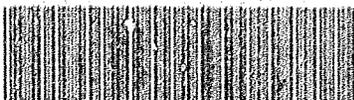


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PRELIMINARY STUDIES OF POLYMERIC MDI  
AEROSOLS AND A SUBACUTE (2-WEEK)  
INHALATION TOXICITY STUDY OF  
POLYMERIC MDI IN RATS

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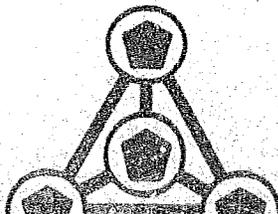
At the request of: International Isocyanate Institute  
Inc., New Canaan CT 06840, Conn.,  
U.S.A.

Project number: B 81-2478

Start of the studies: May 18, 1982

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Date: September, 1983



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## LIST OF ABBREVIATIONS

polymeric MDI	= Desmodur 44 V 20
monomeric MDI	= 2,4'-diphenyl methane diisocyanate + 4,4'-diphenyl methane diisocyanate
MDA	= 4,4'-diamino diphenyl methane
PHI	= phenyl isocyanate
HPLC	= high performance liquid chromatography

## SUMMARY

1. Preliminary studies were performed dealing with the analyses, the characteristics and reproducibility of MDI aerosols in an inhalation chamber. These studies were followed by a subacute inhalation toxicity study of polymeric MDI, during which groups of 10 male and 10 female rats were exposed to atmospheres containing aerosols of the test material at levels of 0, 2, 5 or 15 mg/m<sup>3</sup> for 6 hours/day, 5 days/week during a period of 2 weeks. Symptomatology, body weights, lung weights and gross pathology were used as criteria to disclose possible adverse effects.
2. During the 2-week inhalation toxicity study concentrations of polymeric MDI in the test atmospheres were monitored by means of a QCM cascade and, once daily, determined by HPLC.  
The mean actual concentrations of polymeric MDI as determined by means of the QCM cascade were 2.18 ± 0.23, 4.88 ± 0.77 and 13.55 ± 1.30 mg/m<sup>3</sup> for the low-, mid- and high-level group, respectively. There were large discrepancies between the values obtained by the QCM cascade and those found by means of HPLC.  
No MDA and no PHI could be detected in the test atmospheres.
3. Severe respiratory distress was observed in male and female rats exposed to 15 mg polymeric MDI/m<sup>3</sup>. Male rats exposed to 5 mg/m<sup>3</sup> showed similar but much less severe signs.
4. Seven out of 10 males and one out of 10 females exposed to 15 mg polymeric MDI/m<sup>3</sup> died before the end of the study.
5. Severe growth retardation was observed in male and female rats exposed to 15 mg polymeric MDI/m<sup>3</sup> and slight growth retardation in males exposed to 5 mg/m<sup>3</sup>.
6. Lung-to-body weight ratios were higher in all test groups than in controls. There was a positive dose-response relationship.

7. Gross pathological examination failed to reveal changes which could be ascribed to the test material.
8. Based on the marginal increase in lung-to-body weight ratio 2 mg polymeric MDI/ $\text{m}^3$  of air is an effect level.

PRELIMINARY STUDIES OF POLYMERIC MDI AEROSOLS AND A SUBACUTE (2-WEEK)  
INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

## 1. INTRODUCTION

At the request of the International Isocyanate Inc. U.S.A., a research program is performed concerning the toxicological properties of inhaled polymeric MDI. Within the scope of this program a subacute (2-week) inhalation toxicity study in rats was carried out to enable the selection of the dose levels of MDI to be used in a subsequent subchronic inhalation toxicity study in rats. The dose levels for this 2-week study were selected on the basis of the results obtained from an one-week fur deposition study with polymeric MDI (Report V 82.049, August 1982). In this study the concentration of the aerosol was measured indirectly by determination of oil-red which was added to the test material. During the 2-week study described in the present report the concentration of polymeric MDI in the atmosphere was determined among others by a QCM cascade. Therefore, it was necessary to perform a preliminary study dealing with the relation between values obtained by the QCM cascade and the oil-red method.

In addition, two other preliminary studies with polymeric MDI were carried out. One to get information on the distribution of the MDI aerosol in the exposure chamber under conditions corresponding with those prevailing during the inhalation toxicity studies with this test material. The other probe study was performed to investigate the reproducibility of the polymeric MDI aerosol. Both the three preliminary studies and the 2-week inhalation toxicity study are described in the present report.

## 2. MATERIAL AND METHODS

### 2.1 Test material

A sample of polymeric MDI (Desmodur 44 V 20) was received from Bayer AG, Leverkusen, FRG, in May, 1982. Desmodur 44 V is a viscous ( $\eta = 200 \pm 40$  mPas), dark brown liquid with the following composition as specified by Bayer AG:

NCO - content	$30 \pm 2$ % (w/w)
hydrolysable chlorine	$\leq 0.3$ % "
total chlorine	$\leq 0.8$ % "
chlorobenzenes	$\leq 0.015$ % "
phenyl isocyanate	$\leq 0.005$ % "
content of monomeric MDI	$52 \pm 3$ % "
content of sediment	0.01 % "

### 2.2 Exposure chambers

H 1000 multitiered inhalation chambers manufactured by Hazleton Systems Inc., U.S.A., were used. 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  $2.5 \text{ m}^3$ .

