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**INDUSTRIAL HEALTH FOUNDATION, INC.**

A NONPROFIT ORGANIZATION FOR THE ADVANCEMENT OF HEALTHFUL WORKING CONDITIONS

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March 21, 1994

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Chemical Information Division  
Office of Toxic Substances  
Room E-108  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

RE: Notice of Substantial Risk Under TSCA 8(e) for Methylenechloride  
(MEK) when Administered by Inhalation to Rats and Mice in an  
Oncogenicity Study

EPA Docket No. 8EHQ-0990-1063

Attention: Section 8(e) Coordinator

Dear Sir:

As indicated in our Section 8(e) submission of March 18, 1994, reporting  
on findings in rats, a copy of the final report is being forwarded by UPS,  
together with a copy of this letter.

If there are any questions, please contact me.

Sincerely,

*William E. Rinehart*

William E. Rinehart, Sc.D.  
President

(Agent for the MEK Testing Group)

WER:la

EPA-OTS



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# PHARMACO::LSR

STUDY NO. 89-8243R

AN INHALATION ONCOGENICITY STUDY  
OF METHYLETHYLKETOXIME IN RATS AND MICE

PART II - RATS

Final Report

VOLUME I OF VI

Submitted to: Industrial Health Foundation, Inc.  
34 Penn Circle West  
Pittsburgh, Pennsylvania 15206

Attn: Dr. William E. Rinehart

Date: February 14, 1994

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# PHARMACO :: LSR

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PROJECT NO. 89-8243R  
AN INHALATION ONCOGENICITY STUDY  
OF METHYLETHYLKETOXIME IN RATS AND MICE

PART II - RATS

ABSTRACT

This study, conducted for the Industrial Health Foundation, Inc., was designed to evaluate the oncogenic effects of Methyleneethylketoxime (MEKO, CAS No. 50-29-7), when administered by whole-body inhalation as a vapor to Fischer 344 rats (80/sex/group) and CD-1 mice (60/sex/group). This report presents the results from the rat portion of the study. The mouse results are presented in a separate report. The test substance was administered for 6 hours per day, 5 days per week, for approximately 26 months at target concentrations of 0, 15, 75 and 375 parts per million (ppm) of air. Exposures commenced 9 October 1990 and were completed on 22 December 1992. Exposure levels were analyzed hourly using an infrared spectrophotometer (IR). MEKO exposure levels and the presence of methyl ethyl ketone (MEK) were determined using gas chromatography four times during the study. Particle size distribution measurements of any background aerosol were made daily for the first two weeks and monthly thereafter. Detailed physical examinations were conducted on all animals pretest and weekly thereafter. Ophthalmoscopic examinations were performed on all animals pretest, at Month 18 and terminal sacrifice and for all animals sacrificed at Months 3 and 12. Body weight measurements were recorded once pretest, weekly through Week 13, monthly through Week 113 and just prior to sacrifice. Hematology and clinical chemistry parameters were evaluated for up to 10 animals/sex/group sacrificed at Months 3, 12 and 18 and at study termination. Differential white blood cell counts were analyzed for all survivors at Months 12 and 18 and at termination of the study. Following approximately 3, 12 and 18 months of exposure, up to 10 animals/sex/group were sacrificed, selected organs were weighed and organ/body and organ/brain weight ratios calculated. Following approximately 26 months of exposure, all survivors were sacrificed, selected organs were weighed and organ/body and organ/brain weight ratios calculated. Histopathological evaluation of selected tissues was performed for all Group I and IV animals and sentinel animals and all animals in Groups II and III which were found dead or sacrificed in a moribund condition prior to study termination. In addition, the eyes, liver, lungs, nasopharyngeal tissues, ovaries, spleen and testes were examined for all animals in Groups II and III.

The cumulative mean MEKO exposure concentrations as determined by IR were  $15 \pm 1$ ,  $75 \pm 2$  and  $374 \pm 10$  ppm, respectively. Particle size distribution determinations indicated no significant test substance aerosol was present in the exposure chambers.

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At termination of the study, in the control group survivorship was 34% in the males and 60% in the females. There was no difference in survivorship among any of the exposure groups including control.

There were no physical observations which were considered MEKO related except for opacities. Ophthalmoscopic examinations of the animals found a dose-related increase in cataracts and a treatment-exaggerated incidence of corneal dystrophy. The dystrophic changes seen in the 374 ppm group were far more severe than in other groups. This increase was probably the result of MEKO exaggerating a strain-related condition already present.

Mean body weights and body weight gains from study initiation were significantly elevated by exposure to MEKO in both the males and females. After 13 weeks of exposure, the 374 ppm males were 13% heavier than the control males and the females were 4% heavier.

At the 3 month sacrifice in the 374 ppm group, methemoglobin was elevated in the males from 0.4 to 1.2%; hemoglobin was decreased 4%; erythrocytes were decreased 7%; mean corpuscular volume was increased 2%; mean corpuscular hemoglobin concentration was decreased 4%; platelets were increased 25% and leukocyte counts were increased 6%. Similar effects were seen in the females. The differences were still statistically significantly different at 12 months in the 374 ppm group but tolerance or adaptation seemed to occur for the effects. Most were no longer significantly different by 18 months in the males or 24 months in both sexes.

MEKO-related increases in absolute and relative organ weights were seen in the liver, spleen and testes. At 3 months in the 374 ppm group, liver weights were elevated about 18% and spleen weights were elevated by about 33%. Tolerance or adaptation occurred and the liver and spleen differences decreased over time. However, the increase in testes weight did not. At study termination the 374 ppm group's testes weighed 82% more than the control group's.

Treatment-related macroscopic findings were not observed at 3 or 12 months. At 18 months an increased incidence of red/tan discoloration of the liver and enlarged testes in treated animals appeared to be treatment related. In the chronic study (24 months and all unscheduled deaths), an increased incidence of red/tan discoloration and nodules/masses of the liver, enlarged testes, and opacity of the eyes in treated animals; and reduced incidence of enlarged spleens in animals of Group IV appeared to be treatment related.

There were a number of treatment-related microscopic findings. Congestion of the spleen with pigment in reticuloendothelial cells and extramedullary hematopoiesis appeared to be treatment related in the 374 ppm animals at the 3 month, 12 month and 18 month sacrifices. However, at the terminal sacrifice these findings were masked by the high incidence of mononuclear cell leukemia in animals other than the 374 ppm animals and could not be evaluated. Findings which appeared treatment related at 12 and 18 months and in the chronic study were seen in the liver and nasal turbinates. The liver changes were increased incidence of basophilic foci and hepatocellular vacuoles and decreased incidence of hyperplasia/proliferation of the biliary duct and peribiliary fibrosis. The turbinate changes were degenerative changes of olfactory epithelium eosinophilic/basophilic material/erythrocytes in the lumen of nasal turbinate sections 2,3,

and 4: and a decrease in the incidence of eosinophilic droplets in olfactory epithelium in treated animals. Further findings which appeared treatment related only in the chronic study animals were seen in the liver and eyes. The liver changes were increased incidence of hepatocellular carcinoma and adenoma and spongy hepatitis. The eye changes were eosinophilic material and inflammatory cells in the anterior chamber, mineralization and neovascularization of the cornea and cataracts.

In conclusion, under the exposure conditions of this study, MEXO was a liver carcinogen in the male rat at 75 ppm.

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I. INTRODUCTION:

This study was conducted for the Industrial Health Foundation, who was acting as an agent for the following companies: AlliedSignal, Inc., Aceto Corp., Akzo Chemicals, Inc., Huels America, Inc., Dussek Campbell Ltd., Troy Corp., and Mooney Chemicals, Inc. This study was designed to evaluate the oncogenic effects of methylethylketoxime when administered by whole-body inhalation to Fischer 344 rats (80/sex/group) and CD-1 mice (60/sex/group). This report presents the results from the rat portion of the study. The mouse results are presented in a separate report. The test substance was administered for 6 hours per day, 5 days per week, for approximately 26 months at target concentrations of 0, 15, 75 and 375 parts per million (ppm) of air.

Species and strain of test animal, method and route of test substance administration and target exposure levels were determined by the sponsor.

This study was mandated by an Environmental Protection Agency test rule issued 13 September 1989 under TSCA section 4(a) and was performed in compliance with 40 CFR Part 798.3300 oncogenicity test standard and the 40 CFR part 792 Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. The facilities of Pharmaco LSR Inc., Toxicology Services North America, and this study were conducted in accordance with the requirements and recommendations of the Animal Welfare Act (P.L. 89-544 as amended by P.L. 91-579 and P.L. 94-279), and other applicable federal, state and local laws, regulations and policies.

This study was conducted at Pharmaco LSR Inc. (formerly Bio/dynamics, Inc.), Toxicology Services USA, Mettlers Road, East Millstone, New Jersey 08875-2360. All raw data, specimens, the original study protocol, the original final report and a sample of the test substance are stored in the Archives of Pharmaco-LSR.

II. MATERIALS AND METHODS:

A. Study Dates:

Study Initiation Date: 21 September 1990  
(Date Study Director signed the protocol)

Receipt of Test Animals: 25 September 1990

Initiation of Exposures: 9 October 1990  
(Experimental Start Date)

Termination of Exposures: 18, 21 and 22 December 1992

Necropsy: 21, 22 and 23 December 1992

Study Completion Date: Date final report is signed by Study Director.

B. Test Substance:

Methylethylketoxime

Supplier: AlliedSignal Inc.  
Allied Fibers  
P.O. Box 761  
Hopewell, Virginia 23860

Dates Received: 19 September 1990 (4 containers)

Lot No.: 911002

C.A.S. No.: 96-29-7

Concentration: 99.9% Active Ingredient

Description: Clear colorless to light yellow liquid;  
hardly discernible ethereal aroma.

Analysis: The synthesis, fabrication, and/or  
derivation of the test substance have  
been documented by the supplier.

Physical Properties: The nature of the test substance and its  
solubility, melting/boiling point, vapor  
pressure and flammability have been  
documented by the supplier.

Stability and Purity: The stability and purity of the test  
substance over the duration of the study  
were documented by the Department of  
Metabolism and Analytical Chemistry at  
Pharmaco LSR Inc. These analyses were  
performed using standards supplied by the  
sponsor. (See Appendix A-9).

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II. MATERIALS AND METHODS (cont.):

B. Test Substance (cont.):

Storage: The test substance was stored in 55 gallon drums away from ignition sources. A nitrogen headspace was maintained in the drum as the test substance was being dispensed.

Sample Retention: An archival sample of approximately 10 milliliters of test substance is stored in the Archives of Pharmaco LSR Inc.

Disposition: All remaining containers of the test substance will be returned to the sponsor.

C. Test Animal:

Rat

Strain: Fischer 344

Justification for Animal Selection: Standard laboratory animal for inhalation toxicity studies. The Fischer 344 rat was used due to its availability and due to the existing historical data base for comparative evaluation.

Number of Animals:

Received: 767 total (383 males, 384 females)

Placed on Test: 640 total (320 males, 320 females)

Supplier: Charles River Breeding Laboratories, Inc. Kingston, New York 12484

Date of Birth:

Males: 28 August 1990

Females: 28 August 1990

Date Received: 25 September 1990

Age at Receipt (approx.): 4 weeks

Age at Initiation of Exposure (approx.): 6 weeks

0-0-1-5

II. MATERIALS AND METHODS (cont.):

C. Test Animal (cont.):

Weight at Initiation of Exposure (grams):

	<u>Mean</u>	<u>Range</u>
Males:	101.6	63.6-203.2
Females:	84.5	51.8-133.6

Acclimation Period:

Animals were acclimated for approximately 2 weeks (25 September through 8 October 1990). All animals received a physical examination by the staff veterinarian during the acclimation period.

