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March 7, 1985

INIT 07/14/94



Dana Cazzulino
Dynamac Corporation
11140 Rockville Pike
Rockville, MD 20852

Dear Dana:

Please find enclosed for your ITC evaluation the International Isocyanate Institute (III) sponsored 13-week inhalation study reports, Sub-Chronic (13-Week) Inhalation Toxicity Study of Polymeric MDI Aerosol in Rats (Part B1) and (Part B2). These reports are final reports from the laboratory but are still considered draft reports by the Institute awaiting final approval by the III Board of Directors. The studies were initiated by the III to determine the sub-chronic toxicity of MDI aerosol and to derive data to select exposure levels for a lifetime study. The second 13-week study (B2) was conducted to supplement the data in B1 which did not demonstrate a clear adverse-effect level.

For your information, exposure for the lifetime study is projected to be initiated second quarter 1985. Target levels are 0, 0.5, 2 and 6 mg/m³.

Sincerely yours,

J. P. Lyon

J. P. Lyon
Manager, Industrial Toxicology

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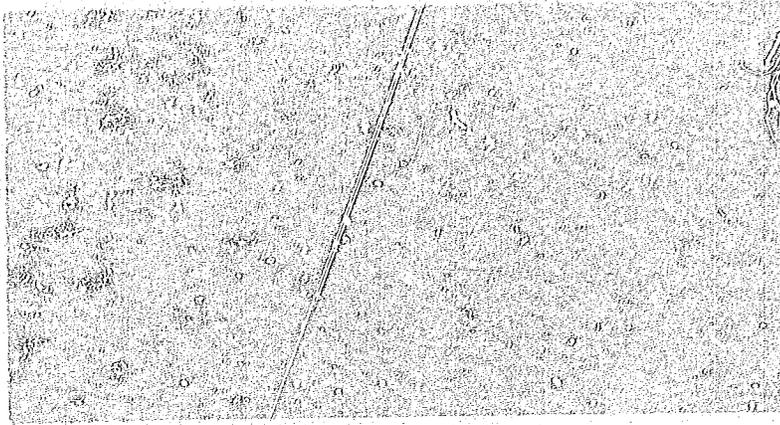
United States Geological Survey

DRAFT

FIGURE

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Scale



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division for nutrition and
food research tno

p.o. box 380
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Report no. V 83.290/220758

SUB-CHRONIC (13-WEEK) INHALATION TOXICITY
STUDY OF POLYMERIC MDI AEROSOL IN RATS (Part B1)
(final report)

DRAFT

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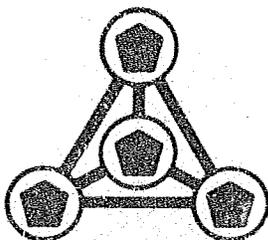
At the request of : International Isocyanate
Institute Inc., New Canaan
CT 06840, Conn., U.S.A.

Project number : B 82-0758

Start of the study : December 28, 1982

End of the study : March 28, 1983

Date : January, 1985



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LIST OF ABBREVIATIONS

polymeric MDI	= Deamodar 44 V 20
monomeric MDI	= 4,4'-diphenylmethanediisocyanate
MDA	= 4,4'-diaminodiphenylmethane
PHI	= phenylisocyanate
NR = nitroreagent	= N-(4-nitrobenzyl)-N-n-propylamine
MDI-NR	= urea derivative of MDI and nitroreagent
PHI-NR	= urea derivative of PHI and nitroreagent
HPLC	= high performance liquid chromatography
SD	= standard deviation
SEM	= standard error of the mean
NAD	= no abnormalities detected

SUMMARY

1. A sub-chronic inhalation toxicity study with polymeric MDI was performed, by exposing groups of 15 male and 15 female Wistar rats to test atmospheres containing an aerosol of the test material at levels of 0.2, 1 or 5 mg/m³ air for 6 hours/day, 5 days a week during a period of 13 weeks. Symptomatology, body weight gain, haematology, biochemistry, urine analyses, organ weights and gross- and microscopic pathology were used as criteria to disclose possible adverse effects.
2. The actual concentrations of polymeric MDI aerosol in the test atmospheres as determined by QCM cascade were 0.20, 1.04 and 5.03 mg/m³ air.
3. Transient, slight growth retardation was observed in males exposed to 5 mg/m³ air.
4. Haematology, blood chemistry and urine analyses did not show treatment-related effects.
5. There were no significant differences in organ weights between test and control groups.
6. Gross examination at autopsy did not reveal changes which could be ascribed to the test material.
7. Histopathological examination revealed yellow material in the respiratory tract of rats exposed to 5 mg/m³ air.
8. It was concluded that under the conditions of the present study no clear adverse-effect level was determined.

SUB-CHRONIC (13-WEEK) INHALATION TOXICITY STUDY OF POLYMERIC MDI AEROSOL
IN RATS (PART B1)

1. INTRODUCTION

At the request of the International Isocyanate Institute Inc. U.S.A. , a research program was performed to investigate the toxicity of inhaled polymeric MDI. The present study was carried out to provide data on the sub-chronic inhalation toxicity of the test material and to enable the selection of the concentrations to be used in a chronic inhalation study. The sub-acute inhalation toxicity of the test material had already been examined in a previous 2-week study in rats at levels of 2, 5 and 15 mg/m³ air (CIVO report no V 82.308/212478). From the results of that study it appeared that the test material at a level of 15 mg/m³ air induced growth retardation, severe respiratory distress, mortality and increased lung-to-body weight ratios, while at the lowest level only slightly increased lung-to-body weight ratios were found. On the basis of the results of this previous study the dose levels for the present study were selected.

2. MATERIAL AND METHODS

2.1 Test material

Samples of polymeric MDI (Desmodur 44 V 20) were received from Bayer AG, Leverkusen, FRG, in aluminum bottles each containing about 1 kg of the test material, in September, 1982. Desmodur 44 V 20 is a viscous ($\eta = 200 \pm 40$ mPas), dark brown liquid with the following composition as specified by Bayer AG:

content of monomeric MDI	52 ± 3 %	"
NCO - content	30 ± 2 % (w/w)	"
hydrolysable chlorine	≤ 0.3 %	"
total chlorine	≤ 0.8 %	"
chlorobenzenes	≤ 0.015 %	"
phenyl isocyanate	0.004 ± 0.001 %	"
content of sediment	≤ 0.01 %	"

2.2 Animals

Sixty male and sixty female SPF-bred Wistar rats (Cpb:WU, Wistar random) were purchased from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, the Netherlands. They were delivered on December 7, 1982. On arrival the rats weighed 35-50 g and were 22 ± 1 days old. They were acclimatized for a period of 21 days. Just prior to the start of the study the animals were allocated randomly, according to computer randomization listings to four groups. Each group, composed of 15 males and 15 females, was coded by a letter and colour. Within each group the individual animals were identified by an earmark and a computer reference number. At the start of the study the animals had an age of 6 weeks.

2.3 Exposure chambers

Animals were exposed in H 1000 multitiered inhalation chambers manufactured by Hazleton Systems Inc., USA. The chambers have been constructed of stainless steel with glass doors on two sides. This allows observation of the animals during exposure. The capacity of the chambers is about 2.3 m^3 . The animals were housed individually in wire mesh stainless steel cages. The total air flow through the inhalation chambers ranged from 50-60 m^3/hour . The temperature in the chambers was 19.5 ± 1 °C. The relative humidity could be maintained between 40 and 60 % during the major part of the exposures. Under extreme weather conditions slightly higher or lower values, which were still considered acceptable, have been recorded.

2.4 Maintenance

The rats were exposed to the test material for 6 hours a day, 5 days a week during a period of 13 weeks. During exposure the animals did not have access to food or water.

During the non-exposure periods the animals were housed in wire mesh, stainless steel cages, which were suspended in an open rack in an animal room. Each cage accommodated 5 males or 5 females. The temperature in the animal room was 22 ± 1 °C, the relative humidity 30-70 % and the number of air changes was c. 15/hour.

After exposure the animals were provided ad libitum with the Institute's stock diet for rats and unfluoridated tap water from an automatic drinking water system.

The nutrient composition of the diet and the levels of the various contaminants, as determined periodically in batches of the stock diet and in samples of drinking water, are shown in annexes 1, 2 and 3.

2.5 Generation of the test atmosphere

Polymeric MDI aerosol was generated using the institute's stainless steel/glass air nebuliser.