### 2.3 Generator of the test atmospheres

The polymeric MDI aerosols were generated by means of a stainless steel/glass air nebuliser. A cyclone was placed behind the nebuliser to remove large particles to ensure that, upon entering the inhalation chamber, 95 % of the particles was smaller than  $5 \mu\text{m}$ .

## 2.4 Preliminary studies

### 2.4.1 Study into the distribution of polymeric MDI aerosol in the inhalation chamber

An aerosol of polymeric MDI was led into the inhalation chamber during one period of 6 hours. The total airflow through the chamber was  $36 \text{ m}^3/\text{hour}$ .

The chamber was fully laden with cages, pans, and 120 individually housed adult Cpb:WU, Wistar Random rats.

To determine the concentration of polymeric MDI, test atmosphere samples were taken with a Berkley model C-1000A QCM Cascade Airborne Particle Size Analyzer (QCM cascade), using a flow rate of 250 ml/minute (fig. 1). The sampling locations in the chamber are given in fig. 2; they were coded 1, 2, 3, 4, 5 and 6.

Two series of samples were taken, one series in duplo 2.5 hours after the start of the exposure period and the other after 5 hours.

### 2.4.2 Two-day study into the control and reproducibility of polymeric MDI aerosol in the inhalation chamber

An aerosol of polymeric MDI was generated from a nebulizer and led into the inhalation chamber during 6 hours/day, for 2 days. Initially, the total air flow through the chamber was  $40 \text{ m}^3/\text{hour}$ . The nebuliser was operated in such a way that a concentration of about  $5 \text{ mg polymeric MDI}/\text{m}^3$  air could be expected. The chamber was fully laden with cages and pans.

To determine the concentration of the polymeric MDI atmosphere, samples were taken from one location in the chamber with the QCM cascade. To adjust the aerosol concentration in the atmosphere, the total air flow through the chamber was either increased or decreased depending on the concentration values determined by the QCM cascade.

During the second day additional atmosphere samples were taken for determination of the monomeric MDI concentration by HPLC. The polymeric MDI aerosol concentration was calculated from the content of monomeric MDI. One of these samples was analysed for MDA content (for method and analyses see report V 82.050).

#### 2.4.3 Two-day study into the relation between polymeric MDI concentrations measured by a QCM cascade and those obtained by oil-red measurements

Aerosols of polymeric MDI in the inhalation chamber were generated at concentrations of  $2 \text{ mg/m}^3$  and  $15 \text{ mg/m}^3$  during 6 hours per day, for 2 days. The total air flow through the chamber was  $40 \text{ m}^3/\text{hour}$ . The concentration of the aerosol in the chamber was simultaneously determined by the QCM cascade and by the oil-red method (see addendum to protocol for preliminary atmosphere generation, analytical, and acute and sub-acute inhalation toxicity studies of polymeric MDI, chapters 8 and 2 respectively).

On the basis of the concentration data, obtained by the QCM cascade and oil-red measurements, the relation between these two methods was calculated.

### 2.5 Sub-acute (2-week) inhalation toxicity study

#### 2.5.1 Test animals

Forty male and forty female SPF-bred Wistar rats (Cpb:WU, Wistar Random) were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, the Netherlands on May 25, 1982. They weighed 35-50 g on arrival and were  $22 \pm 1$  day old. The animals were allocated randomly, according to a computer randomization listing, to four groups, each one consisting of 10 males and 10 females.

### 2.5.2 Maintenance

During exposure all rats were housed individually in wire mesh stainless steel cages. The total air flow through the inhalation chambers ranged from 48 to 56 m<sup>3</sup>/hour. The temperature during the exposures was 19.5 ± 0.5 °C. The relative humidity in the exposure chambers varied from 50 to 60 % during the first three exposures. In this period about 75 % of the humidity readings were above the upper limit of 55 % mentioned in the protocol. To reduce the relative humidity, air supplied by the ventilation system was partly replaced by dry air from the compressed-air system. From that point of time the relative humidity in the inhalation chambers did not fall below 44 % and did not rise above 56 %. After each exposure the animals were returned to their living cages, 5 males or 5 females per cage. These cages were of stainless steel and had wire-mesh bottoms and front sides; they were suspended in an open rack in an animal room. The temperature and relative humidity in this room were maintained at 21 ± 1 °C and 45-70 % respectively.

During exposure the animals were deprived of food and water. During the non-exposure periods the animals were fed the Institute's stock diet and bottled tap water ad libitum

### 2.5.3 Dose levels

The selected dose levels of polymeric MDI in the various test atmospheres were 0, 2, 5 and 15 mg/m<sup>3</sup>.

### 2.5.4 Test atmosphere control

The determination of the concentration and particle size distribution of polymeric MDI in the test atmospheres were performed by means of a QCM cascade. From each test atmosphere about 10 samples were taken per day. The sampling periods were 2, 1 and 0.5 sec for the low-, mid- and high-dose levels, respectively.