Sentinel Control:

A sentinel control group of 20 animals per sex were housed in the same racks and exposed along with the control animals. Five animals per sex were sacrificed after approximately 0, 6, 12 and 18 months for macroscopic, microscopic, histopathological (See page 20) and virological (see page 12) examination in order to monitor the health status of the animals.

D. Selection:

More animals than required for the study were purchased and equilibrated. Animals considered unsuitable for the study on the basis of pretest physical examinations, outlying body weight or ophthalmoscopic examination were eliminated prior to random selection for group assignment.

E. Group Assignment:

Animals considered suitable for study were distributed into 4 groups of 80 animals per sex by a computerized random sort program so that body weight means for each group were comparable. Groups were assigned to control and exposure groups randomly.

F. Animal Identification:

Each rat was identified with a tail tattoo bearing its unique Pharmaco LSR Inc. animal number. In addition, each exposure was identified with the animal number.

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## II. MATERIALS AND METHODS (cont.):

## G. Experimental Outline:

Group	Test Exposure Level <sup>a</sup> (ppm)	Number of Animals											
		On-Test (M/F)	Clinical Laboratory Studies <sup>b</sup>				Blood Smears		Necropsy				Histo- pathology <sup>c</sup> (M/F)
			Mo. 3 (M/F)	Mo. 12 (M/F)	Mo. 18 (M/F)	Term. (M/F)	Mo. 12 (M/F)	Mo. 18 (M/F)	Mo. 3 (M/F)	Mo. 12 (M/F)	Mo. 18 (M/F)	Term. (M/F)	
I (Control)	0	80/80	10/10	10/10	10/10	10/10	60/60	49/46	10/10	10/10	10/10	17/30	80/80
II (Low)	15	80/80	10/10	10/10	9/10	10/10	59/60	49/45	10/10	10/10	9/10	19/29	80/80
III (Mid)	75	80/80	10/10	10/10	9/10	10/10	58/60	44/46	10/10	10/10	9/10	14/30	80/80
IV (High)	375	80/80	10/10	10/9	9/10	10/10	59/59	47/48	10/10	10/9	9/10	22/39	80/80

<sup>a</sup>Exposures were conducted for 6 hours/day, 5 days/week for approximately 26 months.

<sup>b</sup>Hematology and clinical chemistry parameters were evaluated for up to 10 animals/sex/group sacrificed at Month 3, Month 12 and Month 18 and at study termination.

<sup>c</sup>Histopathological evaluation of selected tissues was performed for all Group I and IV and sentinel animals and all animals in Groups II and III which were found dead or sacrificed in a moribund condition prior to study termination. In addition, the eyes, liver, lungs, nasopharyngeal tissues, ovaries, spleen and testes were examined for all animals in Groups II and III.

## H. Husbandry:

During Non-Exposure  
Periods:

Currently acceptable practices of good laboratory husbandry were followed, e.g., Guide for the Care and Use of Laboratory Animals: DHHS Publication No. (NIH) 85-23 Revised 1985. Pharmaco LSR Inc. is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

## Housing:

Animals were doubly housed in suspended stainless steel wire mesh cages during the first week of the acclimation period and were individually housed thereafter.

## Food:

ad libitum; standard pellet laboratory diet (Purina Rodent Laboratory Chow<sup>®</sup> Brand Animal Diet #5002). Fresh food presented twice weekly.

## Water:

ad libitum; by automated watering system (Elizabethtown Water Company).

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

During Non-Exposure  
Periods (cont.):

Contaminants: There are no known contaminants reasonably expected to be found in food or water which would interfere with the results of this study. Batch certification of feed analyses, provided by Purina, and monthly water analyses, provided by Elizabethtown Water Company, are maintained on file at Pharmaco LSR Inc.

Environmental  
Conditions: Approximately 12 hour light/dark cycle (6 AM to 6 PM) via automatic timer. Temperature and relative humidity were monitored and recorded twice daily and maintained, to the maximum extent possible, within the range presented below.

Temperature: Desired: 18-26°C  
Actual: 22±1°C ( $\bar{X}$ ±SD)

Humidity: Desired: 40-70%  
Actual: 54±12% ( $\bar{X}$ ±SD)

During Exposure Periods:

Housing: Animals were individually housed in stainless steel, wire mesh cages within 10,000 liter glass and stainless steel exposure chambers (see Figure 1).

Food: None

Water: None

II. MATERIALS AND METHODS (cont.):

H. Husbandry (cont.):

During Exposure  
Periods (cont.):

Environmental  
Conditions:

Chamber temperature and relative humidity were monitored and recorded at least 12 times during exposure. Oxygen content was maintained at >19%.

Temperature:	<u>Range</u>	<u>Mean±SD</u>
Desired:	18-26°C	
Actual:	16-31°C	22±1°C
Humidity:	<u>Range</u>	<u>Mean±SD</u>
Desired:	40-70%	
Actual:	7-79%	48±8%

I. Test Substance Administration  
and Chamber Operation:

Route: Inhalation, administered into the breathing zone of the animals as a vapor.

Justification of Route of Administration: Inhalation was chosen as the route of administration to simulate potential exposure during manufacture and/or use.

Target Exposure Levels: 0 (control), 15, 75 and 375 ppm.

Justification of Exposure Levels: The target exposure levels of 15, 75, and 375 ppm were selected based upon the previously conducted range-find studies (Bio/dynamics, Inc. Study Nos. 90-8249 and 90-8278).

Frequency of Exposure: Daily (6 hours per day, 5 days per week for approximately 26 months).

Number of Exposures: 556, 557 or 558

Dates of Exposure: 9 October 1990 - 18, 21 or 22 December 1992

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Animal Rotation: The placement of each animal within the chamber was rotated weekly to assure uniform exposure of the animals.

Pre-Study Trials: Prior to the start of the study, chamber validation trials were conducted to ensure homogeneity of the test atmosphere and to determine reproducibility over a 5 day period of target concentrations for the various exposure levels. The results of these trials are maintained in the data.

Chamber Operation: The glass and stainless steel exposure chambers (Figure 1) in which the animals were exposed had a total volume of 10,000 liters. The exposure chambers were operated slightly negative and the airflow rate, N2 flow rate from the test substance generation apparatus, total chamber flow rate, percent oxygen, time for air change and time for equilibration (T99) for each group are summarized below:

Group	Flow Rate (lpm)			O2 (%)	Air Change (min.)	T99 (min.)
	N2	Air	Total			
I	80	2060	2060	a	4.8	22.3
II	40	2230	2230	21	4.5	20.6
III	70	2080	2080	20	4.8	22.1
IV	80	2010	2010	20	5.0	22.9

<sup>a</sup>Analysis not performed because of technician oversight.

This chamber size and airflow rate were adequate to maintain the oxygen concentration above 19% with an animal loading of less than 5% of the chamber volume. The chamber was exhausted through a coarse filter, a HEPA filter, a charcoal filter and into a MOCO

## II. MATERIALS AND METHODS (cont.):

### I. Test Substance Administration and Chamber Operation (cont.):

Chamber Operation (cont.): incinerator. A Carbtrol® charcoal drum was attached, via 2" flex tubing, to the exhaust pipe of each chamber as a back-up in case of incinerator shut down.

Recordings of chamber temperature, relative humidity, airflow rate and static pressure were made approximately every half-hour during exposure. See Figure 1 and Appendix A for equipment details.

#### Test Substance Preparation:

The test substance was used as received.

#### Exposure Procedure:

##### Group I (Control):

Heated nitrogen (approximately 150°F) was delivered through a Whitey® metering valve, via 1/4" Teflon® tubing, to a Dwyer® flowmeter regulated by a Nupro® metering valve and then into the turret of the exposure chamber. The nitrogen flow rate was the same as used in Group IV. The animals remained in the exposure chamber for 30 minutes following the exposure to simulate chamber clearing, using conditioned air at the same airflow rate used during exposure. See Figure 1 and Appendix A for equipment details.

##### Group II (15 ppm):

An appropriate amount of test substance was placed into a 5 gallon Protectoseal® laboratory can and connected to a FMI fluid metering pump (RPG-6 pump with an 1/8" piston) with an initial pump setting of 50%.<sup>1</sup> A nitrogen bag was attached to the can to maintain a nitrogen headspace within the can while allowing the test substance to be pumped from the can. The test substance was fed from the pump, via 1/8" Teflon® tubing, into the liquid inlet at the top of the counter current

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<sup>1</sup>A 100% FMI pump setting is rated at 0.4 ml/min for a 1/8" piston.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group II (cont.):

volatilization generator. The volatilization generator had a coiled glass rod insert heated internally by a nichrome heating element (approximately 100°F). The temperature of the heating element was controlled by a STACO variable autotransformer. Nitrogen delivered through a Whitey® metering valve heated by a Lindberg® Compact tube furnace (to approximately 130°F) branched into a generation flow and a purge flow. The generator nitrogen flow was delivered, via 1/4" Teflon® tubing, to a Dwyer® flowmeter regulated by a Nupro® metering valve and into the bottom of the counter current unit where it flowed counter to the test substance within the generator for volatilization of the test substance. The purge nitrogen flow was delivered, via 1/4" Teflon® tubing, to a Dwyer® flowmeter regulated by a Nupro® metering valve and into the center of the heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating element of oxygen. As a result, the heating and volatilization of the test substance was diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. A Sentry digital alarm module was used to monitor the temperature of the nitrogen stream leaving the volatilization generator. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon® tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure. See Figures 1 and 2 and Appendix A for details.

## II. MATERIALS AND METHODS (cont.):

### I. Test Substance Administration and Chamber Operation (cont.):

#### Exposure Procedure (cont.):

##### Group III (75 ppm):

An appropriate amount of test substance was placed into a 5 gallon Protectoseal<sup>®</sup> laboratory can and connected to a FMI fluid metering pump (RPG-6 pump with an 1/4" piston) with an initial pump setting of 35%.<sup>2</sup> A nitrogen bag was attached to the can to maintain a nitrogen head space within the can while allowing the test substance to be pumped from the can. The test substance was fed from the pump, via 1/8" Teflon<sup>®</sup> tubing, into the liquid inlet at the top of the counter current volatilization generator. The volatilization generator had a coiled glass rod insert heated internally by a nichrome heating element (approximately 100°F). The temperature of the heating element was controlled by a STACO variable autotransformer. Nitrogen delivered through a Whitey<sup>®</sup> metering valve heated by a Lindberg<sup>®</sup> Compact tube furnace (to approximately 140°F) branched into a generation flow and a purge flow. The generator nitrogen flow was delivered, via 1/4" Teflon<sup>®</sup> tubing, to a Dwyer<sup>®</sup> flowmeter regulated by a Nupro<sup>®</sup> metering valve and into the bottom of the counter current unit where it flowed counter to the test substance within the generator for volatilization of the test substance. The purge nitrogen flow was delivered, via 1/4" Teflon<sup>®</sup> tubing, to a Dwyer<sup>®</sup> flowmeter regulated by a Nupro<sup>®</sup> metering valve and into the venter of the heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating element of oxygen. As a result, the heating and volatilization of the test substance was diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. A Sentry digital alarm module was used to monitor the temperature of the nitrogen stream

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<sup>2</sup>A 100% FMI pump setting is rated at 1.6 ml/min for a 1/4" piston.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group III (cont.):

leaving the volatilization generator. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon<sup>®</sup> tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure. See Figures 1 and 2 and Appendix A for details.