Prior to use the amount of polymeric MDI needed for the aerosol generation was filtered through a glass G4 filter under vacuum, to remove clots, if any, from the liquid to assure a trouble-free operation of the nebuliser.

At week 1, 5, 7, 9 and 13 polymeric MDI samples were taken before filtration, immediately after filtration and after having been used for the generation of the aerosol. The samples were analysed by Bayer AG, Leverkusen, FRG, for monomeric MDI-, NCO- and PHI-content.

The results as provided by Bayer AG are presented in table 1.

Non-respirable particles in the aerosol were eliminated in a cyclone so that 95 % of the particles were smaller than 5 μm . The aerosol was continuously passed through a delivery system, constructed of 8-cm PVC pipe with a length of 8 m. Appropriate amounts of polymeric MDI aerosol were withdrawn from the delivery system by small air jet pumps and mixed with the main air flow before entering the chambers giving the desired concentration of test material within each chamber.

2.6 Exposure levels

The selected exposure levels of polymeric MDI in the various test atmospheres were 0.2, 1.0 and 5 mg/m^3 air.

2.7 Test atmosphere control

To determine the concentrations of the test material in the inhalation chambers four different analytical methods were used.

2.7.1 Cascade impaction

The total mass and particle size distribution of the aerosols were determined by a Berkeley QCM cascade.

The method of sampling is described in CIVO report no. V 82.308.

The frequency of determinations as stated in the protocol was as follows:

5 times daily during week 1- 4

4 times daily during week 5- 8

3 times daily during week 9-13

In view of the unreliability of the light scattering readings, probably as a result of slight changes in the particle size distribution, it was decided to continue the number of 5 determinations a day with the QCM cascade during the whole study.

2.7.2 Photometry

Total isocyanate and amine content in the test atmospheres were determined by photometry. The concentrations were calculated from these data. The photometric method is described in annex 4 to this report.

The frequency of determinations was:

3 times daily during week 1- 4

2 times daily during week 5- 8

1 time daily during week 9-13

2.7.3 HPLC

In week 1, 3, 5, 7, 9, 11 and 13 one sample was taken from each test atmosphere for the determination of the content of monomeric MDI, PHI and MDA.

The methods used concerning the determination of MDI, PHI and MDA are described in annex 5 to this report.

2.7.4 Light scattering

The steady state of the exposure levels was continuously monitored by Simulin aerosol light scattering photometers.

Light scattering readings were used as an expedient to adjust the concentrations of the test atmospheres.

In addition, light scattering readings were used to calculate the aerosol concentration of the test atmospheres. For this purpose light scattering readings were recorded at 20-minute intervals. The total exposure period of each test group was divided by the 5 QCM determinations into 5 sub-periods. Thus each sub-period was covered by one determination of the concentration by the QCM cascade. Simultaneously with each QCM determination the light scattering was read. This reading represented the same concentration as determined with the QCM cascade. By comparing this light scattering reading with those readings recorded at 20-minute intervals during the respective sub-periods the concentrations of polymeric MDI could be calculated (see table 2). These calculated concentrations are only a reflection of the values obtained by the QCM cascade.

2.8 Observations

2.8.1 Clinical observations

All animals were visually inspected for clinical symptoms and behaviour just before and just after exposure.

During the weekends the inspections were performed only once a day.

The animals were examined for:

- mortality
- behavioural status
- respiratory signs
- skin abnormalities
- changes of eyes and mucous membranes
- bleedings from the various orifices
- changes in excretory products

2.8.2 Body weights

Body weights of the individual animals were recorded just prior to the start of the study and then weekly.

2.8.3 Haematology

Examinations were performed in 10 predesigned rats/sex of each group in blood samples collected from the tip of the tail in week 13.

The following haematological parameters were examined:

Parameter	Method	Reference
red blood cells	Coulter Counter ZF	Manual of Coulter Electronics Ltd., Harpenden, England, March 1977
white blood cells	do.	do.
haemoglobin	cyanmethaemoglobin using Lyzerglobin TM reagents (J.T. Baker Chemicals BV., Deventer, the Netherlands)	Manufacturers manual
packed cell volume	micro haematocrit	Helleman, P.W. a.o. Haematologie. Elsevier, Amsterdam, 1973, p. 27
differential white blood cell count	microscopic examination of stained blood smears according to Pappenheim	Gorter, E. and W.C. de Graaff. Klinische Diagnostiek, 7th ed. H.E. Stenfert Kroese N.V., Leiden, 1955, part I, p. 34
prothrombin time	Normotest, method for capillary citrate blood Nyegaard & Co. As., Oslo, Norway	Manufacturers manual

2.8.4 Blood chemistry

Determinations were carried out in blood samples taken from the abdominal aorta at autopsy of 10 predesigned rats/sex/level. The samples were collected in plastic tubes containing c. 30 μ l of a heparine solution (5000 IU/ml). The samples were centrifuged at 2000 rpm for 15 minutes using Sure-Sep dispensers (General Diagnostics) for good separation of the plasma. Glucose was determined in blood samples collected from the tip of the tail after overnight fasting. The following measurements were made:

Parameter	Method	Reference
albumin	Coulter Kem-O-Lab with bromocresol green reagents	Coulter test methodology sheet; Albumin, February 1978. Based on Doumas, B.F., a.o., Clin. Chim. Acta <u>31</u> (1971) 87
alkaline phosphatase (ALP)	Coulter Kem-O-Lab with Accuzyme TM II reagents and thymolphthalein mono phosphate as substrate	Coulter test methodology sheet; ALP, November 1981. Based on Roy, A.V., Clin. Chem. 16 (1970) 431
glutamic-oxalacetic transaminase (GOT)	colorimetric INT/diaphorase, Coulter Kem-O-Lab with Accuzyme TM II reagents	Coulter test methodology sheet; GOT (AST) November 1981. Based on Wilkinson, J.H., Aminotransferase isoenzymes, 2nd ed. J.P. Lippincott Co., Phil. USA, 1970, p. 224
glutamic-pyruvic transaminase (GPT)	colorimetry, INT/diaphorase, Coulter Kem-O-Lab with Accuzyme TM II reagents	Coulter test methodology sheet; GPT (Alt), November 1981. Based on Wiobleski, F. and J.S. la Due, Proc. Soc. Exp. Biol. Med. 91 (1956) 569

Parameter	Method	Reference
urea	Coulter Kem-O-Lab with urease reagent	Coulter test methodology sheet; Urea (Bun), November 1981. Based on Creno, R.J., a.o., Am. J. Clin. Path. 54 (1970) 828
total protein	Coulter-Kem-O-Lab with modified biuret reagents	Coulter test methodology sheet; TP, March 1979. Based on Gornall, A.G., J. Biol. Chem. 177 (1949) 751
creatinine	AutoAnalyzer	Technicon AutoAnalyzer method N II-b
total bilirubin	diazotized sulphanilic acid	Jendrassik, L. and P. Grof, Biochem. Z. 297 (1938) 81
calcium (Ca)	o-cresolphthalein- complexone, Boehringer Mannheim, GmbH kit no. 204-382	Ray Sarkar, B.C., and U.P.S. Chauhan, Anal. Biochem. 20 (1967), 155
potassium (K)	Electrolyte-2-Analyzer TM (Beckman Instruments)	Manual of Beckman Electro- lyte-2-Analyzer TM, Brea, Cal. 92621, USA, May 1981
sodium (Na)	do.	do.
inorganic phosphate	colorimetric, using Boehringer Mannheim GmbH kit no. 124-974	Zilversmit, D.B. and K. Davis, J. Lab., Clin. Med. 35 (1952) 55
gamma-glutamyl transferase	colorimetric method using Boehringer Mannheim GmbH Kit no. 125-954	Persijn, P.G. and W. van der Slik, J. Clin. Chem. Clin. Biochem. 14 (1976) 421

Parameter	Method	Reference
glucose (blood)	hexokinase using Glucquant reagents, from Boehringer Mannheim GmbH Kit no. 245-178	Schmidt, F.H. in: E.F. Pfeiffer a.o. Handbuch des diabetes mellitus, Bd. 2. J.F. Lehmanns Verlag, Munchen, 1971, p. 938

2.8.5 Urine analysis

Urine analyses were performed in overnight urine samples of 10 predesigned rats/sex/group in week 13. Urine was collected during the last 16 hours of a period of 24 hours during which the animals were deprived of food and water.