In addition, once a day one sample was taken from each of the test atmospheres for the determination of the MDI and PHI concentration by means of HPLC.

Once a week the MDA concentration was measured by HPLC in each of the test atmospheres.

All methods and analytical procedures concerning the determination of PHI are described in full detail in our report V 82.050.

#### 2.5.5 Conduct of the study

Three groups of rats, each one consisting of 10 males and 10 females, were exposed to atmospheres containing aerosols at concentrations of 2, 5 or 15 mg polymeric MDI/m<sup>3</sup> respectively for 6 hours per day during two periods of five successive days. The first exposure was on Monday, June 7, 1982; the last exposure was terminated in the early afternoon of Friday, June 18, 1982. Another group of 10 males and 10 females served as sham-controls.

The animals were observed for clinical symptoms before and after exposure, but during the weekend only once a day.

Body weights were recorded just prior to the start of the exposure to the test substance and on day 3, 6, 8 and 10, and just prior to sacrifice.

Autopsy was started two hours after termination of the last exposure. The surviving rats were sacrificed randomly. The rats were killed by exsanguination from the abdominal aorta under ether anaesthesia, and then examined grossly for pathological changes.

Lungs, trachea, larynx and nose of all rats were preserved in a 4 % neutral aqueous phosphate buffered solution of formaldehyde. The lungs of rats killed terminally were weighed, and then fixed by intratracheal infusion of the fixative under 10 cm water pressure.

### 2.5:6 Statistical analysis of the results

The statistical analysis of body weights and absolute lung weights was carried out using analysis of co-variance followed by the Dunnett test. Body weight gain data were evaluated by using the Mann/Whitney U-test. The lung-to-body weight ratios were compared by analysis of variance and the Dunnett test.

## 3. RESULTS

### 3.1 Preliminary studies

#### 3.1.1 Distribution of polymeric MDI aerosol in the inhalation chamber

The results obtained are presented in table 1.

The mean concentration of polymeric MDI in the atmosphere was  $2.9 \text{ mg/m}^3$  of air. When the variation in the concentration is allowed to be  $\pm 20 \%$ , the range of concentration may vary from  $2.3 \text{ mg/m}^3$  of air to  $3.5 \text{ mg/m}^3$  of air.

In that case the data of table 1 show that three concentration values were out of limit; i.e. the values 3.9, 3.7 and 2.2, which were the results of samples taken after 2.5 hours at different locations. The concentrations at the different sample ports determined 5 hours after starting the expose showed only a very slight variation ( $2.6 - 2.9 \text{ mg/m}^3$ ).

### 3.1.2 Control and reproducibility of polymeric MDI aerosol concentrations

Concentrations and the corresponding air flows are summarized in table 2.

The concentration of polymeric MDI during the first measurements were different from the expected concentration of about  $5 \text{ mg/m}^3$ . The concentration of the aerosol, however, could be well controlled by increasing or decreasing the total air flow.

### 3.1.3 Relation between polymeric MDI concentration values determined by the QCM cascade and by HPLC analysis

During the control and reproducibility study of polymeric MDI aerosols, additional atmosphere samples were taken for analysis by HPLC. The results of these analyses and those obtained by the QCM cascade are presented in table 3. From these results it appeared that the concentrations as analysed by the HPLC were mostly higher than the values measured by the QCM cascade.

The relation between QCM cascade and HPLC values varied from 0.61 to 1, and was on average 0.81.

### 3.1.4 Relation between polymeric MDI concentration values determined by the QCM cascade and by oil-red measurements

The results of the measurements and the relation between values obtained by the QCM cascade and the oil-red method are presented in table 4.

Concentration values as measured by the oil-red method are slightly higher than those determined by the QCM cascade. The relation between oil-red values and those obtained by the QCM cascade varied from 1.10-1.49 at concentrations of about 2 and  $15 \text{ mg/m}^3$ . On average this relation was 1.20 as based on five series of measurements.

### 3.2 Sub-acute (2-week) inhalation toxicity study

#### 3.2.1 Concentrations of polymeric MDI, MDA and PHI in the test atmospheres

Mean daily concentrations of polymeric MDI in the test atmospheres are presented in table 5.

The dose levels of this study (0, 2, 5 and 15 mg/m<sup>3</sup> air) were selected on the basis of a previous fur-deposition study (see report V 82.049). During that study the concentrations of polymeric MDI were determined by the oil-red method. As a consequence of the results of the preliminary studies (see 3.1.4) the concentrations in the 2-week study which were determined by QCM cascade had to be corrected with the oil-red/QCM cascade ratio. This was done by multiplication of the mean concentrations of polymeric MDI obtained by the QCM cascade with a factor of 1.20.

After correction with this factor the mean daily atmospheres of polymeric MDI in the different test atmospheres during the entire study were  $2.18 \pm 0.23$  mg/m<sup>3</sup>,  $4.88 \pm 0.77$  mg/m<sup>3</sup> and  $13.55 \pm 1.30$  mg/m<sup>3</sup>, respectively.