Group IV (375 ppm):

An appropriate amount of test substance was placed into a 5 gallon Protectoseal<sup>®</sup> laboratory can and connected to a FMI fluid metering pump (RPG-20 pump with an 1/4" piston) with an initial pump setting of 42%.<sup>3</sup> A nitrogen bag was attached to the can to maintain a nitrogen headspace, within the can while allowing the test substance to be pumped from the can. The test substance was fed from the pump, via 1/8" Teflon<sup>®</sup> tubing, into the liquid inlet at the top of the counter current volatilization generator. The volatilization generator had a coiled glass rod insert heated internally by a nichrome heating element (approximately 100°F). The temperature of the heating element was controlled by a STACO variable autotransformer. Nitrogen delivered through a Whitey<sup>®</sup> metering valve heated by a Lindberg<sup>®</sup> Compact tube furnace (to approximately 150°F) branched into a generation flow and a purge flow. The generation or nitrogen flow was delivered, via 1/4" Teflon<sup>®</sup> tubing, to a Dwyer<sup>®</sup> flowmeter regulated by a Nupro<sup>®</sup> metering valve and into the bottom of the counter current unit where it flowed counter to the test substance within the generator for volatilization of the test substance. The purge nitrogen flow was

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<sup>3</sup>A 100% FMI pump setting is rated at 1.35 ml/min for a 1/8" piston.

II. MATERIALS AND METHODS (cont.):

I. Test Substance Administration and Chamber Operation (cont.):

Exposure Procedure (cont.):

Group IV (cont.):

delivered, via 1/4" Teflon<sup>®</sup> tubing, to a Dwyer<sup>®</sup> flowmeter regulated by a Nupro<sup>®</sup> metering valve and into the venter of the heated coiled glass insert (at a flowrate of 2 lpm) to purge the heating element of oxygen. As a result, the heating and volatilization of the test substance was diluted to below its lower explosive limit prior to mixing with air in the exposure chamber. A Sentry digital alarm module was used to monitor the temperature of the nitrogen stream leaving the volatilization generator. The test atmosphere was directed into the turret of the exposure chamber which housed the animals, via 1/2" Teflon<sup>®</sup> tubing. The animals remained in the chamber for 30 minutes following the exposure to allow the chamber to clear, using conditioned air at the same air flowrate used during the exposure. See Figures 1 and 2 and Appendix A for details.

J. Exposure Chamber Sampling:

1. Chamber Sampling:

a. MEKO MIRAN<sup>®</sup> Analyses:

Samples for determination of Methyleneketoxime (MEKO) exposure levels were analyzed using a MIRAN<sup>®</sup> 1A Ambient Air Analyzer equipped with a Omniscrite Recorder strip chart recorder. The test atmosphere was drawn through 1/4" Teflon<sup>®</sup> tubing and a particulate filter and then into the MIRAN<sup>®</sup>. A General Electric pump was used to draw the samples through the MIRAN<sup>®</sup>. In line was a Dwyer flowmeter, a Marsh vacuum gauge and a Nupro<sup>®</sup> metering valve. Samples were withdrawn hourly during exposure from the normal sampling portal (designated H-1 in Figure 1). The resultant absorbance was read off of a Micronta<sup>®</sup> LCD Bench Top digital

II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling (cont.):

1. Chamber Sampling (cont.):

a. MEKO MIRAN®

Analyses (cont.): multimeter. The exposure levels were determined by comparison of the resultant absorbance to a calibrated response curve constructed using the same instrument settings. (See Appendix A for equipment details.)

b. MEKO Gas Chromatography Analyses:

Gas chromatographic confirmation of the MEKO exposure levels were conducted after approximately 6, 12, 18 and 24 months of exposure. Two midjet glass impingers were filled with 15 mls of methanol. The two impingers were connected in tandem using glass elbow connectors. A calibrated mass flowmeter, equipped with a Nupro® metering valve, was attached to the outlet of the second impinger. Air was drawn through the system by a Thomas air pump at a calibrated flow rate of 1.00 lpm for 8 minutes (Groups I and II), .200 lpm for 10 minutes (Group III) and .200 lpm for 5 minutes (Group IV) as measured by a Galab timer. The sample air was exhausted into the in-house exhaust system. After sample collection, additional methanol was added, as needed, to bring the samples back up to 15 mls. The samples were then placed in labelled scintillation vials and the vials were transferred to the Metabolism and Analytical Chemistry Department of Pharmaco LSR, Inc. for analysis. (See Appendix A for equipment details.)

c. MEK Chromatography Analyses:

Gas chromatographic evaluation of the MEK exposure levels were conducted after approximately 6, 12 and 18 months of exposure. A Thomas air pump was used to draw sample air from the chamber. The flow rate<sup>4</sup> was measured by Mass flowmeter

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<sup>4</sup>The flow rates were .204 lpm (Group I), .201 lpm (Group II), .163 lpm (Group III) and .127 lpm (Group IV).

0-0 2 5

II. MATERIALS AND METHODS (cont.):

J. Exposure Chamber Sampling (cont.):

1. Chamber Sampling (cont.):

c. MEK Chromatography  
Analyses (cont.):

and adjusted by a Nupro<sup>®</sup> metering valve. The chamber atmosphere was drawn through 1/4" Teflon<sup>®</sup> tubing into two charcoal tubes connected in tandem. The tubes were fed through the H-11 sampling port to sample the chamber air and air was exhausted back into the chamber. The sample time was 240 minutes as measured by a Galab timer. The charcoal tubes were then transferred to the Metabolism and Analytical Chemistry Department of Pharmaco LSR, Inc. for analysis. (See Appendix A for equipment details.

2. Chamber Distributions: Distribution samples were taken prestudy and after approximately 6, 12, 18 and 24 months of exposure. Samples were taken using a MIRAN<sup>®</sup> 1A Ambient Air Analyzer from the H-4, H-6, H-7, H-9, H-12, H-14, H-15 and H-17 sampling portals within the chamber. See Figure 1 for location of sampling portals and B-7 for results.

3. Particle Size  
Distribution  
Analysis:

Particle size distribution measurements were performed daily for the first two weeks and monthly thereafter for chamber air using a TSI Aerodynamic Particle Sizer. An IBM PS/2 Model 30 computer was used to program the system to the appropriate settings prior to sampling. The particle size distributions were calculated by the computer and printed out on a EPSON LQ-500 dot matrix printer based on the amount of material collected by the particle sizer. See Appendix A for equipment details.

4. Nominal Concentration: The nominal concentration was determined by weighing the generation apparatus containing the test substance before and after the exposure and dividing the difference in these weights by the total volume of air delivered during exposure (volumetric flow rate times total exposure time).

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II. MATERIALS AND METHODS (cont.):

K. Physical Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:

Twice daily, once in the morning and once in the afternoon.

Detailed Physical Examinations:

Pretest and weekly thereafter.

L. Ophthalmoscopic Examination:

Ophthalmoscopic evaluations were performed at the time intervals listed below. Examinations were performed by Lionel F. Rubin, V.M.D., Diplomate, American College of Veterinary Ophthalmologists. See Appendix A for Methodology and References.

Number of Animals:

Performed on all animals pretest, Month 18 and at terminal sacrifice. Performed on all animals sacrificed at Months 3 and 12.

Time Intervals

Pretest: 2 October 1990  
Month 3: 10 January 1991  
Month 12: 9 October 1991  
Month 18: 7 and 24 April 1992  
Termination: 16 December 1992

M. Body Weight:

Once pretest, weekly through Week 13, monthly thereafter and prior to termination. See Appendix A for Methodology and References.

N. Laboratory Studies:

At scheduled intervals blood was obtained via a tail nick in unanesthetized animals. See Appendix A for Methodology and References.

Number of Animals:

Performed on all survivors at the time intervals listed below:

Time Intervals

Month 12: 14 through 18 October 1991  
Month 18: 13, 14, 15, 16, 20, 21, 22 and 23 April 1992  
Termination: 21, 22 and 23 December 1992

II. MATERIALS AND METHODS (cont.):

N. Laboratory Studies (cont.):

Parameter Evaluated: differential leukocyte counts

Blood was also obtained via venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anesthesia. Animals were fasted overnight prior to blood collections and were not exposed until after samples were collected. Number of Animals: Performed on up to 10 animals/sex/group at Months 3, 12 and 18 and at Termination.

Time Interval

Month 3: 10 January 1991  
Month 12: 10 October 1991  
Month 18: 13 and 14 April 1992  
Termination: 21, 22 and 23 December 1992

Parameter Evaluated:

Hematology: methemoglobin concentration  
hemoglobin concentration  
hematocrit  
erythrocyte count  
reticulocyte count  
platelet count  
mean corpuscular volume (calculated)  
mean corpuscular hemoglobin (calculated)  
mean corpuscular hemoglobin concentration (calculated)  
total and differential leukocyte counts  
erythrocyte morphology

Clinical Chemistry:

serum glutamic oxaloacetic transaminase  
serum glutamic pyruvic transaminase  
alkaline phosphatase  
lactic acid dehydrogenase  
blood urea nitrogen  
fasting glucose  
cholesterol  
triglycerides  
total protein  
albumin  
globulin (calculated)  
creatinine  
uric acid  
total bilirubin  
sodium

II. MATERIALS AND METHODS (cont.):

N. Laboratory Studies (cont.):

Parameter Evaluated (cont.):

Clinical Chemistry  
(cont.):

potassium  
chloride  
calcium  
inorganic phosphorus  
creatin kinase  
gamma glutamyl transpeptidase

Sentinel Animals:

A virology screen was performed on up to 5 animals per sex pretest and at Months 6, 12 and 18. The serum samples were analyzed by Charles River Laboratories, Inc. The virology data was used to document any viral antibody titers present in the study animals at the designated monitoring intervals. Assays included:

Sendai virus (SEN)  
Pneumonia virus of mice (PVM)  
Reovirus type 3 (REO-3)  
Mycoplasma pulmonis (MPUL)  
Rat coronavirus/Sialodacryoadenitis virus (RCV/SDA)  
Kilman rat virus (KRV)  
Toolan's H-1 virus (H-1)

O. Postmortem:

Gross Postmortem  
Examination:

Performed on all animals found dead, dying accidentally, sacrificed in a moribund condition or killed at the scheduled sacrifice intervals. Examinations included the external surface, all orifices, the cranial cavity, carcass, the external surfaces of the brain and spinal cord, nasal cavity, the thoracic, abdominal and pelvic cavities and their viscera and cervical tissues and organs. Animals were fasted prior to scheduled sacrifices.

II. MATERIALS AND METHODS (cont.):

0. Postmortem (cont.):

Necropsy:

Month 3: 10 January 1991  
 Month 12: 10 October 1991  
 Month 18: 13 and 14 April 1992  
 Termination: 21, 22 and 23 December 1992  
 Sacrifice Method: Exsanguination while under ether anesthesia.