The following determinations were made:

Parameter	Method	Reference
appearance	visual inspection	
volume	callibrated tubes	
density	using a Bellingham & Stanley refractometer	
pH	L-Combur-5-test strips Boehringer Mannheim GmbH, FRG	Manufacturers manual
protein		
occult blood		
glucose		
ketones		
microscopy of the sediment (pooled samples)	microscopic examination after centrifugation at 1500 rpm for 3 minutes	

2.8.6 Pathology

All animals were randomly killed on two successive days in week 14 by exsanguination from the abdominal aorta under ether anaesthesia, autopsied and examined for gross pathological changes.

Organs or samples of organs or tissues listed below were preserved in a 4 % aqueous, neutral phosphate-buffered formaldehyde solution.

<u>adrenals</u>	pancreas
aorta	parotid salivary glands
axillary lymph nodes	pharynx
<u>brain</u> (brain stem, cerebrum and cerebellum)	pituitary
caecum	prostate
coagulating glands	sciatic nerve
colon	seminal vesicles
duodenum	skeletal muscle (thigh)
epididymides	skin (flank)
eyes	spinal cord
<u>heart</u>	<u>spleen</u>
ileum	sternum (with bone marrow)
jejunum	stomach
<u>kidneys</u>	sub-maxillary salivary glands
<u>liver</u>	<u>testes</u>
<u>lungs with trachea and larynx</u>	thymus
mammary glands	thyroid with parathyroid
mesenteric lymph nodes	urinary bladder
nose (sections at 4 levels)	uterus (with cervix)
oesophagus	all gross lesions
ovaries	

The underlined organs of all rats killed at the end of the exposure period were weighed.

The lungs were fixed by intratracheal inflation with the fixative under 10 cm water pressure.

Tissues required for microscopic examination were embedded in paraffin wax, sectioned at 5 μ m and stained with hematoxylin and eosin.

Histopathological examination was done on nose, larynx, trachea, lungs, liver and kidneys of all rats of the control- and high-level group.

2.9 Statistical procedure

Body weights were analysed by an analysis of variance followed by an application of the Dunnett test.

Analysis of variance and the Dunnett test were applied to organ weights and haematological and biochemical data.

3. RESULTS

3.1 Concentrations of polymeric MDI, PHI and MDA in test atmospheres

3.1.1 Concentrations of polymeric MDI

Mean daily concentrations of polymeric MDI as determined by QCM, photometry and HPLC are given in tables 3, 4 and 5, respectively.

The overall mean concentrations of the test material in the different inhalation chambers during the study were:

method	polymeric MDI concentration in mg/m ³ air					
	low-level		mid-level		top-level	
	mean	SD	mean	SD	mean	SD
QCM cascade ¹⁾	0.20	0.04	1.04	0.18	5.03	0.72
photometry	0.23	0.11	0.88	0.23	4.67	1.11
HPLC	0.27	0.13	0.68	0.18	5.39	2.33
light scattering ²⁾	0.20	0.03	1.02	0.14	4.86	0.66

1) From the results of the previous studies with polymeric MDI it was decided to consider the concentrations determined by the QCM cascade the most reliable reflection of the polymeric MDI aerosol concentration.

2) Values calculated from QCM cascade and light scattering readings.

The overall mean concentrations of polymeric MDI in the test atmospheres as determined by the various analytical methods, were in rather good agreement at the different levels. However, there was a very wide range in HPLC values especially at the low- and high-level. The low concentration varied from 0.13 - 0.53 mg/m³ and the highest concentration from 3.35 - 9.34 mg/m³. This very inconsistent pattern of polymeric MDI values determined by HPLC was already known from the results of a previous 2-week inhalation study with this material (CIVO-report no. V 82.308). An explanation could not be given this time either. It is, therefore, highly questionable whether any significance can be attached to the quantitative data gathered by the HPLC method. Particle size determinations revealed that more than 95 % of the particles had an aerodynamic diameter smaller than 5 µm.

3.1.2 Concentrations of PHI

Phenyl isocyanate could not be detected in samples of the test atmospheres. The detection limit for PHI is 5 µg/m³ at a level of 0.2 and 1 mg/m³ and 15 µg/m³ at a level of 5 mg/m³.

3.1.3 Concentrations of MDA

Results of MDA analyses are presented in table 6.

Low levels of MDA were detected by HPLC in all samples of the test atmospheres taken after week 3 of the study. The levels, however, were independent of the polymeric MDI concentrations in the test atmospheres. Additional studies on the formation of MDA lead to the conclusion that the MDA levels found in the present study were most likely caused by artifacts (see CIVO report V 84.313/220758).

3.2 Condition and behaviour

No mortality was observed in any of the groups. Clinical observation of the rats did not reveal changes which could be ascribed to treatment. Behaviour was fully comparable in all groups.

3.3 Body weights

Mean body weights and mean weight gain are presented in tables 7 and 8, respectively.

Body weights did not show great differences among the various groups. However, when the body weights were adjusted for initial weights, it appeared that, according to the Dunnett test, there was a small but statistically significant growth retardation in males of the high-level group during the first 3 weeks of the exposure period. During the remainder of the test period body weight gain did not show any treatment-related difference between the test groups and the controls.

3.4 Haematology

Haematological values are presented in table 9.

White blood cell counts were statistically significantly lower in females exposed to 1 or 5 mg polymeric MDI/m³ than in controls. The differences with the controls, however, were not related to the exposure level. Since in addition the differences occurred in one sex only they were considered toxicologically insignificant.

The other haematological parameters showed the usual variation among the different groups, and did not indicate any adverse effect as result of the exposure to the test material.

3.5 Blood chemistry

Biochemical values are presented in table 10.

Statistically significantly higher glucose levels were encountered in males of the highest exposure group when compared with controls. Total protein values were statistically significantly lower in females exposed to 1 or 5 mg polymeric MDI/m³ air than in controls. The differences with the controls did not exhibit a dose-response relationship. In addition both values were within the range of historical control data (58.8-74.0 g/l). Therefore no toxicological significance was attached to this finding.

As compared to controls there was one other statistically significant difference in females exposed to 1 mg/m³, viz. a relatively low inorganic phosphate content. Since in females exposed to 5 mg/m³ this value was similar to that of the controls, the relatively low content in the 1 mg/m³ level was considered an isolated finding unrelated to treatment.

Potassium values in males were unremarkable; females exposed to 5 mg/m³ air, however, showed slightly, but statistically significantly, higher values than did the controls.

3.6 Urine analyses

Data of urine analyses are presented in tables 11 and 12.

Urine production and composition were comparable in all groups both in males and in females.

3.7 Organ weights

Organ weights are presented in tables 13 and 14.

In males exposed to 5 mg polymeric MDI/m³ air both absolute and relative lung weights were slightly higher than in controls. The differences were, however, not statistically significant according to the analyses of variance followed by the Dunnett test. Moreover the lung weights of females exposed to 5 mg polymeric MDI/m³ air were similar to those of control females.

The absolute weights of kidneys and adrenals were slightly higher in males exposed to 5 mg polymeric MDI/m³ air than in control males. However, when expressed in organ-to-body weight ratios these values were not statistically different from those of the controls.

In females no exposure-related differences in organ weights were observed between the test groups and the controls.

3.8 Pathology

3.8.1 Gross examination

Gross examination at autopsy did not reveal any abnormalities that could be related to exposure to polymeric MDI.

3.8.2 Histopathological examination

Histopathological findings are presented in table 15.

Microscopic examination of the liver, kidneys and respiratory tract, including nasal cavity (at 4 levels), larynx, trachea and lungs, did not reveal any specific exposure-related lesions, nor altered incidences or severities of lesions in rats exposed to 5 mg polymeric MDI/m³ air. Alveolar macrophages containing a material, that was yellow in H.E-stained slides, were observed in the lungs of every animal of the high level group. There were also macrophages that did not contain that material. No evidence of any tissue reaction was found in the lungs. In some animals yellow material, not ingested by macrophages, was found in the airspaces of the lungs, either as crystals or as amorphous particles. In some rats, this yellow material was also found as a thin layer on the pharyngeal and/or laryngeal mucosa, without any reaction in the underlying mucosa or submucosal structures.

4. DISCUSSION AND CONCLUSION

The exposure of rats to atmospheres containing polymeric MDI aerosols at levels of 0.2, 1 or 5 mg/m³ air did not reveal clear toxic effects.

An effect, which may be of toxicological significance was a transient, minimal growth retardation in males exposed to 5 mg/m³.

