitum. Animals used for the main study were allowed to acclimate to the laboratory environment for a period of 14 days prior to initiation. These animals weighed in the range of 204 to 315 grams and were 5-6 weeks of age at study initiation.

Experimental Procedure

Dose Range Finding Studies

Topical and intradermal test article dose range finding studies were conducted simultaneously prior to the start of the main study. For both studies, dermal irritation was evaluated according to the Draize system (Draize, 1965).

For the topical range finding study, the test article was diluted with propylene glycol to produce concen-

trations of 25, 50, 75 and 100% (undiluted). The shaved dorsal surface of 4 guinea pigs (nos. 7705031-034) was topically treated with 0.2 ml of each concentration (4 distinct application sites) contained on 2 x 2 cm patches of Whatman filter paper. The patches were occluded using Blenderm[®] tape followed by an elastic bandage overwrap. The animals were unwrapped and the patches were removed after an exposure period of 24 hours. For each treated site, the degree of dermal irritation was evaluated at 24, 48 and 72 hours after removal of the patches.

For the intradermal range finding study, the test article was diluted with propylene glycol and with Freund's complete adjuvant (FCA-50% in distilled water) to produce concentrations of 0.1, 1.0, 3.0 and 5.0%. The shaved dorsal surface of 4 guinea pigs (nos. 7705035-038) was intradermally injected with 0.1 ml of each concentration (8 distinct injection sites) using a 1 ml tuberculin syringe with a 26 gauge needle. For each injection site, the degree of dermal irritation was evaluated at 24, 48, 72, 96, 120 and 144 hours after injection.

Main Study

The experimental design and treatment schedule for the main study is given in Table 1. Specific details not included on this table are discussed below:

Induction: Ten animals were randomly assigned to the test group, the vehicle control group and the positive control group. On day 0, the fur in an area of approximately

4 x 6 cm was clipped from the shoulder region of each animal. Three pairs of intradermal (i.d.) injections were given to each animal (0.1 ml per injection) using a 1 ml tuberculin syringe with a 26 gauge needle. Each pair of injections flanked the animals' dorsal midline. Specific applications are given in Table 1.

On day 7, the area over the shoulder region that received the i.d. injections was again shaved on all animals. Exactly 0.3 ml of the appropriate material (see Table 1) was spread over a 2 x 4 cm patch of Whatman filter paper and then applied to the exposed skin. Each patch was occluded using Blenderm[®] tape followed by an elastic bandage overwrap. The animals were unwrapped and the patches were removed after an exposure period of 48 hours.

Challenge: On day 21, an area of approximately 3 x 3 cm was shaved on both flanks of all animals to expose virgin skin. Exactly 0.2 ml of the appropriate material was spread over a 2 x 2 cm patch of Whatman filter paper and applied to the appropriate virgin skin site (see Table 1). As before, patches were occluded using Blenderm[®] tape followed by an elastic bandage overwrap. After an exposure period of 24 hours, the animals were unwrapped and the patches were removed. Dermal irritation was evaluated by the Draize system at 24 and 48 hours after patch removal.

Daily Observations: Daily observations were recorded for all animals noting appetite, elimination, general appearance and behavior, and any sign of toxic effects. Mortality checks were conducted twice daily.

Animal body weights were determined and recorded on days -7, 0, 7, 14 and 21.

Terminal Sacrifice: At study termination, all animals were sacrificed by exposure to CO₂ gas and then discarded.

Analysis of Data: The percentage of animals in the test group that showed a positive (sensitization) response following the challenge application was calculated. The sensitizing potential of the test article was then classified using the system of Kligman (1966), as follows:

Sensitizing Rate ^a (%)	Sensitizing Potential	
	Grade	Classification
> 0-8	I	Weak sensitizer
9-28	II	Mild sensitizer
29-64	III	Moderate sensitizer
65-80	IV	Strong sensitizer
81-100	V	Extreme sensitizer

^a 0% = non sensitizer.

