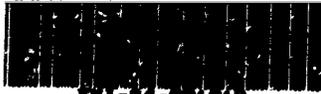


Mobay



771-94-881817
INIT 87/14/94



Mobay
Chemical Corporation



84948888117

FBI-0791-00017

Penn Lincoln Parkway West
Pittsburgh, PA 15205
Telephone: 412/777-2000

July 20, 1982

PIF-323

Martin Greif
Executive Secretary
TSCA Interagency Testing Committee
Environmental Protection Agency (T3-732)
401 M Street, S.W.
Washington, D.C. 20460

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94 JUL 14 AM 9:34

Dear Mr. Greif:

These comments are submitted in response to an ITC Request for Information published in the Federal Register Vol. 47 No. 38 Thursday, February 25, 1982.

The list of candidates to be given priority consideration for the promulgation of a Toxic Substances Control Act Section 4(a) testing rules includes CAS 822-06-0 1,6-diisocyanatohexane ("HDI"). Mobay Chemical Corporation is the only commercial U.S. producer of HDI.

It is Mobay's position that a TSCA Section 4(a) testing rule for HDI is not necessary for the following reasons:

1. Total U.S. production was less than 11 million pounds for the year 1981.
2. More than 99% of the HDI produced for the U.S. market is consumed internally by Mobay to produce higher molecular weight biuret polyisocyanate products. The estimated quantity of HDI which enters the environment during production and use does not exceed 20,000 pounds per year.
3. Industrial hygiene sampling data show that human inhalation exposure during production and use is minimal. Human dermal exposure during production and use is minimized through the use of appropriate protective equipment.

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Martin Greif
Page Two
July 20, 1982

4. Non-occupational human exposure is negligible. Once the coating system is applied and cured the HDI is reacted and no further human contact is possible.
5. The structure of HDI is unrelated to other substances known to present an unreasonable risk of injury to human health or the environment.
6. HDI has been adequately tested for acute and subchronic toxic effects. Mutagenicity testing has been done and results are negative. Mobay has begun toxicity testing on HDI for carcinogenic and other chronic toxic effects.
7. Further testing is not necessary in order to adequately assess the environmental and health effects associated with HDI production and use.

The facts supporting our position that HDI should not be included in the TSCA Section 4(e) Priority List are set forth in detail in the document entitled Response to the Interagency Testing Committee for 1,6-Hexamethylene Diisocyanate which is attached. All necessary testing is completed or in progress and environmental and human exposure to HDI are minimal. We urge the Interagency Testing Committee to withdraw HDI from review for designation to the Section 4(e) Priority List.

Very truly yours,



C. L. H. Howard
Vice President-Scientific,
Environmental and Medical Services

/cp

Attachment

DIR-323

RESPONSE TO THE INTERAGENCY TESTING COMMITTEE
FOR 1,6-HEXAMETHYLENE DIISOCYANATE

I. Identification

CAS Registry Number: 822-06-0

Name: 1,6-Hexamethylene Diisocyanate

Synonyms: HDI, HMDI, 1,6-Diisocyanatohexane, Mondur
HX, Desmodur H

Structural Formula: $O=C=N-(CH_2)_6-N=C=O$

Molecular Formula: $C_8H_{12}N_2O_2$

Molecular Weight: 168.18

II. Physical Properties

Refractive Index: 1.4530₂₀

Viscosity at 25°C: 1.25_{CPS}

Partition Coefficient (Octanol/water): Can not be
measured because HDI reacts with both solvents.

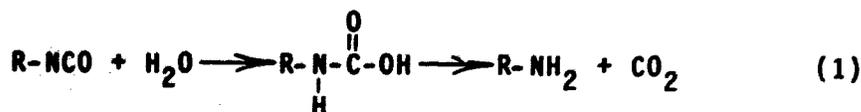
Other Physical Properties: See Mobay material safety
data sheet No. 0213 dated March 1, 1982. (Attachment 1)

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II. Chemical Properties

1. Reaction with Water

Like all isocyanates, 1,6-hexamethylene diisocyanate (HDI) will react with water as shown in the following general reaction scheme:

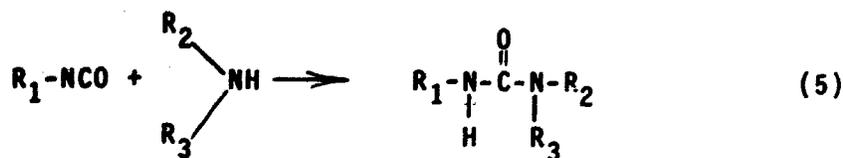


Since HDI is a di-functional isocyanate, polyureas are also formed in addition to the simple N,N'-disubstituted urea shown in equation (2). The rate of reaction of HDI with water to form the amine and CO_2 was determined by Cooper et al (1) to be $0.5 \times 10^{-4} \text{ l mole}^{-1} \text{ sec}^{-1}$ at 100°C .

An example of this reaction which is of great commercial importance is the production of Desmodur N as follows:

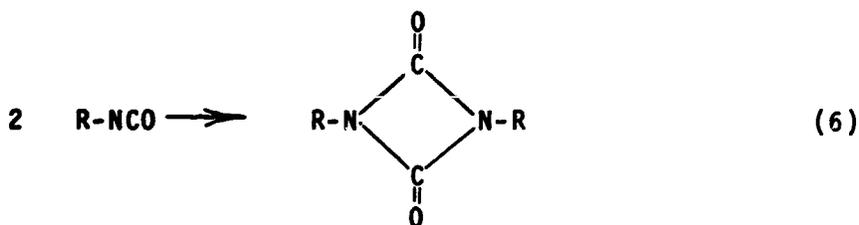
3. Reaction with Amines

HDI will react with primary and secondary amines, yielding the corresponding substituted ureas:

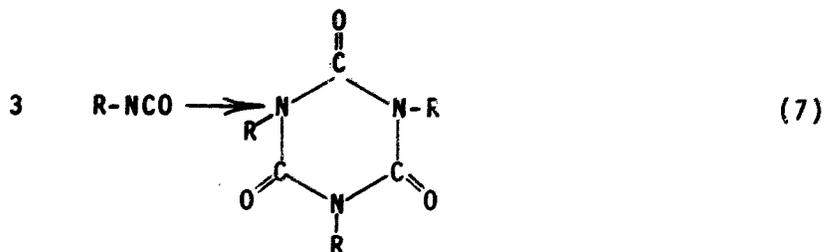


4. Dimer and Trimer Formation

Isocyanates can dimerize slowly to form a uretione; however, aliphatic isocyanates, such as HDI, do not form dimers as readily as aromatic isocyanates. The dimerization reaction is shown in the following equation:



With the appropriate catalyst, HDI will trimerize, forming the following isocyanurate structure:



At elevated temperatures, typically greater than 200°C, isocyanates will trimerize, as shown in equation (7), or form carbodiimides, as shown in equation (8).



III. Production Data

Annual Production: 9 to 11 million pounds

Approximately 2 to 3 million pounds are exported as the monomer. Ninety nine percent of the remainder is consumed internally by Mobay to make higher molecular weight biuret polyisocyanate commercial products. Less than one percent of HDI reaches the United States market as HDI Monomer. This portion is either sold directly as Mondur HX or contained as a residual monomer at less

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than 0.7% in biuret polyisocyanate products. The production process is continuous and closed.

IV. Human Exposure During Production

Possible Routes of Exposure: Inhalation, Dermal
Number of People with Potential for Exposure during 50%
of their Work-Year: Approximately 50
Number of People with Only Occasional Potential for
Exposure During Their Work-Year: Approximately 105

Airborne Concentrations

Of 115 area samples collected in 1981 (some samples taken each month from February through November), 59% showed no detectable airborne HDI using an analytical method sensitive to 0.01 ppm, 20% showed HDI concentrations between 0.01 ppm and 0.02 ppm and 20% showed concentrations greater than 0.02 ppm with the arithmetic mean of this last group of samples being 0.04 ppm. It is important to note that these area samples were collected for the purpose of detecting sources of vapor generation (e.g. leaking pump seals) rather than to evaluate actual personal worker exposures. Thus samplers were often placed right beside a suspected

point of generation and left there much longer than a person would actually stay in that position. Even so, about 80% of the concentrations measured were below the 0.02 ppm Mobay suggested exposure limit for HDI.