The increase in mean blood glucose in males and the increase in mean potassium plasma content in females of the high exposure group was very slight. In addition they occurred in one sex only and no other findings supported these increases. Therefore, if there is any toxicological significance, it is considered to be very small.

The increase in absolute weight of adrenals and kidneys in males exposed to 5 mg polymeric MDI/m³ air was not accompanied by histopathological changes, or by any biochemical effect. In addition the relative weights did not show statistically significant differences with the controls. Therefore no toxicological significance was attached to these findings.

The presence of yellow material in the respiratory tract of all rats exposed to 5 mg polymeric MDI/m³ air was the only histopathological finding which was related to exposure to the test material. The composition of this material is unknown and it was not associated with morphological changes.

During a previous 2-week inhalation study with polymeric MDI (CIWO report no. V 82.308) a clear increase in lung weights was noticed at an exposure level of 5 mg/m³ and to a lower degree at the 2 mg/m³ level. In the present study lung weights were slightly higher only in males exposed to 5 mg/m³ but the differences with the controls were not statistically significant.

This discrepancy might be explained by differences in sensibility for polymeric MDI due to differences in age of the rats at the start of the studies. The rats of the 2-week study were 4 weeks old at the start, while the rats of the present study had an age of 6 weeks.

In conclusion

Under the conditions of the present study no clear adverse-effect level was determined.

5. AUTHENTICATION

This report was prepared by:

Drs P.G.J. Reuzel

date:

21/01/85

Drs M.C. Bosland

date:

January 21, 1985

Drs L.M. Appelman

date:

21/01/85

Dr. A.W.J. de Jong

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January 21, 1985

and approved by:

Dr V.J. Feron

date:

20/01/85

6. RETENTION OF RECORDS AND SPECIMENS

All raw data, specimens and the master copy of the final report are filed in the archives of the Department Toxicology under reference: B82-758, International Isocyanate Institute Inc., polymeric MDI, 13-week inhal. tox. in rats. Formaline-preserved wet tissue specimens and paraffin blocks are stored for a period of 5 years, i.e. till April 1988. The microscopic slides will be retained for a period of 15 years, i.e. till April 1998.

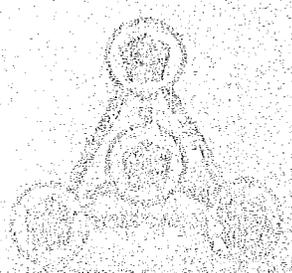
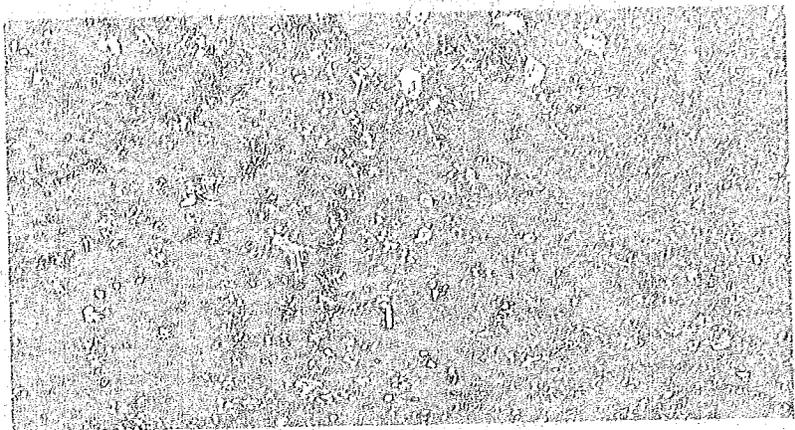
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SUB-CHRONIC (13-WEEK) INHALATION
TOXICITY STUDY OF POLYMERIC
MDI AEROSOL IN RATS (PART B2)
(final report)

DRAFT

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Project number : B 84/0158

Approved by : Dr V.J. Feron

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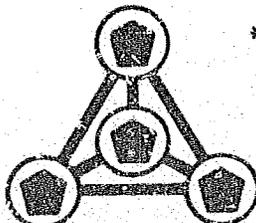
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End of the observation period: June 7, 1984

Study director : Drs P.G.J. Reuzel

Date : January 1985

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LIST OF ABBREVIATIONS

polymeric DI	= Desmodur 44 V 20
monomeric MDI	= 4,4'-diphenylmethanediisocyanate
MDA	= 4,4'-diaminodiphenylmethane
PHI	= phenylisocyanate
HPLC	= high performance liquid chromatography
SD	= standard deviation
SEM	= standard error of the mean
NAD	= no abnormalities detected
ND	= not detectable

SUMMARY

- 1 - A sub-chronic inhalation toxicity study with polymeric MDI in Wistar rats was performed by exposing groups of 30 males and 30 females to test atmospheres containing an aerosol of the test material at levels of 4, 8 or 12 mg /m³ air for 6 hours/day, 5 days a week during a period of 13 weeks.
After the exposure period a part of the animals of each group was held for a post-treatment period of 4 weeks.
Symptomatology, ophthalmology, body weight, haematology, biochemistry, urine analyses, organ weights, gross- and microscopic pathology and lung lavages were used as criteria to disclose possible adverse effects.
- 2 - The mean concentrations of polymeric MDI in the test atmospheres as determined by gravimetry were: 4.07, 8.43 and 12.25 mg/m³ air.
- 3 - Eleven males and 4 females exposed to 12 mg polymeric MDI/m³ air died during the exposure period. Mortality was not observed during the consecutive recovery period.
- 4 - Severe respiratory distress was observed in rats exposed to 12 mg polymeric MDI/m³ air. Similar but clearly less severe signs were observed in rats exposed to 8 mg polymeric MDI/m³ air.
- 5 - Ophthalmoscopy did not reveal changes which could be ascribed to the exposure to MDI aerosol.
- 6 - Body weight gain was statistically significantly depressed in males exposed to 12 mg polymeric MDI/m³ air during the exposure period. There was some reduction in weight gain in males exposed to 8 mg polymeric MDI/m³ air. Weight gain was also depressed in males exposed to 4 and in females exposed to 8 mg polymeric MDI/m³ air during the first week and in females exposed to 12 mg polymeric MDI/m³ air during the first 3 weeks.

During the post-treatment period body weight gain of males exposed to 8 mg polymeric MDI/m³ air recovered completely and that of males exposed to 12 mg polymeric MDI/m³ air for a major part.

- 7 - Haematological examinations were essentially negative.
- 8 - Creatinine values in blood plasma were dose-relatedly increased in females exposed to 8 or 12 mg polymeric MDI/m³ air at the end of the post-treatment period.
- 9 - Urine analyses in treated animals were similar to those of the controls.
- 10 - Relative lung weights were statistically significantly increased in males and females exposed to 8 or 12 mg/m³, at the end of the exposure period. At the end of the post-treatment period relative lung weights of males exposed to 12 mg polymeric MDI/m³ air were still statistically significant higher than those of the controls.
- 11 - Gross examination at autopsy did not reveal changes which could be ascribed to the exposure to the test material.
- 12 - Histopathological examination revealed accumulations of macrophages containing yellow material in the lower respiratory tract and the mediastinal lymph nodes at all exposure levels. Degenerative and hyperplastic lesions of the respiratory- and olfactory epithelium in the nasal cavity, and inflammatory reactions in the pulmonary tissue occurred to a significant degree and incidence at the 8 and even more so at the 12 mg/m³ exposure level. Rats exposed to 4 mg polymeric MDI/m³ air showed similar changes, but to a very minor degree and/or incidence.

At the end of the post-treatment period the histopathological changes still existed but to a lesser degree.

Except for lymphoid depletion in thymus and spleen no distinct treatment-related pathology was observed in animals that died or were killed in extremis. The cause of death could not be explained by microscopic examination.

- 13 - Phagocytosed material was observed in lung macrophages from rats exposed to 4 or 8 mg polymeric MDI/m³ air both at the end of the treatment period and at the end of the post-treatment period. Animals of the 12 mg/m³ group were not examined because of early mortality. At the end of the post-treatment period the phagocytotic capacity was lower in females exposed to 8 mg polymeric MDI/m³ air than in the controls.

- 14 - From the results of the present study it was concluded that inhalation exposure to polymeric MDI at levels of 8 and 12 mg/m³ air for 13 weeks accounted for clear adverse effects in rats and that the no-adverse effect level was lower than, but most probably close to, 4 mg/m³ air.