Organs Weighed, Organ/Body and Organ/Brain Weight Ratios Calculated:

The following organs were weighed for all animals at the scheduled sacrifice intervals. Paired organs were weighed together. See Appendix A for Methodology and References.

adrenals  
 brain  
 kidneys  
 liver  
 lungs  
 ovaries  
 testes  
 spleen

Tissues Preserved:

The following tissues were preserved for all animals. Number in parentheses indicates number of organs/sections preserved.

adrenals (2)  
 aorta (abdominal)  
 bone (femur, sternum)  
 bone marrow smear  
 brain (including medulla/pons, cerebral cortex and cerebellar cortex)  
 clitoral gland  
 esophagus  
 exorbital lacrimal gland  
 eyes (2)  
 gall bladder  
 heart

II. MATERIALS AND METHODS (cont.):

0. Postmortem (cont.):

Tissues Preserved (cont.): intestine  
cecum  
colon  
duodenum  
ileum  
jejunum  
rectum  
kidneys (2)  
larynx  
liver (left lateral and median lobes)  
lungs (mainstem bronchi and trachea)  
lymph nodes (mesenteric, peribronchial)  
mammary gland (right inguinal with skin)  
nasopharyngeal tissues  
nerve (sciatic with biceps femoris)  
ovaries (2)  
pancreas  
penis  
pituitary  
preputial gland  
prostate  
salivary gland (submandibular)  
seminal vesicles  
skin (with mammary gland)  
spinal cord (cervical, mid-thoracic,  
lumbar)  
spleen  
stomach  
testes with epididymides (2)  
thymic region  
thyroid/parathyroids (2)  
trachea  
urinary bladder  
uterus (corpus and cervix uteri)  
vagina  
tissue masses  
gross lesions (including a section of  
normal-appearing portion of same tissue)

II. MATERIALS AND METHODS (cont.):

O. Postmortem (cont.):

Tissues Examined  
Histopathologically:

The following tissues were examined for all Group I and IV and sentinel animals and all animals in Groups II and III which were found dead or sacrificed in a moribund condition prior to study termination. Number in parentheses indicates number of organs/sections examined. In addition, the eyes, liver, lungs, nasopharangeal tissues, ovaries, spleen and testes were examined for all animals in Groups II and III.

adrenals (2)  
aorta (abdominal)  
bone (femur, sternum)  
bone marrow (sternum, femur)  
brain (including frontal cortex and basal ganglia, parietal cortex and thalamus and cerebellum and pons)  
clitoral gland  
esophagus  
exorbital lacrimal gland  
eyes (1)  
heart  
intestine  
  cecum  
  colon  
  duodenum  
  ileum  
  jejunum  
  rectum  
kidneys (2)  
larynx  
liver (2 - from separate lobes)  
lungs (2 - each lobe and mainstem bronchi)  
lymph nodes (mesenteric, peribronchial)  
nasopharyngeal tissue (4)  
nerve (optic, sciatic with biceps femoris)  
ovaries (1)  
pancreas  
pituitary  
preputial gland  
prostate  
salivary gland (submandibular)

II. MATERIALS AND METHODS (cont.):

O. Postmortem (cont.):

Tissues Examined  
Histopathologically  
(cont.):

seminal vesicles  
skin (with mammary gland)  
spinal cord (3)  
spleen  
stomach  
testes with epididymides (1)  
thymic region  
thyroid/parathyroid (1)  
trachea  
urinary bladder  
uterus (corpus and cervix uteri)

Preservatives:

Blood and Bone Marrow  
Smears:

Methanol

All Other Tissues:

10% neutral buffered formalin (eyes, testes and epididymides were placed in Bouin's solution for the initial 48-72 hours and then retained in formalin). Lungs and urinary bladder were infused with formalin to insure fixation.

Stain:

Hematoxylin and Eosin  
Wrights (Gemsa)

See Appendix A for Methodology and References.

P. Statistical Analysis:

Body weight, body weight change from Week 0, hematology and clinical chemistry parameters, organ weights, organ/body and organ/brain weight ratios, survivorship and time to tumor were analyzed. Mean values of all exposure groups were compared to control at each time interval. Statistically significant differences from control are indicated on mean tables of appendices. See Appendix A for Methodology and References.

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II. MATERIALS AND METHODS (cont.):

Q. Protocol Deviations:

The following protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study:

1. Temperature and relative humidity values deviated from the desired range occasionally (see Appendix B-76); no adverse effect on animal health was apparent.
2. Analytical and nominal concentrations for Group III were not within  $\pm 10\%$  of the desired ranges on 27 and 28 February and 1 and 4 March 1991 because of equipment malfunction.
3. Analytical concentrations for some days were based on five samples per day instead of six samples per day because of technician oversight or equipment malfunction.
4. Bulk storage of the test material was in a solvent shed at ambient outside temperature. Prior to administration, the test material was stored at room temperature.
5. Closing drum samples were not taken for drum numbers 4, 5 and 6 because of technician oversight.

III. RESULTS AND DISCUSSION:

A. Chamber Monitoring:

The analytical and nominal concentrations (mean ± standard deviation) of methylethylketoxime (MEKO) for each exposure group are presented in Appendix B, plotted in Figure 3 and are summarized below:

<u>Group</u>	<u>Test Substance Concentration</u>	<u>Target Concentration</u> (ppm)	<u>Analytical Concentration</u> (ppm)	<u>Nominal</u> (ppm)
I	Control	0	0.11 ± 0.79 <sup>a</sup>	-
II	MEKO	15	15 ± 1	15 ± 2
III	MEKO	75	75 ± 2	74 ± 7
IV	MEKO	375	374 ± 10	359 ± 24

<sup>a</sup>During the first few weeks of exposures, MEKO on occasion, appeared to be in the control chamber. These values were subsequently determined to be due to a shift in the baseline produced by differences in room and chamber humidities. Room air was used to flush the spectrophotometer between chamber readings.

The concentration of MEKO as well as MEK (methyl ethyl ketone) was also determined in each chamber four times over the course of the study using a gas chromatographic procedure. The MEKO exposure levels (Appendix B-75) were similar to the chamber concentrations obtained using the infrared spectrophotometer. MEKO was shown to be stable over the duration of the study. In addition, these results show the chamber concentrations of MEK (a possible hydrolysis product of MEKO) were generally less than 1 percent of the MEKO chamber concentrations (3.8 ppm was the maximum in the high exposure chamber).

The mean analytical exposure levels achieved over the duration of this study were very close to the target concentrations. The nominal exposure levels in the mid- and high-exposure levels were approximately five percent lower than the corresponding analytical levels. This slight difference is attributed to interday variations in the chamber airflow. Nominal exposure levels were not

III. RESULTS AND DISCUSSION (cont.):

A. Chamber Monitoring (cont.):

corrected for daily fluctuations in barometric pressure and temperature as these fluctuations had minor impact on these values.

Chamber distribution studies (Appendix B-70) showed there was no significant gradient of the MEKO exposure level within any chamber. The oxygen level was confirmed during chamber trials to be at least 20% in all MEKO exposure chambers. Temperature and humidity values were maintained to the maximum extent possible between 18-26°C and 40-70%, respectively. The occasional excursions from these ranges are presented in Appendix B. These excursions had no apparent effect on the results of this study.

Particle size distributions taken daily for the first two weeks and monthly thereafter (Appendix B) are summarized below.

Group	Test Substance	Average Particle Size Data		
		MMAD ( $\mu\text{m}$ )	GSD	Total Mass Concentration ( $\text{mg}/\text{m}^3$ )
I	Control	2.0	2.4	0.006
II	MEKO	2.3	2.1	0.005
III	MEKO	2.6	2.8	0.006
IV	MEKO	2.3	2.1	0.006

These data show a similar particle size and concentration of background particles among all of the chambers including control. Therefore, there is no indication of any MEKO aerosol within the MEKO exposure chambers.

B. Mortality:

The survivorship data is presented in Appendix C-1 and C-2 and plotted in Figures 4-1 and 4-2. Percent survival at termination of the study after 20 months of exposure is summarized on the following page.

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III. RESULTS AND DISCUSSION (cont.):

B. Mortality (cont.):

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Group	Month 26	
	Percent Survival Males	Females
I	34	60
II	37	58
III	27	60
IV	43	76

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Survivorship was better in the females than in the males but survivorship analyses (Appendix L) found no significant difference in survivorship among the exposure groups.

C. Physical Observations:

Physical observations (Appendix D) which were noted occurred sporadically. There were no physical observations which were considered MEKO-related effects except for opacities which were elevated near study termination in the 374 ppm group.

D. Ophthalmology:

No compound-related ocular abnormalities (Appendix E) were noted in the Months 3 and 12 interim sacrifices. At Month 18, an increased number of cataracts was seen in both sexes in the 374 ppm interim sacrifice group. To further evaluate this, all surviving animals in all groups were examined and the increased incidence of cataracts was confirmed. At the terminal sacrifice, there was a MEKO dose-related increase in cataracts and treatment-exaggerated increase in corneal dystrophy in both sexes. The dystrophic changes seen in the 374 ppm group were far more severe than in other groups. This increase was probably the result of MEKO exaggerating a strain-related condition already present. The incidence of corneal dystrophy and cataracts as found by the ophthalmoscopic examinations at the terminal sacrifice are presented on the following page.

### III. RESULTS AND DISCUSSION (cont.):

#### D. Ophthalmology (cont.):

<u>Incidence of Corneal Dystrophy</u>			
<u>Group</u>	<u>Males</u>	<u>Females</u>	<u>Total</u>
I	13/17 (76%)	26/31 (84%)	39/48 (81%)
II	19/20 (95%)	31/31 (100%)	50/51 (98%)
III	14/16 (88%)	26/32 (81%)	40/48 (83%)
IV	20/22 (91%)	40/40 (100%)	60/62 (97%)

## = Number found over number examined.

<u>Incidence of Cataract of All Types</u>			
<u>Group</u>	<u>Males</u>	<u>Females</u>	<u>Total</u>
I	9/17 (53%)	14/31 (45%)	23/48 (48%)
II	3/20 (15%)	14/31 (45%)	17/51 (33%)
III	12/16 (75%)	19/32 (59%)	31/48 (65%)
IV	14/22 (64%)	33/40 (83%)	47/62 (76%)

## = Number found over number examined.

#### E. Body Weights:

Mean body weights and gains from Week 0 (Appendix F and Figure 5) were significantly elevated by exposure to MEKO in all groups in the males. After 13 weeks of exposure, the 374 ppm males were 13% heavier than the controls. This dose-related increase in body weight, relative to controls persisted over most of the study and is considered to be MEKO related.

The female weights were also elevated in the MEKO exposure groups but the increase was smaller. At 13 weeks, the 374 ppm females were 4% heavier than the controls and the differences disappeared later in the study. Therefore, the weight differences in the females is of unclear relevance to MEKO exposure.

### III. RESULTS AND DISCUSSION (cont.):

#### F. Laboratory Studies:

##### 1. Hematology:

Many hematology parameters (Appendices G and H) in both the males and females were significantly affected by exposure to 374 ppm of MEKO throughout the duration of this study and sporadically at 75 ppm. At the 3-month interim sacrifice in the males, relative to control values, methemoglobin was elevated from 0.4 to 1.2%; hemoglobin was decreased 4%; erythrocytes were decreased 7%; mean corpuscular hemoglobin was increased 2%; mean corpuscular volume was increased 6%; mean corpuscular hemoglobin concentration was decreased 4%; platelets were increased 25% and leukocyte counts were increased 6%. Similar effects were seen in the females.