Exposure Controls:

1. Closed system production minimizes exposures.
2. Walk-in hoods are used at drumming stations.
3. Exhaust hoods are fitted over sampling ports to control vapors when liquid quality assurance samples are taken.
4. Personal protective equipment including slicker suits, boots, gloves, visors, goggles and full face supplied air respirators are available and used as needed.

V. Uses

Greater than 99% of the HDI produced for the U.S. market is consumed internally at Mobay to manufacture higher molecular weight biuret polyisocyanates. (See Part II, 1. and Attachments 2, 3 and 4) These resins are used by Mobay's customers to formulate polyurethane paint systems for automobile refinishing, industrial maintenance, marine coatings, and other similar markets

which require such high performance coating systems. At time of manufacture the HDI biuret polyisocyanate resins contain less than 0.7% free HDI monomer. This percentage has been shown to slowly rise to as high as 1.6% during storage. Since these resins are mixed with other paint components before the paint is applied, the free HDI monomer percentage in the paint systems at time of application is usually less than 0.5%.

VI. Human Exposure During Use

Approximately 204,000 people worked in auto body repair shops in the United States in 1981⁽²⁾. About 75% of these shops used some paints which contain small amounts (<0.5%) of HDI. Thus 153,000 workers in such shops have a potential for minimal exposure to HDI. In addition there are approximately 50,000 other industrial workers who have a potential for minimal exposure to HDI.

Field industrial hygiene surveys conducted during the spray-application of HDI-containing paint systems revealed the following:

Airborne HDI

<u>Concentration</u>	<u>Number of</u>	
<u>Range (ppm)</u>	<u>Samples</u>	<u>Percentage</u>
0.04	1	1
0.015 to 0.02	11	9.2
0.010 to 0.0149	10	8.4
0.005 to 0.0099	16	13.4
less than 0.005	<u>81</u>	<u>68.0</u>
	119	100.0

These data were gathered during six different surveys during the period 1978 to 1982. Each of the six surveys involved spray application of polyurethane paints containing small amounts of residual HDI. These data suggest that although the potential number of exposed workers is rather high, exposure to airborne concentrations exceeding Mobay's suggested exposure limit (0.02 ppm) is unlikely. This would be expected based on the small percentage (<0.5%) of HDI monomer present in the paint at time of application.

In addition to conducting such industrial hygiene surveys, Mobay's industrial hygienists and product safety specialists are involved with writing and up-dating of material safety data sheets (Attachments 1

and 4) precautionary labels (Attachment 5) and general safety literature (Attachment 6) designed to inform our customers of the precautions they should take in the processing and use of our products.

Vii. Environmental Release During Production

There are only two production units which are potential sources of environmental release. One is the HDI production unit (See Attachment 7) and the other is the biuret polyisocyanate production unit (See Attachment 8).

A. HDI Production Unit

Release to Air

All of the process off-gases from this unit are collected by a common vent system and sent to the HCl production unit. There, the HCl is recovered and the remaining off-gases are incinerated after first passing through a carbon adsorption unit. Before entering the common vent system some of the off-gases pass through a counter current scrubber.

The production facility is equipped with a spot vent system to reduce exposure to the personnel. This system includes a totally enclosed hood and a small portable hood both of which vent through a scrubber. This system operates only during collection of liquid quality assurance samples. Thus air emission of HDI from this source is negligible.

There are two 30,000 gallon and one 80,000 gallon HDI storage tanks. The total annual air emission from these three tanks (calculated using approved EPA methods)⁽³⁾ is 11 pounds.

Release to Wastewater

Because of the chemical reactivity between isocyanates and water, the process is designed so that the only wastewater streams are from the off-gas scrubbers and clean-up operations. The water from the process sewers is sent to an NPDES permitted wastewater treatment plant where it is treated by neutralization, aeration, clarification and carbon adsorption.

HDI would probably not be found in any scrubber water since it would react with water to form the amine or urea.

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All the rain water falling in the process area is collected and treated in a NPDES permitted wastewater treatment plant.

Solid/Liquid Waste

The waste still bottoms from the HDI production unit is a high viscosity residue which is collected in a 2500 gallon holding tank. It is then pumped into a tank trailer for transportation to an on-site liquids incinerator. During incineration the residue is pumped directly from the trailer to the incinerator to avoid emissions during the transfer operations.