SUB-CHRONIC (13-WEEK) INHALATION TOXICITY STUDY OF POLYMERIC MDI AEROSOL IN RATS (PART B2)

1. INTRODUCTION

At the request of the International Isocyanate Institute Inc., U.S.A. a research program is performed to investigate the toxicity of inhaled polymeric MDI aerosol. During a previous sub-acute (2-week) inhalation study in rats with polymeric MDI a level of 15 mg/m³ air accounted for growth retardation, severe respiratory distress, mortality and increased lung-to-body weight ratios (CIVO report no. V 82.308/212478). A subsequently performed sub-chronic study in rats (CIVO report no. V 83.290/220758) with levels up to 5 mg polymeric MDI/m³ air did not result in any clear adverse effect. To provide adequate data on the sub-chronic inhalation toxicity of polymeric MDI and to enable the selection of the concentrations to be used in a chronic toxicity study in rats an additional sub-chronic inhalation study was performed.

2. MATERIAL AND METHODS

2.1 Test material

Samples of polymeric MDI (Desmodur 44 V 20) were received from Bayer AG, Leverkusen, FRG, in aluminum bottles each containing about 1 kg of the test material in January 1984. Desmodur 44 V 20 is a dark brown liquid.

The composition of the test material and analytical data of the test material as specified by Bayer AG are presented in annex 1.

2.2 Animals

One hundred and twenty male and one hundred and twenty female SPF-bred Wistar rats (Cpb:WU, Wistar Random) were purchased from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, the Netherlands. They were delivered on January 17, 1984. On arrival the rats weighed 35-50 g and were 22±1 days old. The animals were checked for overt signs of ill health and anomalies. Next they were kept for an acclimatization period of 23 days. Just prior to the start of the study the animals were allocated randomly, according to computer randomization listings, to four groups. Each group, composed of 30 males and 30 females, was coded by a letter and a colour. Within each group the individual animals were identified by an earmark and a computer reference number. At the start of the study the animals had an age of 6 weeks.

2.3 Exposure chambers

Animals were exposed in H 1000 multitiered inhalation chambers manufactured by Hazleton Systems Inc., USA. The chambers have been constructed of stainless steel with glass doors on two sides. This allows observation of the animals during exposure. The capacity of each chamber is about 2.3 m³. The animals were housed individually in wire mesh stainless steel cages. The total air flow through the inhalation chambers was determined by measuring the air velocity in the exhaust pipes using an anometer (Schiltknecht type 442). The total air flows ranged from 42-51 m³/hour. The temperature and relative humidity were monitored during the exposures by means of a PC 6604 thermo-hygrometer (Novasina). The temperature in the chambers was 19.5±1°C. The relative humidity could be maintained between 40 and 60% during the major part of the exposures. Under extreme weather conditions higher or lower values have been recorded (min. 31%, max. 66%).

2.4 Frequency and duration of administration of polymeric MDI

The rats were exposed to the test material for 6 hours a day, 5 days a week during a period of 13 weeks. After the exposure period 15 rats/sex/group were kept for a post-treatment period of 4 weeks. From the group of rats exposed to 12 mg polymeric MDI/m³ only 10 males and 10 females were kept for the post-treatment period because of considerable mortality during the exposure period.

2.5 Maintenance

During exposure the animals had no access to food or water. After exposure the animals were provided ad libitum with the Institute's stock diet for rats and unfluoridated tap water from an automatic drinking-water system. The nutrient composition of the diet and the levels of the various contaminants, as determined periodically in batches of the stock diet and in samples of drinking water, are shown in annexes 2, 3 and 4.

During the non-exposure periods the animals were housed in wire mesh, stainless steel cages, which were suspended in an open rack in an animal room. Each cage accommodated 5 males or 5 females.

The temperature in the animal room was 22±1°C, the relative humidity 40-70% and the number of air changes c. 12 times/hour.

2.6 Generation of test the atmospheres

Polymeric MDI aerosol was generated using the institute's stainless steel/glass air nebuliser, which was provided with a baffle to eliminate non-respirable particles from the aerosol so that 95 % of the particles were smaller than 5 µm. The aerosol was continuously passed through a delivery system, constructed of 3-cm PVC pipe with a length of 8 m. Appropriate amounts of polymeric MDI aerosol were withdrawn from the delivery system by air movers and mixed with the main air flow before entering the chambers giving the desired concentration of test material

within each chamber. The test atmospheres entered the top of the chambers and exited through an exhaust tube at the bottom of the chamber. The exhaust air was passed through a filter to remove the test material.

2.7 Exposure levels

The selected exposure levels of polymeric MDI in the various test atmospheres were 4, 8 and 12 mg/m³ air.

2.8 Test atmosphere control

The total mass of the aerosols was to be determined by a Berkeley QCM cascade. Just prior to the start of the study it appeared that the QCM cascade did not function correctly and had to be put out of use. Since at the levels applied gravimetry is a very reliable method to monitor the concentrations it was decided to apply this method until the QCM cascade could be used again. The QCM cascade was repaired and equipped with new crystals. In use again it appeared that especially at the 8 and 12 mg/m³ level the concentrations obtained were irreproducible and frequently considerably lower than those obtained by gravimetry. For this reason it was decided to continue the monitoring of the concentrations by gravimetry.

2.8.1 Gravimetry

Samples of test atmospheres were drawn through glass fibre filters (Sartorius SM 13430). The filters were weighed just before and after sampling. From the increase in weight and the volume of the test atmosphere drawn through the filter the concentration could be calculated.

The frequency of determination of each atmosphere was at least 5 times each exposure day.

2.8.2 Cascade impaction

The particle size distribution of the aerosols was determined by a Berkeley QCM cascade. The method of sampling is described in CIVO Report no. V 82.308/212478.

The frequency of determination of each test atmosphere was once each exposure day, except for the first 7 exposure days during which the QCM cascade impactor was in repair.

2.8.3 Photometry

The polymeric MDI content of the test atmospheres was also determined by photometry. The photometric method is described in CIVO Report no. V 82.290/220858.

The frequency of determination of each test atmosphere was once each exposure day.

2.8.4 HPLC

In week 1, 3, 5, 7, 9, 11 and 13 a sample was taken from each test atmosphere and drawn through a solution of nitroreagent in toluene. The solution was split up into two equal parts. Both parts were analysed for PHI and MDA content, one part by CIVO and the other by Bayer A.G. The methods used by CIVO for the determination of PHI and MDA are described in CIVO Report no. V 82.290/220758.

Analytical method Le 2085/075/020/40 was used by Bayer for MDA determinations. The method for PHI determinations used by Bayer are

described by: Keller, J., Dunlap, U.L. and Sandridge, R.L., Anal. Chem. 46 (1974), 1845-1846).

2.8.5 Light scattering

The steady state of the exposure levels was continuously monitored by Simslin aerosol light scattering photometers.

2.9 Observations

2.9.1 Clinical observations

All animals were visually inspected for clinical symptoms and behaviour twice a day. During the weekends the inspections were performed only once a day.

The animals were examined for:

- mortality
- behavioral status
- respiratory signs
- skin abnormalities
- changes of eyes and mucous membranes
- bleedings from the various orifices
- changes in excretory products.

2.9.2 Ophthalmological examinations

Ophthalmoscopy was performed using a Heine ophthalmoscope. The examinations were carried out prior to the start of the study in 30 animals/sex of the control- and high-dose group and at the end of the treatment period in 20 animals/sex of the control- and high-dose group.

2.9.3 Body weights

Body weights of the individual animals were recorded just prior to the start of the study and then weekly.

2.9.4 Haematology

Examinations were performed in 10 predesigned rats/sex of each group in blood samples collected from the tip of the tail in week 13 and 17.

The following haematological parameters were examined:

Parameter	Method	Reference
red blood cells	Coulter Counter ZF	Manual of Coulter Electronics Ltd., Harpenden, England, March 1977
white blood cells	do.	do.
haemoglobin	cyanmethaemoglobin using Lyzerglobin TM reagents (J. T. Baker Chemicals BV., Deventer, The Netherlands)	according to the manufacturers manual
packed cell volume	micro haematocrit	Helleman, P.W. a.o. Haematologie. Elsevier, Amsterdam, 1973, p. 27

differential white blood cell count	microscopic examinat- ion of stained blood smears according to Pappenheim	Corter, E. and W.C. de Graaff. Klinische Diagnostiek, 7 th ed. H.E. Stenfert Kroese N.V., Leiden, 1955, part I, p. 34
prothrombin time	Normotest, method for capillary citrate blood Nyegaard & Co. As, Oslo, Norway	Manufacturers manual

2.9.5 Blood chemistry

Determinations were carried out in blood samples taken from the abdominal aorta at autopsy of 10 predesigned rats/sex/level in weeks 14 and 18. The samples were collected in plastic tubes containing c. 30 µl of a heparin solution (5000 IU/ml). The samples were centrifuged at 2000 rpm for 15 minutes using Sure-Sep dispensers (General Diagnostics) for good separation of the plasma. Glucose was determined in blood samples collected from the tip of the tail after overnight fasting.