The differences were still statistically significantly different at 12 months in the 374 ppm group but tolerance or adaptation seemed to occur for the effects. Most were no longer significantly different by 18 months in the males or 24 months in both sexes.

##### 2. Clinical Chemistry:

The only clinical chemistry parameter (Appendix I) which was consistently affected by MEKO exposure was alanine aminotransferase (ALT) in the males. This enzyme was significantly decreased at the 3, 12 and 18 month interim sacrifices by as much as 63% (12 months). There was also a trend for decreased aspartate aminotransferase (AST) in the males but it was only significantly decreased at 12 months (54%). The toxicological significance of a decrease in these enzymes is not clear.

##### 3. Sentinel Virologies:

Virological analyses of the sentinel animals (Appendix M) at Months, 0, 6, 12 and 18 found no seroconversion for any virus evaluated.

III. RESULTS AND DISCUSSION (cont.):

G. Terminal Organ and Body Weights, Organ/Body Weight and Organ/Brain Weight Ratios:

MEKO-related increases in absolute and relative organ weights were seen in the liver, spleen and testes (Appendix J). In the liver, at the Month 3 interim sacrifice, there was a dose-related increase which was statistically significant in the 374 ppm group. In the 374 ppm group, the absolute liver weights in the males and females were increased relative to control group weights by 23 and 15%, respectively. The weights were still elevated at 12 months but adaptation appeared to take place. At the Month 18 sacrifice, there was no longer any significant differences. At study termination the liver weights were again significantly elevated in the males (40%) relative to control weights. However, the interpretation of this increase in liver weights is confounded by the presence of masses in the liver. The absolute and relative liver weights of animals without any macroscopic evidence of tumors were not significantly different from control values.

The absolute and relative spleen organ weights were increased at the Month 3 and 12 interim sacrifices. At Months 3 and 12 the absolute spleen weights in the 374 ppm group were about 33% greater than the control group spleen weights in both the males and females. Similar to the liver weight effect, adaptation was seen. By study termination there was no significant difference in the spleen weights relative to control weights.

The absolute and relative testes weights were statistically significantly elevated in the 374 ppm group at the Month 3, 18 and terminal sacrifice intervals. At Months 3 and 12 the increase was small but by study termination the 374 ppm testes weights were 82% greater than the control group weights. There was no microscopic correlate associated with this increase in testes weights.

III. RESULTS AND DISCUSSION (cont.):

G. Terminal Organ and Body Weights, Organ/Body Weight and Organ/Brain Weight Ratios (cont.):

Other statistically significant increases in organ weights, or ratios, relative to control were also seen. But, because these did not occur in the high level exposure group, or they only occurred in one sex at one sacrifice interval, they were considered to be spurious.

H. Macroscopic and Microscopic Postmortem Observations:

1. Macroscopic Postmortem Observations (Appendix X):

3 and 12 Month Sacrifices

Macroscopic changes observed either occurred sporadically or with similar incidence in treated and control animals and were not considered to be related to treatment.

18 Month Sacrifice

Tan/red discoloration of the liver was observed in 2/9 male and 2 /10 female rats in Group III and 6/9 male rats of Group IV and appeared to be treatment-related. An increase in the incidence of enlarged testes was observed in male rats from Groups III and IV.

Terminal Sacrifice

Opacity of the eye and red/tan discoloration of the liver occurred with greater incidence in males and females of Group IV. In the males of Group IV, an increased incidence of nodules/masses of the liver and enlarged testes were observed. A reduced incidence of several findings was observed in treated animals compared to the control animals. These included surface irregularity of the eyes and liver, and enlarged spleens (Group IV animals only), and soft and small testes in all treated male groups.

Other macroscopic findings in each interval of the study occurred sporadically and did not appear to be related to treatment.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Postmortem Observations (cont.):

2. Microscopic Postmortem Observations:

3 Month Sacrifice

Increased incidence of congestion of the spleen occurred only in treated males with a greater severity in Group IV males and females; this correlates with significant increases in the splenic organ weights in the high-dose male and female rats. The findings of pigment in reticuloendothelial cells and extramedullary hematopoiesis also occurred with greater severity in Group IV males and females.

12 Month Sacrifice

There was an increase in the severity of congestion in the spleen of male and female rats in Groups II, III and IV; this roughly correlated with increased in the spleen weights in females. In addition, there was an increase in the severity of pigment in reticulendothelial cells in males of Groups III and IV and an increase in the severity of extramedullary hematopoiesis in females in Group IV.

In the liver, the incidence and severity of basophilic foci and vacuoles in hepatocytes were increased in male rats in Group IV. Also in the liver, there was a decrease in the incidence of hyperplasia/proliferation of the biliary duct in male and female rats in Group IV and a decrease of peribiliary fibrosis in male rats in Group IV.

Olfactory degeneration, characterized by thinning of the layers of olfactory epithelium in the dorsal meatus, was observed with greater incidence and severity in turbinate sections 2, 3 and 4 of male and female rats in Group IV; and in some of the turbinate sections in Group III animals. Accumulations of eosinophilic/basophilic material/erythrocytes in the nasal lumen occurred

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Postmortem Observations (cont.):

2. Microscopic Postmortem Observations (cont.):

12 Month Sacrifice (cont.)

with greater incidence and severity in turbinate sections 2 and 4 of females in Groups III and IV. A decrease in the incidence of eosinophilic droplets in the olfactory epithelium was observed in turbinate sections 2, 3 and 4 of animals in Groups II, III and IV.

At one year there seemed to be a slight increase in the incidence of interstitial cell adenomas of the testes in males in Groups III and IV.

18 Month Sacrifice

Treatment-related findings in the spleen were seen in females. There was increased severity of congestion in Groups II, III and IV; greater severity of pigment in reticuloendothelial cells in Group IV; and greater severity of extramedullary hematopoiesis in Group IV.

There was an increase in the severity of basophilic foci and of hepatocyte vacuoles in the liver of male rats in Group IV. In addition, in the liver, there were decreased incidence of peribiliary fibrosis and hyperplasia/proliferation of the biliary duct in males of Group IV.

There were treatment-related degenerative changes present in turbinate sections 2 and 3 of male and female rats in Group IV, and in turbinate section 4 of males in Group IV. There was increased severity and incidence of eosinophilic droplets in the olfactory epithelium in turbinate sections 2, 3 and 4 of males and females in Group I. There was an increase in the incidence and severity of eosinophilic/basophilic material/erythrocytes in the nasal lumen of turbinate sections 3 and 4 of males in Group IV, in turbinate section 3 of females in Group III, and in turbinate section 4 of males and females in Group IV.

0 0 4 3

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Postmortem Observations (cont.):

2. Microscopic Postmortem Observations (cont.):

Terminal Sacrifice

There was a decreased incidence of mononuclear cell leukemia in Group IV male and female animals; with an increase in incidence of this finding in Group I, II and III animals. This depression of tumor rates has been seen with aniline<sup>5</sup> which can also cause methemoglobinemia. Methemoglobinemia was seen in this study in males and females of Group IV at the 3 month sacrifice but was not observed at other times in this study. The findings in the spleen that previously appeared to be treatment-related (congestion, extramedullary hematopoiesis and pigment in reticuloendothelial cells) could not be evaluated in those animals with leukemia, because the tumor cells so effaced the architecture of the organ.

The treatment-related findings in the liver included hepatocellular carcinoma in male Group IV rats, a dose-related increase in hepatocellular adenoma in males of Groups III and IV, an increase in the incidence of basophilic foci in male and female rats of Group IV (with increased severity in males of Groups II, III and IV and in females of Group IV), a slight increase in the incidence of spongiosis hepatis in males of Groups II, III and IV (with an increased severity in Groups III and IV), and slight increases in the incidence of intracytoplasmic vacuoles in male rats of Groups III and IV and in females of Group IV. There was a decrease in the incidence of two findings in the livers of males and females of Group IV, these were peribiliary fibrosis and hyperplasia/proliferation of the biliary duct.

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<sup>5</sup>Pathology of the Fischer Rat, Academic Press, 1990, p.374.

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Postmortem Observations (cont.):

2. Microscopic Postmortem Observations (cont.):

Terminal Sacrifice (cont.)

Degeneration of the olfactory epithelium, primarily in the dorsal meatus of turbinate sections 2, 3 and 4, were present in males and females of Group IV with less severe findings in Group III and even less severe in Group II turbinates. The changes consisted of a thinning of the olfactory epithelium with some loss of apical cytoplasm and fewer layers present, usually in a focal distribution. There were also accumulations of eosinophilic/basophilic material/erythrocytes in the nasal lumen of turbinate sections 2, 3 and 4 in males and females primarily in Groups III and IV. In addition, there was a decrease in the incidence of eosinophilic droplets in olfactory epithelium in turbinate sections 2, 3 and 4 in the animals of treatment groups.

In the eyes, there was an increase in the incidence of eosinophilic material and inflammatory cells in the anterior chamber of males in Group IV, an increase in the incidence of corneal mineralization and neovascularization in male and female rats in Groups III and IV, and an increase in the incidence and severity of cataracts in the eyes of males and females in Group IV.

The enlarged testes observed macroscopically in Group IV males could not be explained by the presence of interstitial cell adenomas which were observed with similar incidence in the treated and control animals.

Other microscopic findings in each interval of the study occurred sporadically and did not appear related to treatment.

### III. RESULTS AND DISCUSSION (cont.):

#### H. Macroscopic and Microscopic Postmortem Observations (cont.):

##### 2. Microscopic Postmortem Observations (cont.):

##### Terminal Sacrifice (cont.)

There appears to be no NOEL (no observable effect level) on the basis of olfactory degenerative changes. With the historical incidence of liver adenomas in Fischer 344 rats in the range of 3-4% (see the table on below), the presence of 2/51 in Group II males is not clearly related to treatment.

Liver Adenoma									
	Historical Control Data						89-8243 <sup>a</sup>		
	R-57	R-59	R-60	R-61	R-92	R-99	II	III	IV
No. Examined (♂/♀):	69/70	79/80	70/70	75/75	50/50	50/50	51/50	51/50	51/51
Adenoma (♂/♀)									
Total Number	0/0	1/1	0/0	0/0	0/0	3/0	2/0	5/2	18/4
Percent	0/0	1.3/1.3	0/0	0/0	0/0	6/0	3.9/0	9.8/4.0	35.3/7.8

<sup>a</sup>Includes the animals killed at terminal sacrifice and all unscheduled deaths.

##### 3. Time to Tumor Analyses:

Time to tumor analyses (Appendix L) were performed when 5 percent of the animals in any group had a specific type of tumor. The tumor type, incidence and statistical significance for the male and females are presented on the following pages.

0 1 4 5

## III. RESULTS AND DISCUSSION (cont.):

## H. Macroscopic and Microscopic Postmortem Observations (cont.):

## 3. Time to Tumor Analyses (cont):

Tumor Type	Male Tumor Incidence			
	Control	15 ppm	75 ppm	374 ppm
Adrenals Pheochromocytomas	9	8	10	14
Liver Adenomas	0	2	5 <sup>*F</sup>	18 <sup>**F+</sup>
Liver Carcinomas	0	0	1	12 <sup>**F+</sup>
Combined Liver Adenomas and Carcinomas	0	2	6 <sup>**F+</sup>	27 <sup>**F+</sup>
Lymphoreticular Mononuclear Cell Leukemia	16	19	19	4
Mammary Gland Fibroadenoma	2	2	4	9 <sup>*</sup>
Mesentery Mesothelioma	1	1	0	4
Pituitary Adenoma	36	18	30	24
Pituitary Carcinoma	1	4	1	0
Skin Fibroma	1	3	7	2
Testicular Adenoma	50	54	57	58
Thyroid Adenoma	12	5	2	10
Thyroid Carcinoma	0	0	1	4
Total Examined	80	80	80	80

Combined groupings reflect animals with at least one tumor of the type mentioned, animals with more than one tumor type cause the incidence of combined incidence to be less than the sum of the individual incidences.