The concentrations of polymeric MDI determined by HPLC and the concentrations determined by the QCM cascade in samples taken simultaneously are presented in table 6.

The atmospheres were sampled with the QCM cascade at the beginning and frequently also at the end of the period during which a sample was taken for analysis by HPLC. The differences between concentrations determined by the two methods were rather large. At the lowest dose-level, lower as well as considerably higher values were obtained by HPLC analysis than by means of the QCM cascade. At the mid-dose level more values were similar, but also at this level a few concentrations determined by HPLC were considerably higher than those found with the QCM cascade. At the level of 15 mg/m<sup>3</sup> most values determined by HPLC were lower - in a few cases even more than 50 % - than the concentrations obtained by the QCM cascade. Particle size distribution measurements showed that more than 95 % of the particles were smaller than 5 µm (table 7).

MDA and PHI could not be detected in any of the atmosphere samples.

### 3.2.2 Clinical signs and mortality

At the highest exposure level behaviour and condition of the animals were severely affected, and 7 males and 1 female died. The first rat died shortly after the fourth exposure. During the first three days no abnormalities were observed in health or behaviour. On day 4, however, the first clinical changes were noticed. Before exposure movements of the male rats were slower than those of controls. During the exposure they showed slight pilo-erection and an increased respiration frequency. During the further course of the study these signs worsened considerably. The animals became severely dyspnoeic, showed a frequent laboured respiration and mouth breathing.

Other clinical signs were salivation, frequent pawing of the nose and bleeding from the nares. Swollen abdomens were observed in many males towards the end of the study. Slight diarrhoea was seen at day 6 only. Similar but less severe effects were observed in females exposed to 15 mg polymeric MDI/m<sup>3</sup>.

Signs of irritation of the respiratory tract could also be observed in rats exposed to 5 mg polymeric MDI/m<sup>3</sup>. These animals were restless and slightly dyspnoeic, pawed their noses and showed some pilo-erection. None of these animals died. Behaviour or health of rats were not visibly affected at the exposure level of 2 mg polymeric MDI/m<sup>3</sup>.

### 3.2.3 Body weights

Rats exposed to 15 mg polymeric MDI/m<sup>3</sup> showed severe depression in weight gain. In fact these animals hardly grew, whereas the controls gained nearly 100 % body weight (tables 9 and 10).

Also in males of the mid-level group weight gain was statistically significantly lower than that of the controls.

Females exposed to 2 mg polymeric MDI/m<sup>3</sup> of air showed lower weight gain than did controls. Since at the higher exposure level of 5 mg/m<sup>3</sup> of air weight gain was about similar to that of the controls the differences between female controls and females of the low-level group were considered to be toxicologically insignificant.

### 3.2.4 Lung weights

Mean absolute lung weights were decreased in the top-level group and slightly increased in males of the low-level group as compared to the controls (table 11). When the lung weights were expressed relative to the body weights, it appeared that the mean lung weights in all test groups were higher than those of the controls. There was a positive dose-response relationship both in males and females; the differences were statistically significant only in rats exposed to 15 or 5 mg polymeric MDI/m<sup>3</sup>.

### 3.2.5 Gross pathology

At autopsy no gross changes were found that could be ascribed to treatment. The cause of death could not be established in any of the animals that died intercurrently.

## 4. DISCUSSION AND CONCLUSION

The concentration data of polymeric MDI obtained by QCM cascade and by HPLC showed very inconsistent differences. Though, during the preliminary studies the proportional differences between these two methods were relatively small, during the 2-week study large differences were observed. Moreover, any consistency in the differences could hardly be detected. Since the concentration data determined by the QCM cascade were constant, the inconsistency was ascribed to deviating values obtained by HPLC analysis.

So far, no sound explanation is available for these discrepancies in results between the preliminary studies and the 2-week study.

Rats repeatedly exposed to an atmosphere containing an aerosol of 15 mg polymeric MDI/m<sup>3</sup> for a period of two weeks appeared not to gain body weight and showed severe respiratory distress often followed by death, particularly in males. The severe clinical signs of respiratory insufficiency suggest marked changes in this organ system, which might have been responsible for the death of several of these animals. This suggestion is supported by the considerably increased lung-to-body weight ratios found in rats of the high-level group. However, gross examination at autopsy did not reveal pulmonary changes attributable to the test material. Histopathological examination of the lungs is indicated to establish microscopic pulmonary alterations induced by polymeric MDI, if any.

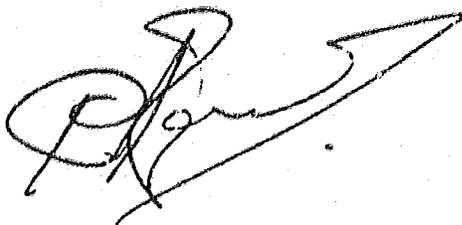
Rats of the mid-level group showed similar but less severe changes than did animals of the high-level group. Growth retardation and slight respiratory distress were observed in males only, whereas increased relative lung weights occurred in both sexes.