The following measurements were made:

Parameter	Method	Reference
albumin	Cobas-Bio centrifugal analyzer with Bromocresol green reagent	According to the Roche manual, May 1982

alkaline phosphatase (ALP)	Cobas-Bio centrifugal analyzer with Baker	N.V.X.C. mededelingenblad 4 (1979) 314
glutamic-oxalacetic transaminase (GOT)/ aspartate amino transferase (ASAT)	Cobas-Bio centrifugal analyzer, kinetic with pyridoxal-5-phosphate using Baker reagents nos 3146 and 3162 at 37°C	N.V.K.C. mededelingenblad 4 (1979) 314
glutamic-pyruvic transaminase (GPT)/ alanine amino transferase (ALAT)	Cobas-Bio centrifugal analyzer, kinetic with pyridoxal-5-phosphate using Baker reagents nos 3147 and 3162 at 37°C	N.V.K.C. mededelingenblad 4 (1979) 314
urea	Cobas-Bio centrifugal analyzer, biuret reaction using reagent from Hoffmann-la Roche kit no. 0713228	According to the Roche manual, June 1981
total protein	Cobas-Bio centrifugal analyzer; biuret reaction using reagent from Hoffmann-la Roche kit no. 07 1411 9	According to the Roch manual, November 1979
creatinine	Cobas-Bio centrifugal analyzer; Jaffe reaction with Hoffmann-la Roche reagent kit no. 0714216	According to the manufacturers test methodology

total bilirubin	Cobas-Bio centrifugal analyzer with diazotized sulphanilic acid	Jendrassik, L. and P. Grof, Biochem. Z. 297 (1938) 81
calcium (Ca)	Cobas-Bio centrifugal analyzer with Boehringer reagent kit no. 204 382	Ray Sarkar, B.C. and U.P.S. Chauhan, Anal. Biochem. 20 (1967) 155
potassium (K)	Electrolyte-2-Analyzer TM(Beckman Instruments)	Manual of Beckman Electrolyte-2-Analalyzer TM. Brea, Cal. 92621, USA, May 1981
sodium (Na)	Electrolyte-2-Analyzer TM(Beckman Instruments)	Manual of Beckman Electrolyte-2-Analalyzer TM. Brea, Cal. 92621, USA, May 1981
inorganic phosphate	colorimetric, with the Boehringer kit no. 124-974	Zilversmit, D.B. and K. Davis, J. Lab., Clin. Med. 35 (1952), 55
cholesterol	Cobas-Bio centrifugal analyzer with Boehringer reagent kit no. 725242	Deeg, R. and J. Ziegenhorn, Clin. Chem. 28 (1982) 1574

triglycerides

glucose (plasma)	Cobas-Bio centrifugal analyzer, Hexokinase method with Hoffmann-la Roche reagent kit no. 0711004	According to the manufacturers test methodology
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2.9.6 Urine analyses

Urine analyses were performed in overnight urine samples of 10 predesigned rats/sex/group in weeks 13 and 17. Urine was collected during the last 16 hours of a period of 24 hours during which the animals were deprived of food and water.

The following determinations were made:

Parameter	Method	Reference
appearance	visual inspection	
volume	calibrated tubes	
density	using a Bellingham & Stanley refractometer	
pH)	
protein) L-Combur-5-test strips	
occult blood) Boehringer Mannheim	Manufacturers manual
glucose) GmbH, FRG	
ketones)	

microscopy of the sediment (pooled samples) microscopic examination after centrifugation at 1500 rpm for 3 minutes

2.9.7 Pathology

The rats were randomly killed on two successive days in week 14 and in week 18 according to the following scheme:

	Dose level of polymeric MDI in mg/m ³ air			
	0 (controls)	4	8	12
week 14	10/10 ¹⁾	10/10	10/10	9/16
week 18	10/10	10/10	10/10	10/10

¹⁾ males/females

The rats were killed by exsanguination from the abdominal aorta under ether anaesthesia, autopsied and examined for gross pathological changes.

Organs or samples of organs or tissues listed below were preserved in a 4% aqueous, neutral phosphate-buffered formaldehyde solution.

<u>adrenals</u>	nose (sections at 4 levels)
aorta	oesophagus
axillary lymph nodes	ovaries
<u>brain</u> (brain stem, cerebrum and cerebellum)	pancreas
caecum	parotid salivary glands
coagulating glands	pharynx
colon	pituitary
dudodenum	prostate
epididymides	sciatic nerve
eyes	seminal vesicles
<u>heart</u>	skeletal muscle (thigh)
ileum	skin (flank)
jejunum	spinal cord
<u>kidneys</u>	<u>spleen</u>
<u>liver</u>	sternum (with bone marrow)
<u>lungs with mediastinal lymph nodes, trachea and larynx</u>	stomach
jejunum	sub-maxillary salivary glands
mammary glands	<u>testes</u>
mesenteric lymph nodes	thymus
	thyroid with parathyroid
	urinary bladder
	uterus (with cervix)
	all gross lesions

The underlined organs of all rats killed at the end of the exposure or post-treatment period were weighed.

The lungs were fixed by intratracheal inflation with the fixative under 10 cm water pressure.

The lungs were fixed by intratracheal inflation with the fixative under 10 cm water pressure.

Tissues required for microscopic examination were embedded in Paraplast, sectioned at 5 μ m and stained with haematoxylin and eosin.

Histopathological examination was done on:

- all organs and tissues collected of 10 males and 10 females of the control group and of 20 males and 20 females exposed to 12 mg polymeric MDI/m³ air that died or were killed in extremis during the study or killed in week 14,
- all organs and tissues collected of 10 males and 10 females of the control group and of 10 males and 10 females exposed to 12 mg polymeric MDI/m³ air that were killed in week 18,
- nose, larynx, trachea, mediastinal lymph nodes and lungs of all rats of the low- and intermediate groups killed and autopsied in week 14 or 18.

2.10 Lung lavages

At week 14 and 18, 5 animals/sex of the control, low- and intermediate-dose groups were anaesthetized by Euthesate (i.p.) and exsanguinated from the carotid artery. Next the diaphragm was perforated. The trachea was cannulated in situ with a 15-gauge blunt needle. The lungs were instilled with 4 ml sterile saline solution, which was kept in the lungs for 30 seconds before withdrawal. This procedure was repeated twice. Retrieved lavage fluid from the successive lung washings was pooled per animal and centrifuged at 300 g for 10 min. at 15°C and resuspended in cold culture medium (H199 + 10% heat inactivated calf serum). The following parameters were examined:

Cell density (by haemocytometer chamber)

Cell viability (Trypan-blue exclusion method)

Phagocytosing capacity (uptake of latex particles)

Cell survival (Trypan-blue exclusion method after incubation
in CO₂ incubator for 18 h.)

2.11 Statistical procedure

Body weights were analysed by an analysis of co-variance followed by an application of the Dunnett test. Mortality data and incidences of histopathological changes were analysed by the Fisher exact probability test. Analysis of variance and the Dunnett test were applied to the organ weights and haematological and biochemical data.

3. RESULTS

3.1 Concentrations of polymeric MDI, PHI and MDA in test atmospheres

3.1.1 Concentrations of polymeric MDI

Mean daily concentrations of polymeric MDI as determined by gravimetry and photometry are presented in tables 1 and 2, respectively.

The quality of the steady state of the aerosol levels as monitored by Simslin light scattering photometers was rather good. Generally there were only small fluctuations in the readings.

Overall mean concentrations of polymeric MDI as determined by gravimetry and photometry were:

polymeric MDI concentration in mg/m^3 air						
method	low-level		mid-level		top-level	
	mean	SD	mean	SD	mean	SD
gravimetry	4.07	0.30	8.43	0.77	12.25	1.06
photometry	2.90	0.52	6.38	0.98	8.59	1.34
difference (in %)	28		24		30	

The overall mean concentrations of polymeric MDI in the different test atmospheres as determined by photometry were 24-30% lower than those determined by gravimetry. The major part of this difference can be explained as follows.