T or T<sup>+</sup>, statistically significant trend test at p<0.05 or p<0.01.  
F or F<sup>+</sup>, statistically significant Fisher Exact incidence test at p<0.05 or p<0.01.

\* or \*\*, statistically significantly different from control at p<0.05 or p<0.01.

### III. RESULTS AND DISCUSSION (cont.):

#### H. Macroscopic and Microscopic Postmortem Observations (cont.):

##### 3. Time to Tumor Analyses (cont):

Tumor Type	Female Tumor Incidence			
	Control	15 ppm	75 ppm	374 ppm
Liver Adenomas T <sup>+</sup>	0	0	2	4
Lymphoreticular Mononuclear Cell Leukemia T <sup>+</sup>	22	13	10 <sup>*F+</sup>	1 <sup>**F+</sup>
Mammary Gland Fibroadenoma	10	7	9	17
Pituitary Adenoma	36	22 <sup>*F</sup>	27	33
Thyroid Adenoma	7	6	2	8
Uterine Endometrial Polyp	15	10	11	22
Total Examined	80	80	80	80

T<sup>+</sup>, statistically significant trend test at p<0.01.

F or F<sup>+</sup>, statistically significant Fisher Exact incidence test at p<0.05 or 0.01.

or \*\*, statistically significantly different from control at p<0.05 or 0.01.

For the males there were no statistically significant differences in tumor latency nor production between the control and treated groups for adrenal pheochromocytomas, pituitary carcinoma and testicular adenoma. For lymphoreticular mononuclear cell leukemia, pituitary adenoma and thyroid adenoma there was a statistically significant greater incidence in the control group than in the treated groups. For skin fibroma there was a statistically significant difference between the mid-dose and control groups, but no apparent dose response.

The results for liver adenomas and combined liver adenomas and carcinomas show a dose response beyond the 0.01 level, and statistically significant differences between the control and high-dose group by the pairwise tests and the Fisher test of incidence the 0.01 level. There were differences between the control and mid-dose group at least at the 0.05 level by the GBW/KW

III. RESULTS AND DISCUSSION (cont.):

H. Macroscopic and Microscopic Postmortem Observations (cont.):

3. Time to Tumor Analyses (cont):

and Fisher tests (Cox's test was at the 0.06 level for liver adenomas). Tests for liver carcinomas were significant beyond the 0.01 level for trend and test for differences between control and the high-dose group.

Tests for mammary gland adenomas showed a significant dose response ( $p < 0.01$ ) and differences between control and high-dose group. There was a significant dose response when testing mesentery mesothelioma, but no individual differences of a treated group from control. There was a significant dose response when testing thyroid carcinomas but no individual differences of a treated group from control (tests of control versus the high-dose group were significant at  $p < 0.06$  with Fisher's test and 0.07 by the GB/KW test).

For the females there were no statistically significant differences in tumor latency nor production between the control and treated groups for pituitary adenoma, thyroid adenoma and uterine endometrial polyps. For lymphoreticular mononuclear cell leukemia, there was a statistically significantly greater incidence in the control group than in the treated groups.

There was a statistically significant dose response for the survivorship corrected incidence of liver adenomas; individual test indicated differences between the control and high-dose group at  $p < 0.06$ . The trend test for mammary gland fibroadenomas was not significant ( $p < 0.06$ ).

The conclusion is that MEKO is related to tumor production for liver adenomas and carcinomas in male Fischer rats at 26 months. For females there was a weak association with liver adenomas.

IV. CONCLUSION:

Under the conditions of this study, MEKO was a liver oncogen in the male rat at 75 ppm.

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*2/14/94*

Date

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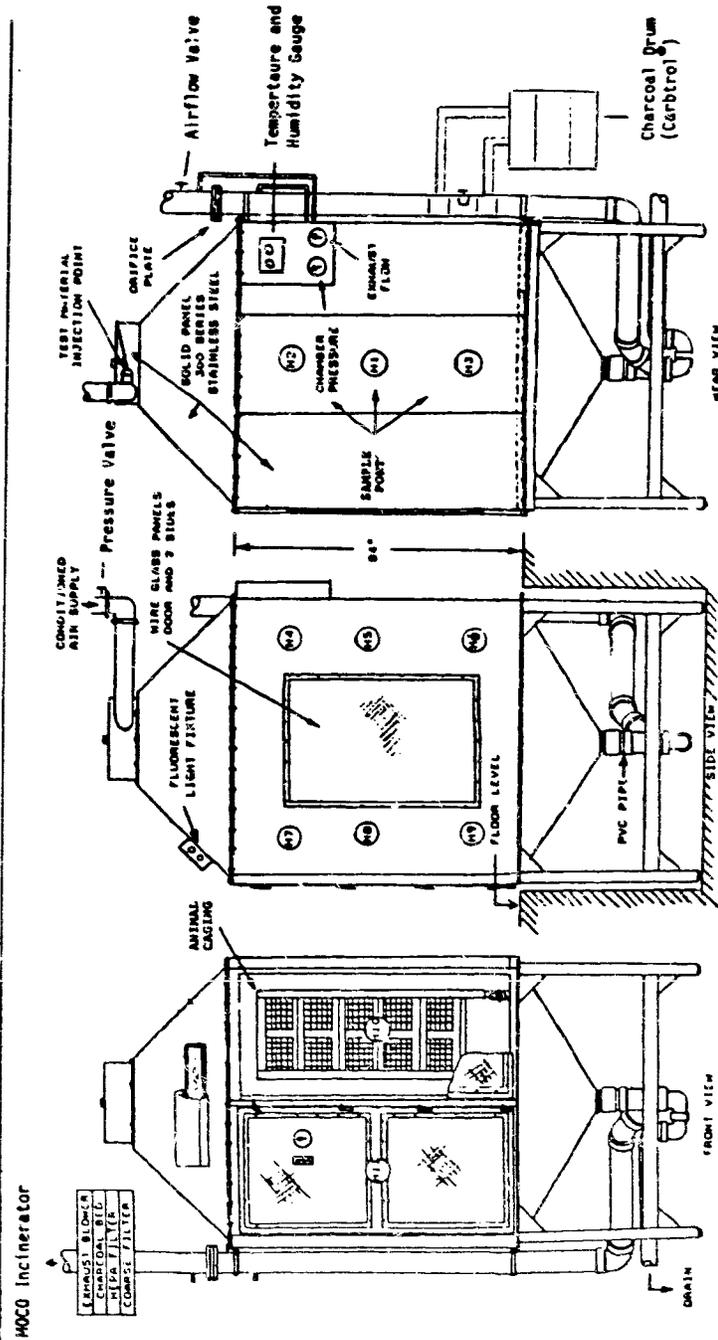
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Date

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F 1-1  
 Figure 1  
 An Inhalation Oncogenicity Study  
 of Methylthioxime in Rats and Mice

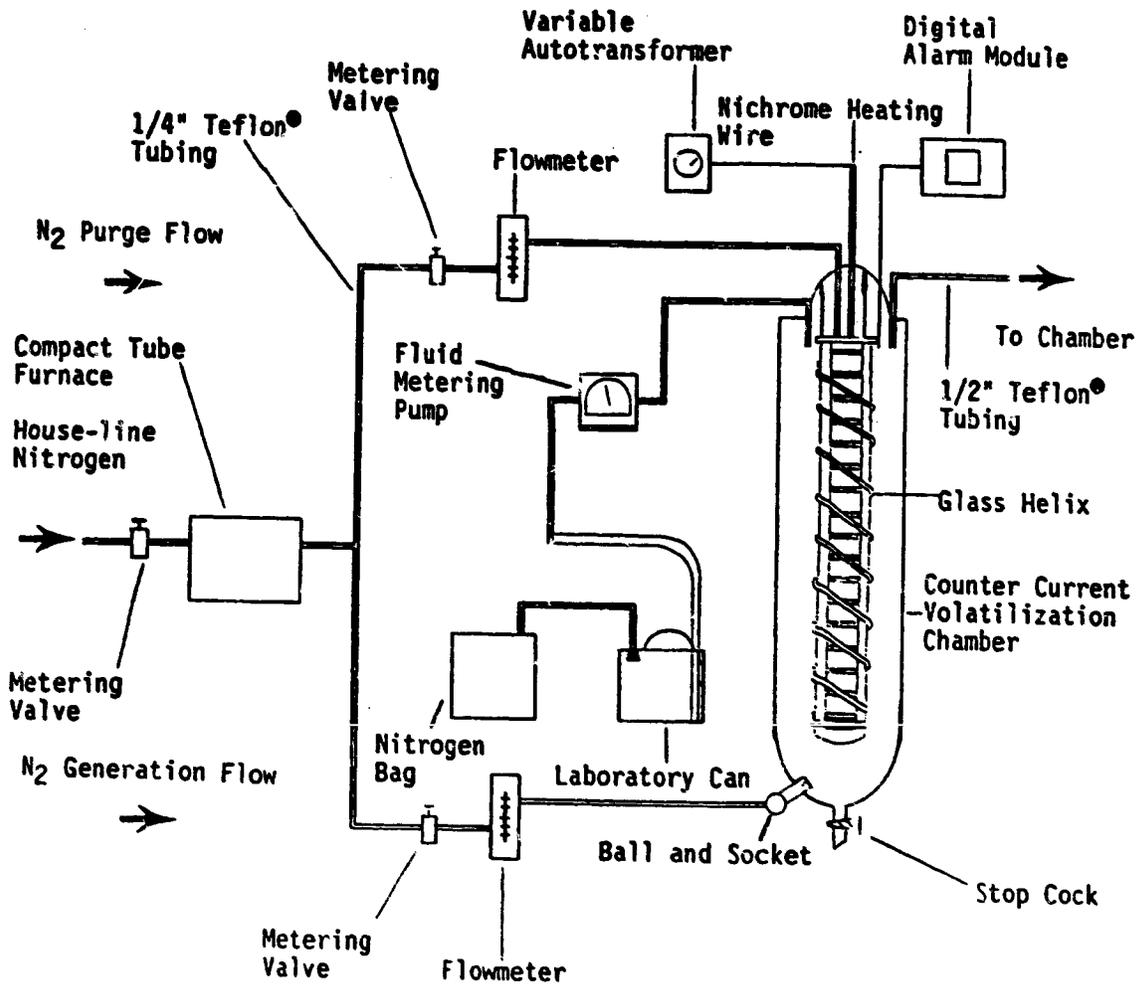
Diagram of 10,000 Liter Chamber



NOTE: This is a stylized drawing of the chambers used. Sample ports H12 - H17 are located on the opposite side of the chamber and correspond to sample ports H4 - H9, respectively.