Exposure to an aerosol of 2 mg polymeric MDI/m<sup>3</sup> resulted only in slightly higher lung-to-body weight ratios, the differences with the controls being not statistically significant. The positive dose response relationship existing for the relative lung weights suggests that these differences with the controls are attributable to treatment. This suggestion has to be considered in future studies.

In summary, based on the marginal increase in lung-to-body weight ratio 2 mg polymeric MDI/m<sup>3</sup> of air is an effect level.

5. AUTHENTICATION

This report was prepared by:



Drs P.G.J. Rauzel

date:

November 8, 1983

and approved by:



Dr V.J. Feron

(Head Department of Biological Toxicology)

date:

November 9, 1983

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 of Biological Toxicology under reference: "B 81-2478, International Isocyanate Institute Inc., polymeric MDI, 2-week inhal. tox. in rats."

QUALITY ASSURANCE UNIT TNO -- P.O.Box 360 , 3700 AJ ZEIST, Netherlands

STATEMENT OF GLP COMPLIANCE

On : Subacute (2-week) inhalation toxicity study of polymeric MDI  
in rats

Report no. : V 83.321/212478

Date : July ,1982.

The study was carried out under conditons of good laboratory practice.  
Within reason there have been no circumstances that might have affected  
the quality and integrity of the study.

Dates and number  
of inspections:

Dates of reports  
to management:

3 June 1982 (1)

4 June 1982

7 June 1982 (1)

7 June 1982

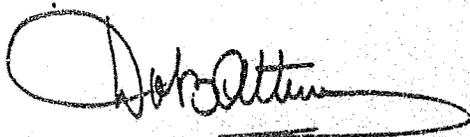
9 June 1982 (1)

9 June 1982

Final report audit:

1-2 July 1982

5 July 1982



Drs D. van Battum  
Quality Assurance Manager

date: 8 July 1982

CIVO/TNO

STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

TABLE 1 - CONCENTRATION OF POLYMERIC MDI IN THE ATMOSPHERE AT DIFFERENT LOCATIONS IN THE INHALATION CHAMBER AND AT DIFFERENT POINTS OF TIME

hours after starting the exposure	concentration of polymeric MDI ( $\text{mg}/\text{m}^3$ ) in samples taken at sampling location					
	1	2	3	4	5	6
2.5	3.1	3.5	2.8	2.9	2.2	2.6
	3.2	3.9	2.7	3.7	2.6	2.8
5	2.7	2.9	2.6	2.6	2.7	2.8

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STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

TABLE 2 - CONCENTRATIONS OF POLYMERIC MDI AEROSOL AT ONE LOCATION DETERMINED BY A QCM CASCADE

sample time (min.) after starting the generation of the aerosol	concentration of polymeric MDI $\mu\text{g}/\text{m}^3$ air)	total air flow rate $(\text{m}^3/\text{hour})$
<u>FIRST DAY</u>		
0	-----	40
45	7.6 ; 7.8	40
95	7.8 ; 7.2	40
125	6.8	47
165	5.5	56
175	4.9 ; 4.6	56
195	4.1 ; 4.4	56
215	4.4 ; 3.9	50
245	5.7 ; 5.3	48
<u>SECOND DAY</u>		
0	-----	50
60	9.1 ; 9.0	50
120	7.4 ; 6.4	56
130	5.8 ; 5.3	54
145	5.2	54
165	4.1	54
180	4.9	61
240	5.3	61
250	5.3 ; 4.6	61
257	5.0 ; 5.7	61
269	4.9 ; 4.6	61
281	4.4	61
290	4.6 ; 4.2	61
307	5.0 ; 5.0	61
317	5.0 ; 6.6	61
335	5.3 ; 4.6	61
345	4.5 ; 4.6	61
355	- ; -	61
360	5.4 ; 4.4	61

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STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

TABLE 3 -- CONCENTRATION OF THE POLYMERIC MDI AEROSOL AT DIFFERENT POINTS OF TIME, AND THE RELATION BETWEEN THE CONCENTRATION DATA OBTAINED BY QCM CASCADE AND HPLC ANALYSIS

sample time after starting the generation (min.)	concentration polymeric MDI (mg/m <sup>3</sup> of air)		QCM cascade/HPLC
	determined by:		
	<u>QCM cascade</u>	<u>HPLC</u>	
250	5.4 ; 4.6	6.72	0.79 ; 0.63
257	5.0 ; 5.7	5.70	0.88 ; 1
269	4.9 ; 4.6	6.04	0.81 ; 0.76
281	4.4	5.42*	0.81
307	5.0 ; 5.0	5.97	0.99 ; 0.99
317	5.0 ; 6.6	5.84	0.86
335	5.3 ; 4.6	7.30	0.73 ; 0.63
355/360	5.4 ; 4.4	7.18	0.75 ; 0.61

\* This sample was also analysed for MDA-content  
No MDA could be detected.