During the previous 13-week inhalation study with polymeric MDI in rats the concentrations determined by photometry were 7% lower than those determined by the QCM cascade at a level of 5 mg polymeric MDI/ m^3 air. Comparison of 74 concentrations simultaneously determined by the QCM cascade and those determined by gravimetry revealed that the QCM cascade values were 7% lower than the gravimetry values at a level of 4 mg polymeric MDI/ m^3 air. Just prior to the start of the present study it was found that there was a dead volume in the valve of the QCM cascade which was not mentioned by the manufacturer. The significance of the dead volume for the results depends upon the sampling time. For the 4 mg/m^3 level it was 8%. This dead space was taken into account during the present study. This means that at a level of 4 mg/m^3 the values determined by the QCM cascade were 8% higher than without correction. In this way 22% of the difference between concentrations determined by gravimetry and those determined by photometry can be explained.

3.1.2 Concentrations of PHI and MDA

The concentrations of PHI and MDA detected are presented in tables 3 and 3a.

In week 7, 50 $\mu\text{g}/\text{m}^3$ air PHI was detected by CIVO in a sample of the test atmosphere that contained 12 mg polymeric MDI/ m^3 air. In the other part of the same sample Bayer detected 8 μg PHI/ m^3 air. In the other weeks no PHI could be detected in any of the samples by CIVO. Bayer, however, detected levels ranging from 8-12 μg PHI/ m^3 in most samples of the test atmosphere containing 12 mg polymeric MDI/ m^3 air and in two samples of the test atmosphere containing 8 mg polymeric MDI/ m^3 air.

In week 11 of the study 5 μg MDA/ m^3 air was detected by CIVO in samples of the control atmosphere and in samples of the test atmospheres containing 4 or 12 mg polymeric MDI/ m^3 air.

Bayer, however, detected 15 μg MDA/ m^3 air in the sample of the control atmosphere and of all test atmospheres. The MDA levels detected both by Bayer and by CIVO did not correlate with the polymeric MDI concentrations. This lack of correlation and the presence of MDA in control samples led to the conclusion that the MDA found in the present study is an artifact.

3.1.3 Aerodynamic diameter

The aerodynamic diameter, determined by QCM cascade, of more than 95% of the particles was smaller than 5 μm . The major part of the mass of particles had a diameter between 2 - 0.4 μm .

3.2 Condition, mortality and behaviour

Mortality figures are presented in table 4.

Severe clinical signs were observed in rats exposed to 12 mg polymeric MDI/m³ air during the exposure period. The first sign usually was a slight serous nasal discharge sometimes accompanied by red-brown crusts around the nares. This was observed in nearly all top-dose rats and was followed by slight to very severe signs of dyspnoea in many rats. These animals showed laboured and rapid breathing, occasionally accompanied by wheezy sounds and or coughing. In addition the animals showed pilo-erection, humpbacked posture, pale mucosae and a flabby belly. Occasionally salivation was observed. The health condition of 11 males and 4 females worsened and they died or had to be killed in extremis. Several of these animals had diarrhoea prior to death.

During the post-treatment period the survivors gradually recovered, though nasal discharge was still observed in most rats of this group at the end of the post-treatment period.

Nasal discharge was also observed in many animals of the intermediate-dose group during both the exposure and the post-treatment period.

No such clinical signs were observed in low-dose animals.

3.3 Ophthalmological examinations

Results are summarized in table 17.

Ophthalmological examinations did not reveal treatment-related changes in animals exposed to 12 mg polymeric MDI/m³ air. The abnormalities observed are common findings in the strain of rats used and their incidences showed the usual variation.

3.4 Body weights

Mean body weights and mean weight gain are presented in tables 5 and 6, respectively.

In male rats growth was clearly more affected by the exposure of the animals to polymeric MDI aerosol than in female rats. During the exposure period body weight gain was statistically significantly depressed in males exposed to 12 mg polymeric MDI/m³ air when compared to the controls. In addition there was reduction in weight gain in males exposed to 8 mg polymeric MDI/m³ air. The differences with the controls, however, were statistically significant only at 7 weekly measurements which were scattered over the exposure period. During the recovery period body weight gain recovered completely in males exposed to 8 and for a major part in males exposed to 12 mg polymeric MDI/m³ air.

Females exposed to 12 mg polymeric MDI/m³ air exhibited a slight transient reduction in weight gain during the first 3 weeks of the exposure period. In addition body weight gain was reduced in females exposed to 8 and in males exposed to 4 mg polymeric MDI/m³ air only during the first week of the study.

3.5 Haematology

Haematological values are presented in table 7.

At the end the post-treatment period but not at the end of the exposure period prothrombin time was slightly lower in females exposed to 12 mg polymeric MDI/m³ air than in the controls. The value was within the normal range of rats of the same strain and age (31.7-39.7 sec). In addition an increase rather than a decrease of the prothrombin time is considered an adverse effect. It is, therefore, very unlikely that this low value has to be considered a delayed effect of the exposure to polymeric MDI aerosol.

The values of the other haematological parameters examined showed a normal variation amongst the different groups and did not indicate any treatment-related effect.

3.6 Blood chemistry

Biochemical parameters are presented in table 8.

Creatinine values were statistically significantly greater in females exposed to 8 or 12 mg polymeric MDI/m³ air than in controls in week 18 only. The differences with the controls were dose-related.

Statistically significantly lower albumin values were encountered in females exposed to 4 or 12 mg polymeric MDI/m³ air when compared with controls at the end of the exposure period. Both values, however, were within the normal range (35.4-44.6 g/l). In addition the differences with the controls did not show a dose-effect relationship. Therefore no toxicological significance was attached to these lower albumin values.

Inorganic phosphate values were statistically significantly greater in males exposed to 8 or 12 mg polymeric MDI/m³ air than in controls in week 18 only. The differences with the controls were not dose-related. When the inorganic phosphate values were compared with those of the historical control values (1.63-2.05 mmol/l), it appeared that the values of the male controls and males exposed to 4 mg polymeric MDI/m³ air were relatively low and those of males exposed to 8 or 12 mg polymeric MDI/m³ air were completely normal.

Therefore these greater phosphate values were considered a fortuitous finding.

At week 18 the blood urea content of female rats exposed to 8 mg polymeric MDI/m³ air was statistically significantly greater than in controls. Since in females exposed to 12 mg polymeric MDI/m³ air this value was similar to that of the controls, the relatively high value in the 8 mg/m³ level was considered an isolated finding unrelated to treatment.

3.7 Urine analyses

Data of urine analyses are presented in table 9.

Urine excretion was slightly higher in females exposed to 4 mg polymeric MDI/m³ air than in the control group at day 88.

The amount was within the normal range (1.1- 2.7 ml). In addition the differences in urine volume between rats exposed to 8 or 12 mg polymeric MDI/m³ air and controls were not statistically significant. Therefore this increase was considered an isolated finding unrelated to treatment. Urine composition was comparable in all groups both in males and females at the end of both the exposure and post-treatment period.

3.8 Organ weights

Organ weights are presented in tables 10 and 11.

There were no statistically significant differences in absolute organ weights between the treated groups and the control groups which could be related to treatment. However, when expressed relative to body weights the values for lung weights in both males and females exposed to 8 or 12 mg polymeric MDI/m³ air were statistically significantly greater than in controls at the end of the exposure period. The differences in relative lung weight with the controls were dose-related in males but not in females. At the end of the post-treatment period lung weights of male and female rats exposed to 8 mg and of females exposed to 12 mg polymeric

MDI/m³ air were comparable with those of the controls, whereas those of males exposed to 12 mg polymeric MDI/ m³ air were slightly lower. Both absolute and relative weights of the other organs showed the common variation amongst the groups and were not affected by treatment.

3.9 Pathology

3.9.1 Gross examination

Gross lesions are presented in table 12.

Discernible differences in type, incidence or severity of gross lesions did not occur between the treated groups and the controls.

3.9.2 Microscopic examination

Histopathological changes are presented in tables 13 and 14.

Treatment-related histopathological changes were found in the nasal cavity, the lungs and the mediastinal lymph nodes in all treated groups.

Thinning of the layer of olfactory epithelium in the posterior part of the nasal cavity was observed in some animals in each of the treated groups but not in the controls. This change was considered a type of atrophy. Incidence and degree increased with increasing dose levels in females but not in males. The difference in incidences of the atrophy was statistically significant in both males and females exposed to 12 mg polymeric MDI/m³ air when compared with the controls. In males exposed to 12 mg and in females exposed 8 or 12 mg polymeric MDI/m³ air the epithelial atrophy was occasionally accompanied by focal hyperplasia of basal cells.