Additionally, dermal irritation scores obtained following the challenge application of the test article to the vehicle control group animals were compared to those scores obtained from the test group animals using an extension of the Mantel-Haenszel Procedure (Mantel, 1963).

RESULTS

Range Finding Studies

The individual and mean dermal irritation scores obtained from the topical range finding study of MEKO are given in Table 2. The individual and mean dermal irritation scores obtained from the intradermal range finding study are given in Tables 3 and 4. Test article, vehicle and positive control material concentrations chosen for use in the main study are specified on Table 1.

Daily Observations and Body Weights

All animals appeared normal and healthy over the course of the study.

Individual and mean body weight data are given in Table 5. As indicated on this table, the mean body weight of the test, vehicle control and positive control groups increased weekly.

Analysis of Data

Individual and mean challenge application scores are given for the test, vehicle control and positive control groups in Tables 6-8, respectively. Additionally, Table 6 gives the percentage of animals in the test article group that exhibited

a positive (sensitization) response. From these data, the sensitizing potential of MEKO was rated as strong-extreme.

CONCLUSION

Allied Corporation, Department of Toxicology sample no. 8211-74A (MEKO) was evaluated for its potential to produce dermal sensitization using the guinea pig maximization test technique. Using the system of Kligman (1966), the sensitizing potential of MEKO was rated as strong-extreme.

The positive control material (DNCB) produced substantial erythema and eschar formation following the challenge application. This reaction indicates that dermal sensitization to DNCB had occurred and that the test system could therefore detect potential dermal allergens.

REFERENCES

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FDOL Study No. 7705

Table 1

Experimental Design and Treatment Schedule
(Main Study)

Group	Animal Nos.	Day 0-Induction Stage 1	Day 7-Induction Stage 2	Day 21-Challenge Application
Test	7705001-010	Site 1) 0.1 ml of 3.0% MEXO in propylene glycol. Site 2) 0.1 ml of FCA ^a . Site 3) 0.1 ml of 1.0% MEXO in FCA ^a .	0.3 mls of 100% MEXO	<u>Left side</u> 0.2 mls of 50% MEXO in propylene glycol. <u>Right side</u> 0.2 mls of 100% propylene glycol.
Vehicle Control	7705011-020	Site 1) 0.1 ml of 100% propylene glycol. Site 2) 0.1 ml of FCA ^a . Site 3) 0.1 ml of 1.0% propylene glycol in FCA ^a .	0.3 mls of 100% propy- lene glycol.	Control group receives same treatment as the test group.
Positive Control	7705021-030	Site 1) 0.1 ml of 0.1% DMCB in propylene glycol. Site 2) 0.1 ml of FCA ^a . Site 3) 0.1 ml of 0.1% DMCB in FCA ^a .	0.3 mls of 0.1% DMCB in propylene glycol.	<u>Left side</u> 0.2 ml of 0.1% DMCB in propylene glycol. <u>Right side</u> 0.2 ml of 100% propylene glycol.

^a FCA was diluted with distilled H₂O (1:1).

^b Determined to be a non-irritating concentration by topical application range finding study.

Table 2
 Individual and Mean Dermal Irritation Scores
 (Topical Application Range Finding Study)

Treatment	Concentration	Animal Number	Hours After Patch Removal		
			24 Erythema	48 Erythema	72 Erythema
MEKO	100% ^a	7705031	1	1	0
		7705032	2	1	0
		7705033	1	0	0
		7705034	0	0	0
		Mean ±S.D.	1.0 ±0.6	0.5 ±0.6	0
	75% ^b	7705031	1	0	0
		7705032	1	0	0
		7705033	0	0	0
		7705034	0	0	0
		Mean ±S.D.	0.5 ±0.6	0	0
	50% ^{b,c}	7705031	0	0	0
		7705032	0	0	0
		7705033	0	0	0
		7705034	0	0	0
		Mean	0	0	0
	25% ^b	7705031	0	0	0
		7705032	0	0	0
		7705033	0	0	0
		7705034	0	0	0
		Mean	0	0	0

^a Concentration chosen for topical induction application on main study.