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STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

TABLE 4 - CONCENTRATION DATA OF POLYMERIC MDI AEROSOL DETERMINED BY A QCM CASCADE OR BY AN OIL-RED METHOD AND THE RELATION BETWEEN THESE VALUES

sample no:	concentration polymeric MDI (mg/m <sup>3</sup> of air)			relation oil-red/QCM
	obtained by			
	QCM cascade		oil-red method	
	individual values	mean value		
1	2.58; 2.58; 2.52	2.56	3.11	1.21
2	2.57; 2.45; 2.65	2.56	3.02	1.18
3	14.8; 13.5; 13.8	14.0	15.4	1.10
4	16.1; 15.5; 13.8	15.1	17.1	1.13
5	13.0; 11.4; 12.7; 13.0	12.5	18.7	1.50

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 STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS

TABLE 5 MEAN DAILY CONCENTRATIONS OF POLYMERIC MDI IN THE TEST ATMOSPHERES

Day of exposure	Concentration (mg/m <sup>3</sup> )								
	groups								
	B (low-level)			C (mid-level)			D (high-level)		
	n <sup>1)</sup>	conc. <sup>2)</sup>	SD <sup>3)</sup>	n	conc.	SD	n	conc.	SD
0 <sup>4)</sup>	12	1.89	0.35	12	3.75	0.59	12	11.43	3.02
1	10	2.07	0.63	11	3.79	0.77	11	10.30	1.53
2	10	1.64	0.57	11	3.92	1.25	11	10.37	1.68
3	11	1.79	0.58	11	4.32	2.45	11	12.23	3.66
4	8	2.12	0.41	10	4.32	1.13	10	13.11	4.13
7	12	1.73	0.28	12	3.82	0.70	12	9.56	2.28
8	9	1.87	0.45	11	3.46	0.64	12	11.73	2.88
9	10	1.61	0.16	11	4.32	1.02	12	12.28	3.16
10	13	1.56	0.61	16	3.40	0.70	16	10.31	1.73
11	12	1.89	0.39	14	5.63	1.62	16	11.62	4.09
Mean daily concentrations		1.82	0.19		4.07	0.64		11.29	1.08
Corrected Mean <sup>5)</sup>		2.18	0.23		4.88	0.77		13.55	1.30

1) n = number of samples

2) conc. = concentration

3) SD = standard deviation of the mean

4) day 0 = first day of exposure

5) After correcting for the oil-red/QCM cascade ratio (x 1.20)

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TABLE 6 CONCENTRATIONS OF POLYMERIC MDI IN TEST ATMOSPHERES DETERMINED BY HPLC AND A QCM CASCADE IN SAMPLES TAKEN SIMULTANEOUSLY

Day of exposure	Dose level (mg/m <sup>3</sup> ) <sup>1)</sup>					
	2		5		15	
	Method <sup>2)</sup>		Method		Method	
	I	II	I	II	I	II
0	1.94*	1.00	4.93*	3.51	11.43*	6.99
1	1.91*	1.32	4.01*	4.63	10.30*	9.20
2	2.60*	1.88	4.08*	2.67	12.70*	10.10
3	2.04*	2.82	4.45*	4.49	15.23*	11.88
4	2.79	6.64	4.21*	7.91	13.95*	10.66
4	2.23	7.22	5.23*	5.99	11.25	9.81
4	2.13	7.60	5.10	7.80	15.59	10.29
7	2.31*	23.86	4.71*	4.92	13.95*	16.21
8	2.13	6.59	4.31*	3.36	17.86*	7.81
9	1.94*	2.62	5.60	4.92	15.90*	6.36
10	1.98*	2.54	4.13*	3.36	11.08*	8.45
11	1.70*	6.38	5.86*	5.67	13.11*	6.36

1) All values have been adjusted for the oil-red/QCM Cascade ratio

2) I = QCM Cascade

II = HPLC

\* = mean value of two determinations, one carried out at the beginning and one at the end of the period during which a sample was taken for HPLC

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 TABLE 7 PARTICLE SIZE DISTRIBUTION OF POLYMERIC MDI AEROSOLS

Dose level	aerodynamic diameter D <sub>50</sub> (µm)	Distribution (%) at day												
		0	1	2	3	4	7	8	9	10	11			
2 mg/m <sup>3</sup>	22.6	0	0	0	0	0	0	0	0	0	0	0	0	0
	10.9	0	0	0	0	0	0	0	0	0	0	0	0	0
	6.4	0	0	0	0	2.9	0	0	0	0	0	0	0	0
	3.3	0	3.3	3.2	3.3	2.9	0	0	0	0	0	0	0	4.5
	1.8	40.9	50.0	51.6	48.4	40.0	59.3	42.1	40.9	50.0	50.0	30.8	27.3	50.0
	1.0	31.8	23.3	25.8	25.8	28.6	25.9	42.1	50.0	30.8	30.8	27.3	27.3	27.3
	0.6	18.2	16.7	19.4	19.4	17.1	14.8	15.8	4.5	15.4	15.4	9.1	9.1	9.1
	0.37	4.5	3.3	0	0	5.7	0	0	4.5	3.8	3.8	9.1	9.1	9.1
	0.20	4.5	3.3	0	0	2.9	0	0	0	0	0	0	0	0
	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0

cont...