Rhinitis was found in some animals exposed to 8 or 12 mg polymeric MDI/m³ air in association with the atrophic changes. In addition, an increase in incidence and severity of nest-like infolds of the respiratory epithelium covering the nasal septum and the nasal turbinates was found in females, but not in males, exposed to 8 or 12 mg polymeric MDI/m³ air when compared with the controls. These increases were related to the dose levels. Nest like infolds consisted of pale columnar cells with periodic-acid-Schiff-negative cytoplasm. They are considered to reflect epithelial hyperplasia (Basrur P.K., T. Harada. Progress. Exp. Tumor. Res., Karger, Basel; 24 (1979) 283-301).

There was a markedly increased incidence of accumulations of macrophages in the mediastinal lymph nodes and the lungs of all test groups as compared with the controls. Most of the macrophages contained a yellow material in the H.E.-stained sections. In lungs the macrophages were found in the alveoli and bronchial tree. In several rats exposed to 8 or 12 mg polymeric MDI/m³ air macrophages were also found in the interstitium of the alveolar septa. Frequently this was associated with an increased incidence of a focal reaction, seen as increased septal cellularity consisting of mainly mononuclear inflammatory cells and fibroblasts. The differences in incidences of macrophage accumulations and of interstitial macrophage infiltrations between controls and the treated groups did not show a dose-relationship.

Incidence and degree of the septal tissue reaction increased with increasing dose levels. The differences in incidence between controls and the test groups were already statistically significant in males exposed to 4 and in females exposed to 8 mg polymeric MDI/m³ air. The epithelium overlying the thickened septa occasionally consisted of cuboidal instead of flat cells.

No evidence of tissue reaction was found in the lymph nodes.

At the end of the post-treatment period changes in the nasal cavity and lungs were still present but mostly to a lesser degree, except for the interstitial macrophage infiltration.

Also in the mediastinal lymph nodes accumulations of macrophages persisted but still without tissue reaction.

Microscopic examination of the other organs did not reveal any treatment-related lesion or treatment-related alteration in incidence or severity of normally occurring lesions.

Animals that died or were killed in extremis did not show distinct, specific pathology except for marked thymic involution and lymphoid depletion in the spleen (see table 15).

These degenerative changes are almost surely the consequence of marked loss of body weight. The cause of death, however, could not be explained by post-mortem or microscopic examination.

3.10 Lung lavages

Results are presented in table 16.

Rats exposed to 12 mg polymeric MDI/m³ air were not used for lung lavages since these animals were needed for pathological examinations.

The phagocytotic capacity of lung macrophages was lower in females exposed to 8 mg polymeric MDI/m³ air than in controls at the end of the post-treatment period.

There were no treatment-related differences in the number of cells in the lavage fluid, or in cell viability and cell survival in the lung lavages between the control group and the groups exposed to 4 or 8 mg polymeric MDI/m³ air.

4. DISCUSSION AND CONCLUSION

The exposure of rats to atmospheres containing polymeric MDI at levels of 4, 8 or 12 mg/m³ air resulted in adverse effects in all treated groups. The effects were only minimal in rats exposed to 4 mg polymeric MDI/m³ air but very severe in rats exposed to 12 mg polymeric MDI/m³ air.

The exposure to 12 mg polymeric MDI/m³ air accounted for a poor health condition of many rats; several of these animals even died or were killed in moribund condition. It was remarkable that mortality occurred only before week 8. From the results of a previous sub-acute inhalation study (CIVO Report no. V 85022/25158) it appeared that younger (4-6 weeks) rats were clearly more sensitive for toxic action of polymeric MDI aerosol than older (6-8 weeks) rats.

Therefore, the absence of mortality after week 7 was most probably due to the fact that the animals had reached an age, at which the animals are less sensitive for the toxic action of polymeric MDI.

The exposure to 8 or 12 mg polymeric MDI/m³ air resulted in reduced body weight gain in males. In females exposed to 12 mg/m³ there was only a transient reduction during the first 2 weeks. The reduction in body weight gain in males exposed to 4 and in females exposed to 8 mg polymeric MDI/m³ air during the first week of the study is considered indicative for an adaptation of the animals to an annoying atmosphere rather than the reflection of a toxic effect of the test material.

Nasal discharge in rats exposed to 8 or 12 mg polymeric MDI/m³ air and signs of slight to severe respiratory distress at the 12 mg/m³ level designated the respiratory tract as target organ system. This was supported by the increase in relative lung weights found in males and females exposed to levels of 8 or 12 mg/m³ and by histopathological changes such as degeneration and hyperplasia of the respiratory and olfactory epithelium in the nasal cavity and inflammatory reactions in the pulmonary tissue at the

end of the exposure period. At the end of the post-treatment period these changes were still present, but generally to a lower degree and incidence than at the end of exposure period.

Atrophy of the olfactory epithelium and basal cell hyperplasia occurred only in the treated groups. These nasal changes occurred relatively infrequently in males and females exposed to 4 or 8 mg polymeric MDI/m³ air, while they were found in a significant incidence only at the 12 mg/m³ level. During the previous 13-week inhalation study with polymeric MDI (CIVO Report no. V 83.290/220758) such changes were not found, though the highest concentration tested was 5 mg/m³ air. Re-examination of the nose of the animals of the previous study confirmed that no compound-related changes of the nasal epithelium were present in these animals. In addition in the present study there was no dose-effect relationship in the incidences of atrophy in males exposed to 4 or 8 mg polymeric MDI/m³ air. This seems to indicate that at a level of 4 to 8 mg/m³ air a minor degree of olfactory epithelial atrophy may or may not occur.

Basal cell hyperplasia of the respiratory epithelium was only found at levels of 8 and 12 mg/m³. Nest-like infolds, another form of hyperplasia, normally occurring in a 20-40% incidence, were increased in incidence and degree in females exposed to 8 and particularly in females exposed to 12 mg polymeric MDI/m³ air.

Lung macrophages containing yellow material were observed in all treated groups. This finding is in accordance with the observations made during the previous 13-week inhalation study with polymeric MDI. The nature of the yellow material is unknown though it is evident that it is related to the inhalation of polymeric MDI aerosol. The presence of these macrophages in the alveoli and in the bronchial tree, but also in the mediastinal lymph nodes indicates that this yellow material is either cleared by macrophages along the ciliary escalator or is taken up through the alveolar epithelium into the lymph vessels to the draining lymph nodes. At the end of the post-treatment period macrophages containing yellow material were still present in the lungs.

In vitro measurements showed that the phagocytotic capacity of the macrophages was reduced in females exposed to 8 mg polymeric MDI/m³ air at the end of the post-treatment period. These findings might indicate that the lung clearance by the macrophages was impaired. The increase in numbers of lung macrophages is a normal response to the presence of increased numbers of particles in the lungs.

The incidence and degree of focal inflammatory changes in the lungs, normally occurring only sporadically, increased with increasing dose levels. The degree in both males and females exposed to 4 mg polymeric MDI/m³ air was very slight, but the incidence in males of this group was rather high.

In the previous 3-month study these changes were not found at the level of 5 mg/m³. However, when the lungs of these rats were re-examined it appeared that this type of pulmonary changes indeed was present, though to a very minor extent.

It was remarkable that in spite of the very severe signs of respiratory distress of some animals that died during the exposure period only very minor changes or even no changes were found in their respiratory tract or in any other organ. Therefore the direct cause of death of these animals remained unclear.

There was a dose-related increase in plasma creatinine values in females exposed to 8 or 12 mg polymeric MDI/m³ air. An increase in creatinine can be explained by an impaired function of the kidneys or by increased muscular activity. For these possibilities no further indications were found. The significance of the increased creatinine values is, therefore, not clear.

In summary the exposure of rats to polymeric MDI aerosol at levels of 4, 8 or 12 mg polymeric MDI/m³ air resulted in effects at all levels tested. The effects depending on age, including mortality, were very severe at the 12 mg/m³ level and much less severe at the level of 8 mg/m³. The changes seen at the level of 4 mg/m³ were very minimal and hardly detectable. These results allow the conclusion that the no-adverse effect level of polymeric MDI aerosol was lower than but most probably close to 4 mg/m³ air.

5. AUTHENTICATION

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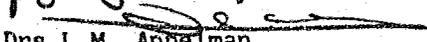
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