^b Diluted in propylene glycol.

^c Concentration chosen for challenge application on main study.

Table 4
Individual and Mean Dermal Irritation Scores
(Intradermal Injection Range Finding Study)

MEKO in FCA ^a (Concentration)	Animal Number	Hours Post Dose										
		24 Erythema	48 Erythema	72 Erythema	96 Erythema	120 Erythema	144 Erythema	Mean	±S.D.			
5%	7705035	2	3	4	4	4	4	4	4	4	2.3	±2.1
	7705036	2	2	2	2	2	2	2	2	2	1	
	7705037	1	1	1	1	1	1	1	1	1	0	
	7705038	2	2	4	4	4	4	4	4	4	0	
	Mean	1.8	2.0	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.3	±2.1
±S.D.	±0.5	±0.8	±1.5	±1.5	±1.5	±1.5	±1.5	±1.5	±1.5	±0.5		
3%	7705035	2	3	2	2	2	2	2	2	2	4	
	7705036	2	1	1	1	1	1	1	1	1	1	
	7705037	1	1	1	1	1	1	1	1	1	0	
	7705038	2	1	1	1	1	1	1	1	1	0	
	Mean	1.8	1.5	1.3	1.3	1.3	1.3	1.3	1.3	1.3	2.3	±2.1
±S.D.	±0.5	±1.0	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5		
1% ^b	7705035	2	2	2	2	2	2	2	2	2	1	
	7705036	1	2	1	1	1	1	1	1	1	0	
	7705037	1	1	1	1	1	1	1	1	1	0	
	7705038	1	1	2	2	2	2	2	2	2	0	
	Mean	1.3	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.3	±1.9
±S.D.	±0.5	±0.6	±0.6	±0.6	±0.6	±0.6	±0.6	±0.6	±0.6	±0.5		
0.1% ^b	7705035	2	2	2	2	2	2	2	2	2	1	
	7705036	1	2	2	2	2	2	2	2	2	0	
	7705037	0	1	1	1	1	1	1	1	1	0	
	7705038	2	2	2	2	2	2	2	2	2	0	
	Mean	1.3	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.3	±0.5
±S.D.	±1.0	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5	±0.5		

^a Freund's complete adjuvant - 50% in distilled water.

^b Concentration of MEKO in FCA chosen for initial intradermal injections on main study.

Table 5

FDM. Study No. 7705

Individual and Mean \pm SD Body Weight Data
(Main Study)

Group	Animal No.	Body Weight (g) on Day				
		-7	0	7	14	21
Test Article Group	7705001	277	259	337	356	405
	7705002	315	341	388	392	438
	7705003	289	322	333	358	391
	7705004	314	358	397	415	467
	7705005	263	288	308	313	345
	7705006 ^a	284	315	338	308	352
	7705007	296	339	368	391	436
	7705008	274	314	341	354	411
	7705009	282	338	362	365	422
	7705010	216	297	337	340	382
	Mean	281.0	317.1	350.9	359.2	404.9
	\pm S.D.	\pm 28.2	\pm 29.5	\pm 27.3	\pm 34.0	\pm 38.5
Vehicle Control Group	7705011	281	318	357	368	409
	7705012	225	293	325	343	401
	7705013	293	301	337	346	392
	7705014	268	314	346	358	429
	7705015	204	217	279	296	334
	7705016	274	302	329	317	365
	7705017	266	293	310	325	343
	7705018	271	300	320	333	377
	7705019 ^b	286	276	341	285	383
	7705020	277	311	331	339	372
	Mean	264.5	292.5	327.5	331.0	380.5
	\pm S.D.	\pm 28.0	\pm 29.1	\pm 21.7	\pm 26.1	\pm 29.1
Positive Control Group	7705021	233	274	302	311	350
	7705022	331	362	403	403	469
	7705023	273	319	347	359	396
	7705024	289	320	368	374	415
	7705025	301	326	322	368	412
	7705026	280	327	361	369	416
	7705027	288	326	368	381	437
	7705028	293	323	340	372	423
	7705029	284	324	345	366	403
	7705030	272	309	347	365	408
	Mean	284.4	321.0	350.3	366.8	412.9
	\pm S.D.	\pm 24.7	\pm 21.4	\pm 27.5	\pm 23.0	\pm 30.2