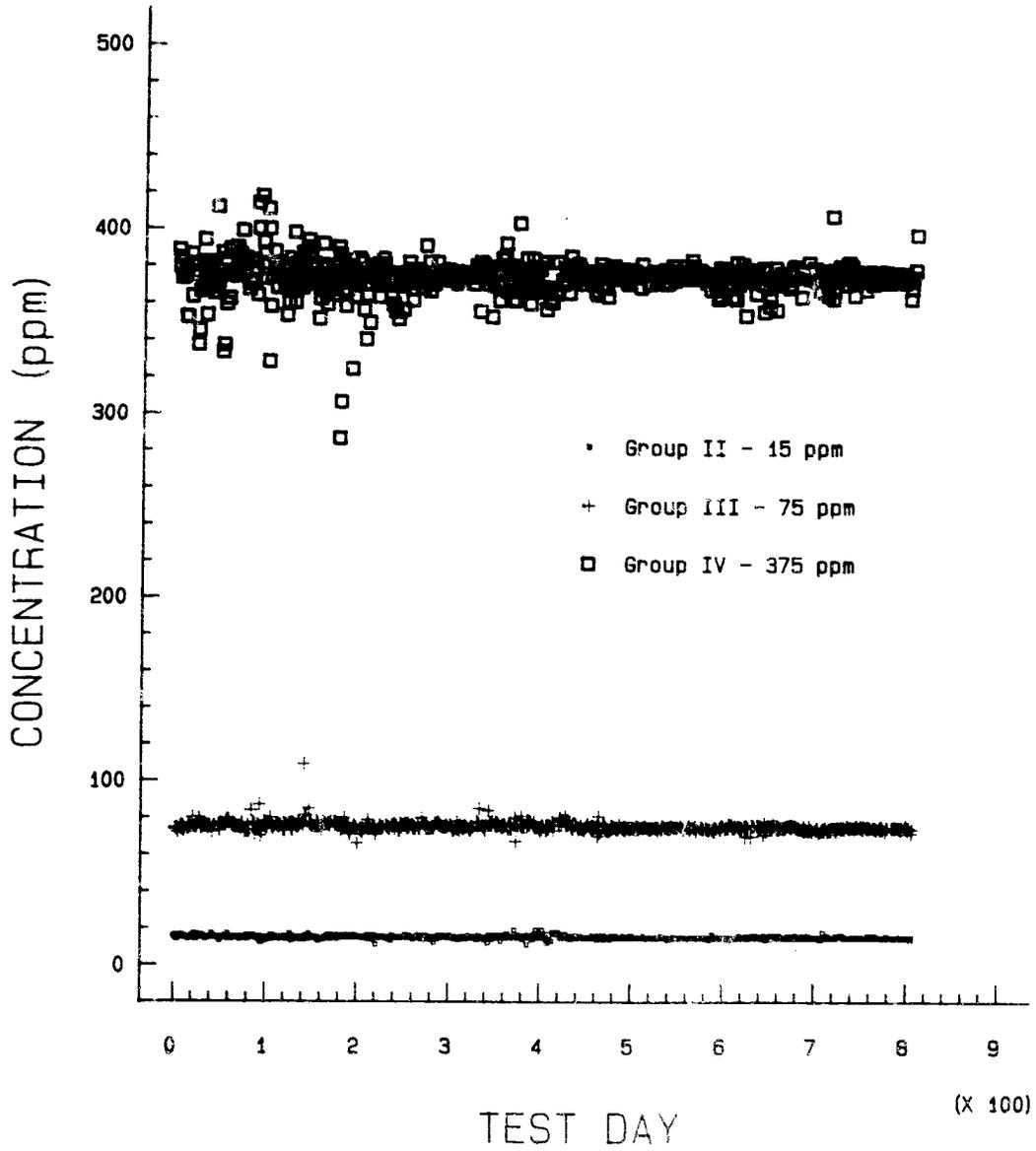
Figure 2  
An Inhalation Oncogenicity Study  
of Methyleneethoxyamine in Rats and Mice

Diagram of Generation System - Groups II through IV



An Inhalation Oncogenicity Study  
of Methylethylketoxime in Rats and Mice

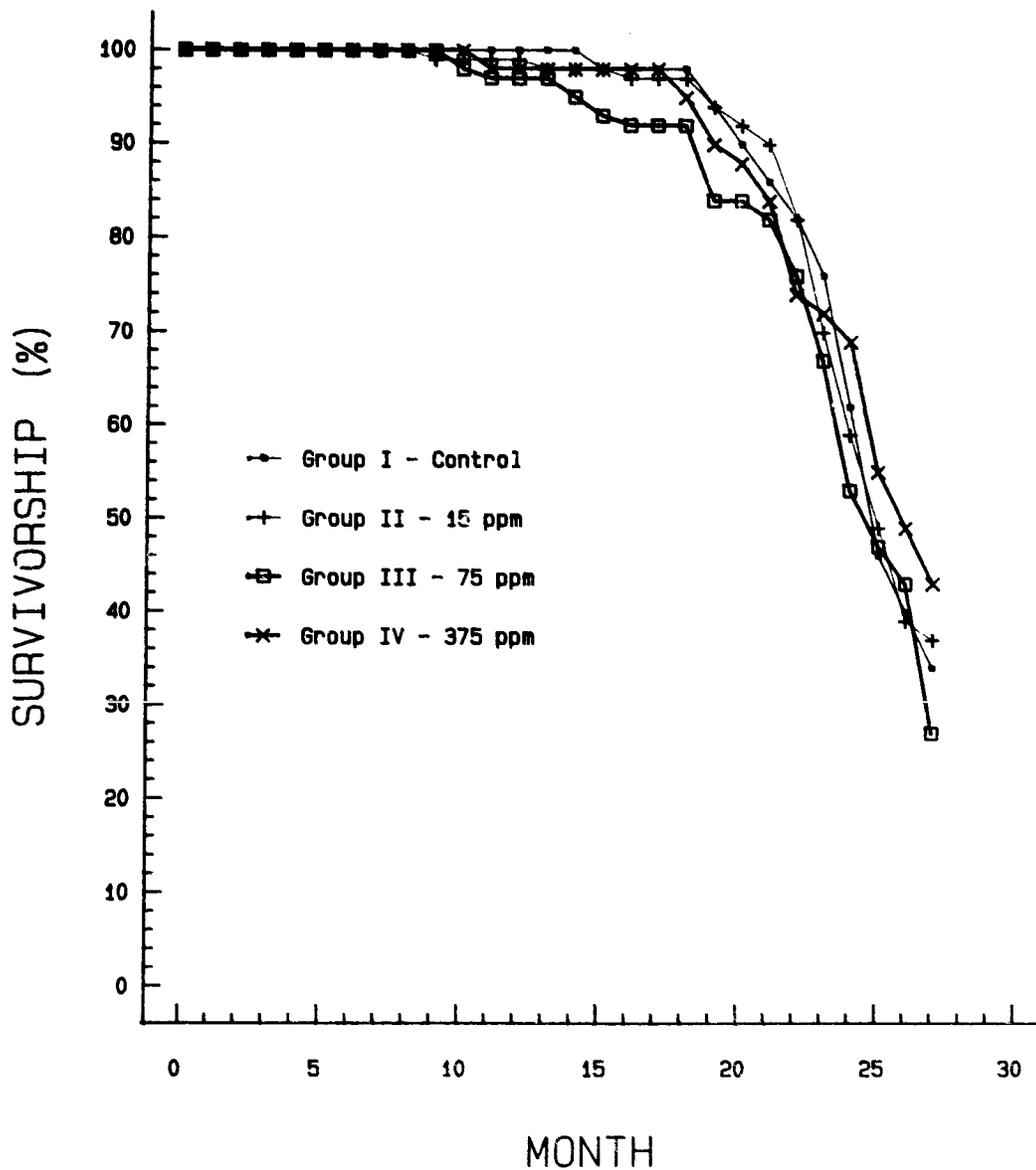
Daily Mean Concentrations



0 0.5 9

Figure 4  
An Inhalation Oncogenicity Study  
of Methylethylketoxime in Rats and Mice

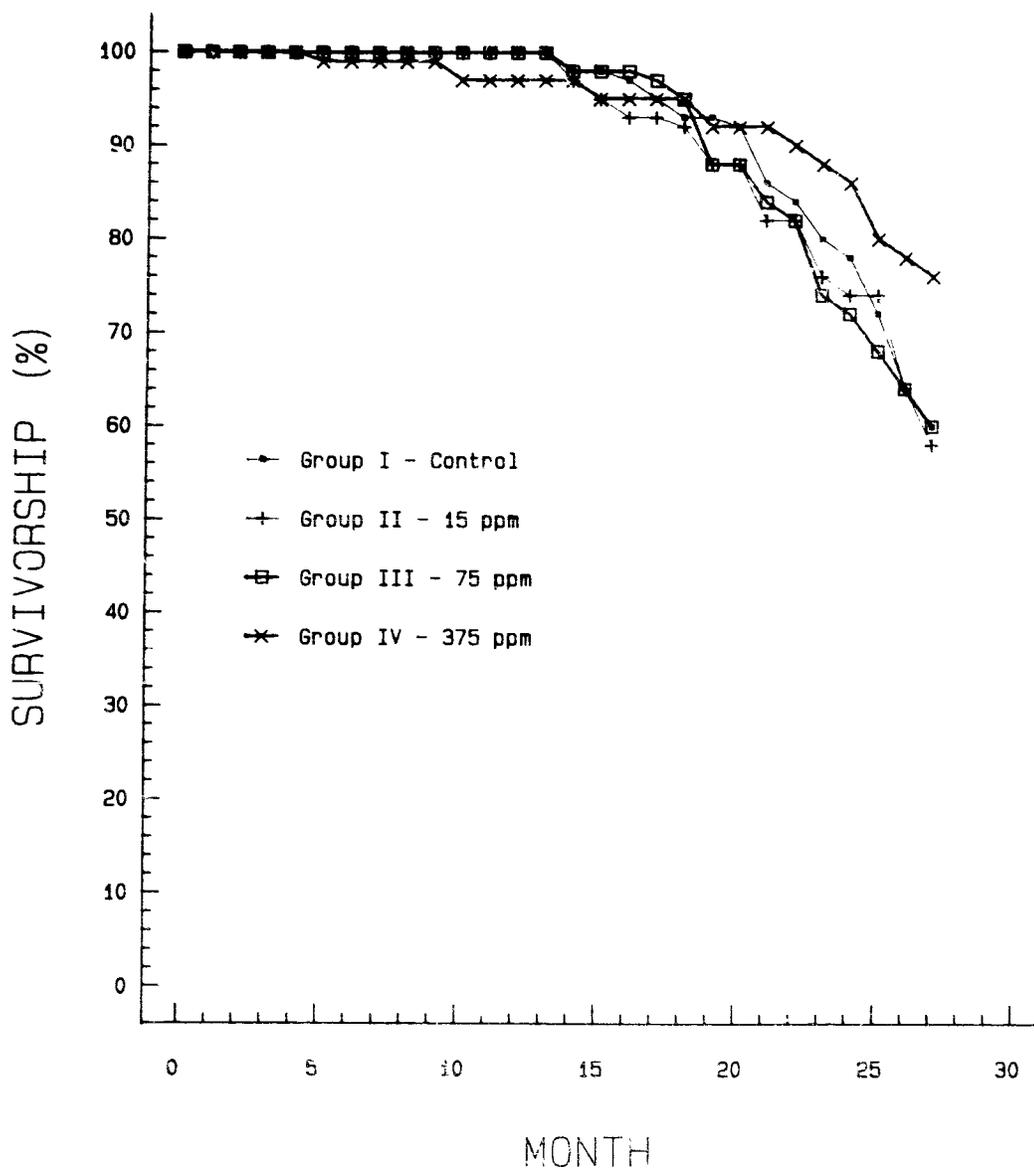
Graphical Representation of Monthly Survival - Male Rats