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 TABLE 7 PARTICLE SIZE DISTRIBUTION OF POLYMERIC MDI AEROSOLS. CONTINUED 1.

Dose level	aerodynamic diameter D <sub>50</sub> (μm)	Distribution (%) at day												
		0	1	2	3	4	7	8	9	10	11			
5 mg/m <sup>3</sup>	22.6	0	0	0	0	0	0	0	0	0	0	0	0	0
	10.9	0	0	0	0	0	0	0	0	0	0	0	0	0
	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0
	3.3	0	6.5	0	0	0	0	0	4.3	2.7	0	3.7	0	0
	1.8	34.6	48.3	28.6	38.5	47.8	46.2	34.8	37.8	41.7	48.1	0	0	0
	1.0	38.5	29.0	42.9	30.8	30.4	34.6	43.5	40.5	41.7	33.3	0	0	0
	0.6	19.2	16.1	21.4	23.1	21.7	15.4	17.4	16.2	12.5	11.1	0	0	0
	0.37	3.8	0	3.6	7.7	0	3.8	0	2.7	4.2	3.7	0	0	0
	0.20	3.8	0	3.6	0	0	0	0	0	0	0	0	0	0
	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0

CONT....



CIVD/TND  
STUDY NO 448 SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC NDI IN RATS

TABLE 8 MORTALITY INCIDENCES

M A L E S

DAY	CONTROL INCIDENCES	2 MG INCIDENCES	5 MG INCIDENCES	15 MG INCIDENCES
4	0	0	0	1
5	0	0	0	1
6	0	0	0	1
7	0	0	0	3
8	0	0	0	5
9	0	0	0	5
10	0	0	0	7
11	0	0	0	7

F E M A L E S

DAY	CONTROL INCIDENCES	2 MG INCIDENCES	5 MG INCIDENCES	15 MG INCIDENCES
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	1
8	0	0	0	1
9	0	0	0	1
10	0	0	0	1
11	0	0	0	1

CIVD/TMD

STUDY NO 448

SUB-ACUTE INHALATION TOXICITY STUDY OF POLYMERIC NDI IN RATS

ID.NO =

TABLE 9

MEAN BODY WEIGHTS

M A L E S

DAY	CONTROL		2 MG		5 MG		15 MG		N
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	
0	66.0	1.4	69.7	2.6	68.4	1.9	69.0	1.5	10
3	81.5	1.6	85.3	3.1	79.0	2.2	67.1**	2.1	10
6	96.7	1.6	101.3	3.5	91.9	2.5	61.6**	3.4	9
8	108.5	1.7	113.6	4.2	101.2*	3.6	71.6**	5.0	5
10	121.3	2.1	127.0	4.6	112.6	5.0	79.0**	8.0	3
11	117.3	2.0	123.5	4.1	108.0	5.2	67.0**	8.3	3

F E M A L E S

DAY	CONTROL		2 MG		5 MG		15 MG		N
	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	
0	70.8	2.9	66.6	2.7	66.7	2.5	62.9	1.7	10
3	83.9	2.9	75.3*	0.9	79.7	2.7	65.8**	2.4	10
6	97.9	3.0	88.4	1.2	91.3	3.1	70.3**	4.3	10
8	105.4	3.2	95.5	0.9	99.2	3.9	75.3**	4.1	9
10	116.9	3.4	106.0	1.2	110.0	3.8	73.5**	4.5	9
11	109.8	2.8	101.3	1.2	102.8	3.4	65.7**	4.2	9

STATISTICS: COVAR + BUNNETT TESTS \* P<0.05 \*\* P<0.01 TWO SIDED

(EXP. UNIT = ANIMAL)



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ID. NO. =

V 83.321

TABLE II MEAN LUNG WEIGHTS AND MEAN LUNG-TO-BODY WEIGHT RATIOS

	BODY WGT		LUNG <sup>1)</sup>		LUNG <sup>2)</sup>	
	MEAN	SEM	(GRAMS)	(GRAMS)	(G/KG)	(G/KG)
CONTROL	117.3	2.0	0.92	0.02	7.84	0.21
	N	10	10	10	10	10
2 MG	123.5	4.1	1.04 <sup>#</sup>	0.04	8.42	0.17
	N	10	10	10	10	10
5 MG	108.0	5.2	0.99 <sup>**</sup>	0.05	9.25 <sup>**</sup>	0.27
	N	10	10	10	10	10
15 MG	67.0	8.3	0.77 <sup>*</sup>	0.04	11.71 <sup>**</sup>	0.95
	N	3	3	3	3	3

1) STATISTICS: MANN WHITNEY U-TEST \* P<0.05 TWO SIDED (EXP.UNIT = ANIMAL)

2) STATISTICS: ANOVA + DUNNETT TESTS \*\* P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