^a Weight loss observed at day 14 is attributable to an inoperative sipper tube which was found on the animals' water bottle.

^b The exact cause of the weight loss observed at day 0 is not known. Experimental manipulation (48 hour occlusive wrap) is considered to be a contributing factor in the loss observed at day 14.

Table 6

FD&L Study No. 7705

**Individual and Mean Challenge Application Erythema Scores
(Test Article Group)**

Treatment	Animal No.	Hours After Patch Removal	
		24	48
50% MEKO in propylene glycol (Test Article)	7705001	2	1
	7705002	1	1
	7705003	1	0
	7705004	2	2
	7705005	1	1
	7705006	0	0
	7705007	1	1
	7705008	1	1
	7705009	2	2
	7705010	1	2
	Mean	1.2 ^a	1.1 ^a
	± S.D.	± 0.6	± 0.7
	Percent of Positive Responses ^b :	90%	80%
	Grade and Classification ^b :	V - Extreme sensitization	IV - Strong sensitization
100% propylene glycol (Vehicle)	7705001	0	0
	7705002	0	0
	7705003	0	0
	7705004	0	0
	7705005	0	0
	7705006	0	0
	7705007	0	0
	7705008	0	0
	7705009	0	0
	7705010	0	0
	Mean	0	0

^a Significantly different from challenge application scores obtained from test article treatment to the vehicle control group (Mantel, 1963).

^b System of Kligman (1966).

Table 7

FDL Study No. 7705

Individual and Mean Challenge Application Erythema Scores
(Vehicle Control Group)

Treatment	Animal No.	Hours After Patch Removal	
		24	48
50% HMD in propylene glycol (Test Article)	7705011	0	0
	7705012	0	0
	7705013	0	0
	7705014	0	0
	7705015	0	0
	7705016	0	0
	7705017	0	0
	7705018	0	0
	7705019	0	0
	7705020	0	0
	Mean	0	0
100% propylene glycol (Vehicle)	7705011	0	0
	7705012	0	0
	7705013	0	0
	7705014	0	0
	7705015	0	0
	7705016	0	0
	7705017	0	0
	7705018	0	0
	7705019	0	0
	7705020	0	0
	Mean	0	0

Table 8

FDRL Study No. 7705

Individual and Mean Challenge Application Erythema Scores
(Positive Control Group)

Treatment	Animal No.	Hours After Patch Removal	
		24	48
0.1% DNCB in propylene glycol (Positive Control)	7705021	2 ^a	3
	7705022	4 ^a	4 ^a
	7705023	3	3
	7705024	4 ^a	4 ^a
	7705025	4 ^a	4 ^a
	7705026	2	4 ^a
	7705027	4 ^a	4 ^a
	7705028	4 ^a	4 ^a
	7705029	4 ^a	4 ^a
	7705030	4 ^a	4 ^a
	Mean	3.5	3.8
	±S.D.	±0.8	±0.4
100% propylene glycol (vehicle)	7705021	0	0
	7705022	0	0
	7705023	0	0
	7705024	0	0
	7705025	0	0
	7705026	0	0
	7705027	0	0
	7705028	0	0
	7705029	0	0
	7705030	0	0
	Mean	0	0

^a Indicates the presence of eschar tissue.