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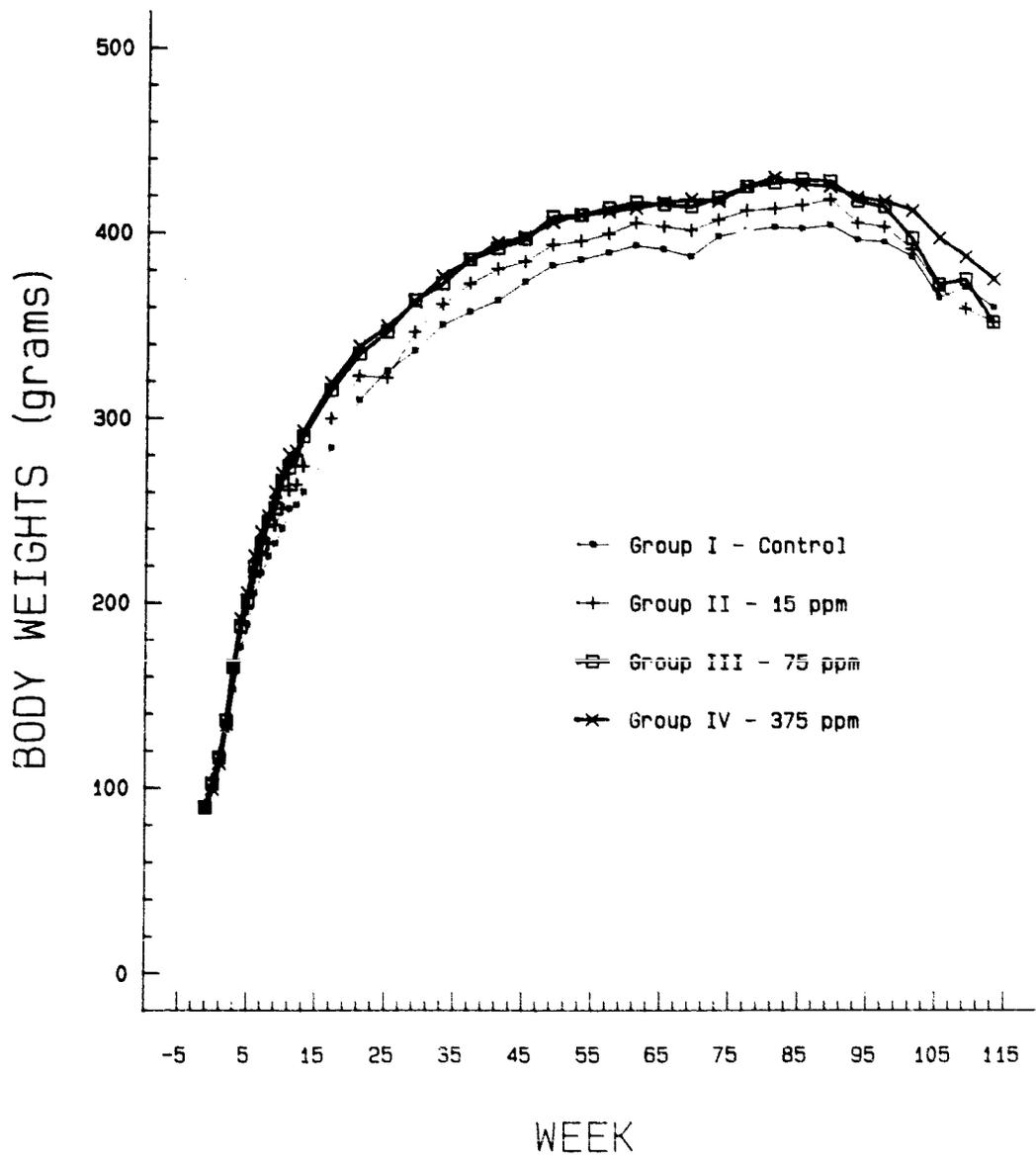
Figure 4 (cont.)  
A: Inhalation Oncogenicity Study  
of Methyleneketoxime in Rats and Mice

Graphical Representation of Monthly Survival - Female Rats



An Inhalation Oncogenicity Study  
of Methylethylketoxime in Rats and Mice

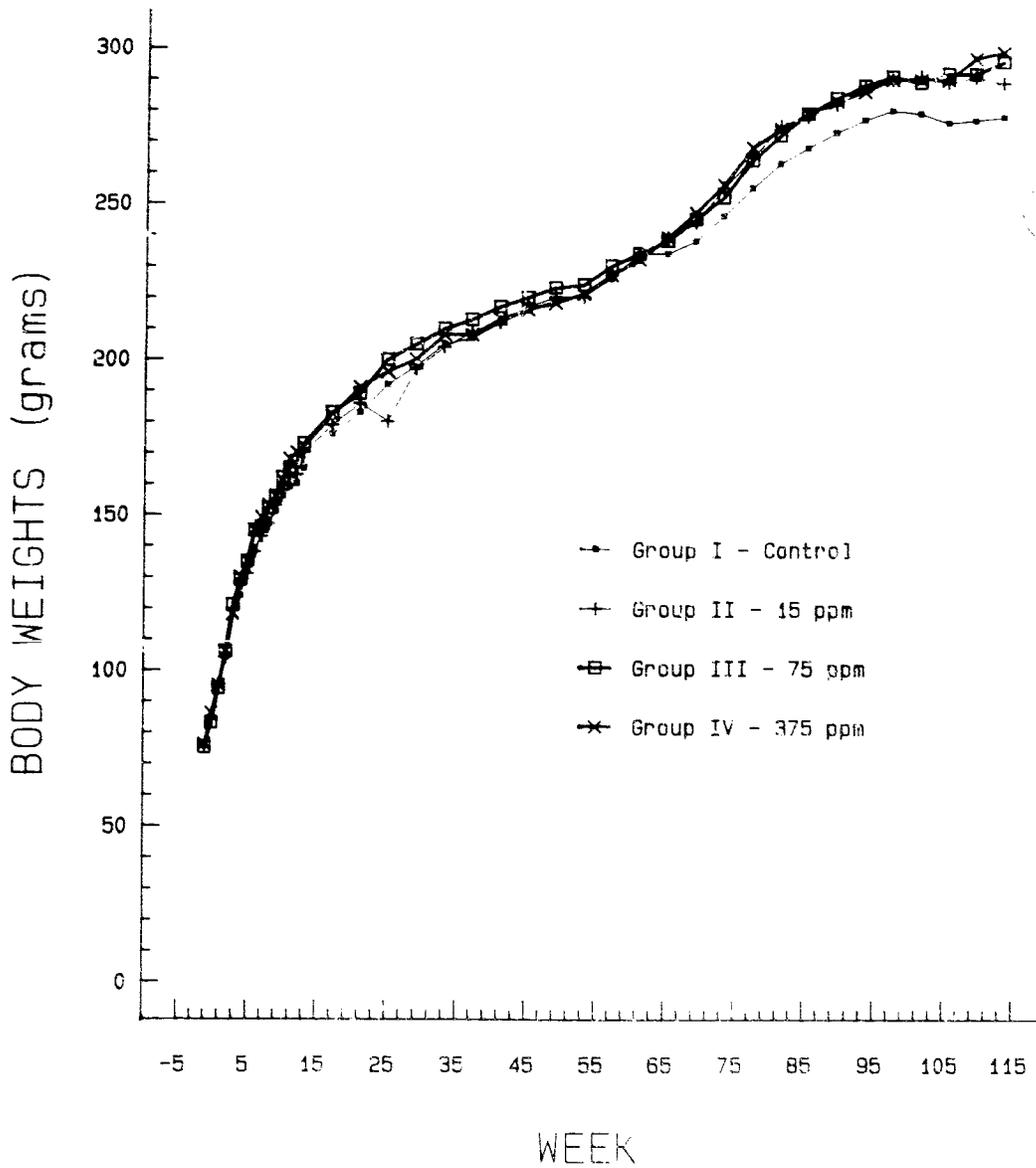
Group Mean Body Weights - Male Rats



10055

An Inhalation Oncogenicity Study  
of Methyl ethyl ketoxime in Rats and Mice

Group Mean Body Weights - Female Rats



## Appendix A

An Inhalation Oncogenicity Study  
of Methyleneketoxime in Rats and Mice

## Methodology and References - General

Parameter	Reference or Description
Physical Examination	Included examination for the following: Discharge - lacrimation, mucoid nasal discharge red nasal discharge, dried red nasal discharge, salivation, chromodacryorrhea, dried material around facial area, ano-genital staining, stool consistency. Neuromuscular Activity - lethargy, hyperactivity, prostration, aggression, irregular gait, splayed, hunched appearance, tremors, convulsions. Respiration - rales, gasping, labored breathing, shallow breathing. General Appearance - rough coat, piloerection, hair loss, foreign material coating, staining, flushing, blanching, cyanosis, body temperature, emaciation, poor condition, tissue masses, nodules, exophthalmia, opacity and/or any unusual observation not included above.
Ophthalmoscopic Examination	Lids, lacrimal apparatus, conjunctiva examined grossly, cornea, anterior chamber, lens, vitreous humor, retina and optic disc examined by indirect ophthalmoscopy. Atropine was used to induce mydriasis.
Body Weight	AND FX6000
Terminal Body Weight (TBW)	Ohaus B 5000. Represents a fasted body weight measured just prior to necropsy.
Organ Weights	Mettler AK-160 - All organs.
Histological Methods	
Stain - Hematoxylin and Eosin	Sheehan, D.C., and Hrapchak, B.B., <u>Theory and Practice of Histotechnology</u> . 2nd Edition. St. Louis: C.V. Mosby Company, 1980, pp.143-144.

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A-2  
Appendix A (cont.)  
An Inhalation Oncogenicity Study  
of Methyl ethyl ketoxime in Rats and Mice

89-8243R

Methodology and References - Inhalation Equipment

Parameter	Equipment	Reference or Description
Chamber Operation	Exposure Chamber	10,000 liter glass and stainless steel chamber; Harford, Aberdeen, MD.
	Incinerator	MOCO Thermal Industries Inc. Romulus, MI, Model 81MZK-452316-.43/.43-309SS
	In-House Filtering System	Pre-filter, HEPA filter and a charcoal filter
	Back-Up Exhaust Air Purifier	Vapor Phase Carbtrol® canisters, Model G-1
Atmospheric Generation	Compound Generator	Crown Glass Co. Inc., Counter-Current Volatilization Unit with coiled glass rod inserts, heated by one nichrome heating element.
	Compound Reservoir	Protectoseal® laboratory can
	Insulation	Owens-Corning Fiberglas® R-19 insulation
	Pumps	Fluid Metering Inc. (FMI), Fluid Metering pump, Model RPG-6 and RPG-20
	Variable Auto Transformer	Staco Energy Products Company, Variable Autotransformer, Type 3PN 1010.
	Furnace	Lindberg® Compact Tube furnace Model No. 55035
	Flowmeter	Dwyer® flowmeter
	Thermometer	T° Sentry digital alarm module Model 110
	Pressure/Vacuum Gauge	Dwyer® Magnehelic® gauge, Model No. 2002