CIVD/TND

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ID.NO

TABLE II MEAN LUNG WEIGHTS AND MEAN LUNG-TO-BODY WEIGHT RATIOS

F E M A L E S

		BODY WGT (GRAMS)	LUNG 1) (GRAMS)	LUNG 2) (G/KG)
CONTROL	MEAN	107.8	0.88	8.05
	SEM	2.8	0.03	0.26
	N	10	10	10
2 MG	MEAN	101.3	0.90	8.89
	SEM	1.2	0.03	0.25
	N	10	10	10
5 MG	MEAN	102.8	0.96	9.40*
	SEM	3.4	0.03	0.27
	N	10	10	10
15 MG	MEAN	65.7	0.69***	10.68**
	SEM	4.2	0.03	0.49
	N	9	9	9

1) STATISTICS: MANN WHITNEY U-TEST \*\*\* P<0.002 TWO SIDED

(EXP.UNIT = ANIMAL)

2) STATISTICS: ANOVA + DUNNETT TESTS

\* P<0.05 \*\* P<0.01 TWO SIDED

(EXP.UNIT = ANIMAL)

FIGURE 1 - SAMPLING METHOD FOR A QCM CASCADE

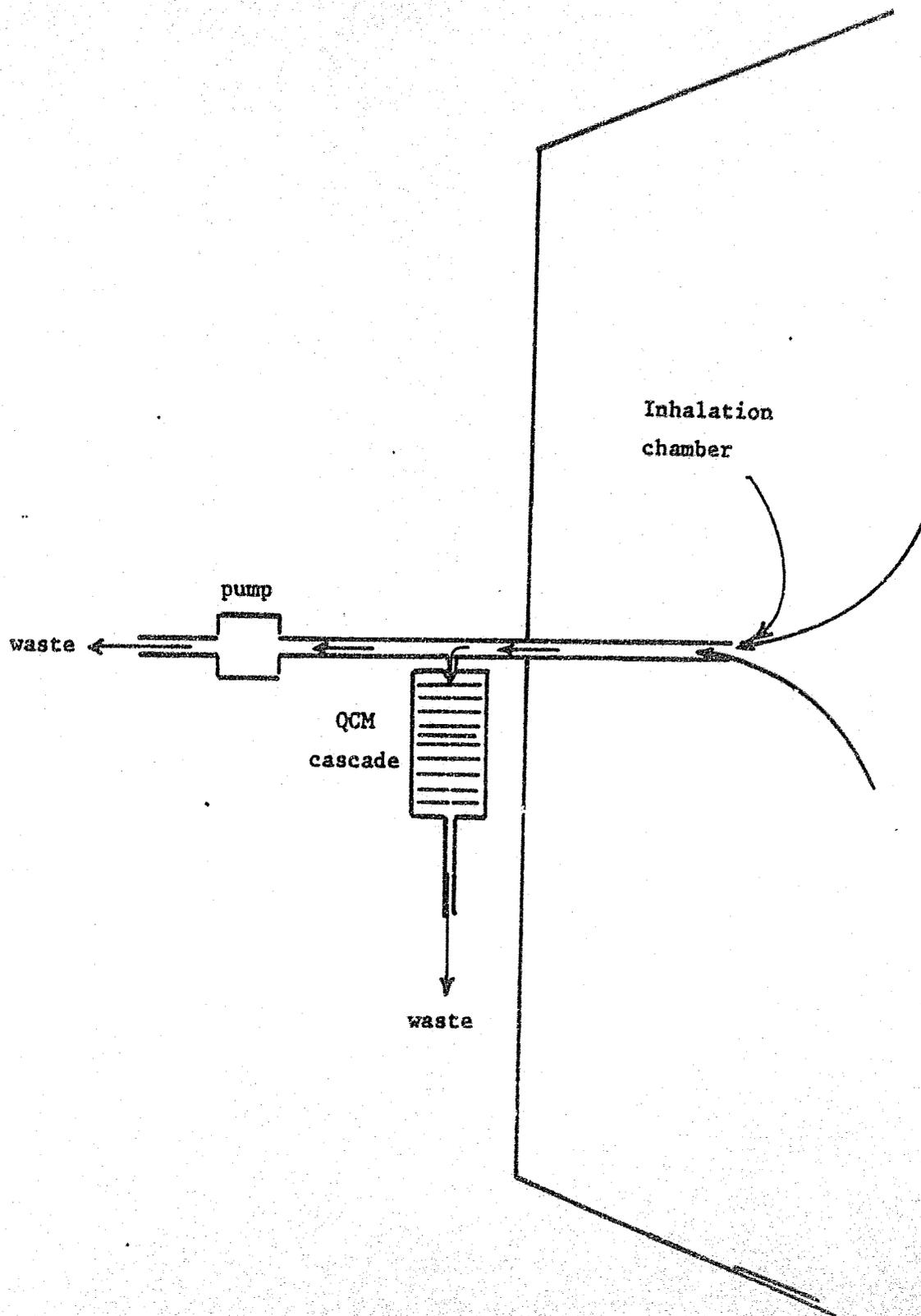


FIGURE 2 - FRONT-SIDE HAZLETON 1000 INHALATION CHAMBER, WITH THE DIFFERENT SAMPLING LOCATIONS