The liquid incinerator typically operates at 900-950°C with a retention time of 1-2 seconds. The incinerator is equipped with a Venturi scrubber with caustic neutralization to treat all off-gases. During 1981 3.33×10^5 Kg of residue was incinerated on-site.

During those times when the on-site incinerator cannot handle all of the residue it is first mixed with wet sand to destroy residual isocyanate. During 1981, 1.27×10^5 Kg of this mixture which is approximately 50 percent sand was shipped off-site to a Class I approved

landfill. Of this quantity 1.4×10^4 Kg was still bottoms which contained up to 20% HDI prior to treatment to destroy residual isocyanate. The remainder is expected to contain very little, if any, residual HDI.

B. Biuret Polyisocyanate Production Unit

Release to Air

All off-gas from the reaction is passed through a natural gas flare which is designed for 100% combustion efficiency. This flare operates at approximately 2000°F and has a diameter of four inches.

There is one other source of HDI air emissions in the biuret polyisocyanate production unit. This is a spot vent system which is similar to the HDI production unit system. (See Section VII, A, Release to Air)

Release to Wastewater

The process is kept free of water because of the high chemical reactivity between isocyanates and water. The only water which might come in contact with HDI is equipment cleanout water. It is treated in the NPDES permitted wastewater treatment plant.

All rainfall falling in the process area is collected and treated in a NPDES permitted wastewater treatment plant.

C. Fugitive Emissions

Fugitive emissions are defined as those emissions not passing through a stack or vent. Fugitive emissions are estimated not to exceed 120 lbs./year for the HDI unit and 100 lbs./year for the biuret polyisocyanate production unit.

VIII. Environmental Release During Use

Greater than 99% of the HDI produced for use in the U.S. reaches the end user in the form of a paint system containing less than 0.5% free HDI monomer. The greatest potential for environmental release of HDI during use of these paints is during their spray application. The industrial hygiene sampling data presented in Section VI readily show that airborne emissions to the environment during spray paint application are negligible.

HDI which would be collected by a waterwash spray booth would be converted to the urea (see Section II) which is essentially non-reactive. Despite attempts to detect HDI emissions from the stacks of waterwash and dry filter spray booths none has been detected.

IX. Persistence and Bioaccumulation

Since HDI reacts with water to form amines and ureas (see Section II) there is very little chance that it will persist in the environment and/or accumulate in the food chain as monomeric HDI.

X. Toxicology

A. Mutagenicity

M. Anderson et. al.⁽⁴⁾ tested HDI and a variety of other isocyanates for mutagenicity in the Salmonella typhimurium microsomal assay system. The authors did not detect any mutagenic activity when HDI was tested against three S. typhimurium strains (TA 100, TA 98 and TA 1537) with or without metabolic activation. No further published or internal studies of the mutagenic potential for HDI were located.

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B. Carcinogenicity, Teratogenicity, and Reproductive Effects

No internal or published reports relevant to the possible carcinogenic, teratogenic or reproductive effects of HDI were located.

C. Acute Toxicity

The NIOSH Criteria Document for Diisocyanates (1978) cites various acute toxicity values from the published literature. (5)

The following report from the Bayer Institute for Toxicology contains results of acute toxicity investigations of HDI:

Kimmerle, G., A. Eben and B. Solmecke:
Desmodur H Toxicological Experiments, Report
No. 2146, July 8, 1970

(A copy of this report is attached as Attachment 9.)

While acute oral, dermal and inhalation toxicity results from HDI administration are reported in the

literature, the following particular values from the report cited above were not reported in the literature.

Acute Inhalation LC₅₀ (Male Wistar II Rats)

(1-hour exposure) = 0.29 mg Desmodur H/liter air (40 ppm)

(4-hour exposure) = 0.15 mg Desmodur H/l (22 ppm)

Five 4-hour exposures = >0.0285 mg Desmodur H/l (>4.2 ppm)

Symptoms of exposure and other details are given in the report.

Acute Oral Toxicity

LD₅₀ (male rats) = 0.913 ml/kg (0.797-1.05 ml/kg)

LD₅₀ (male mice) = 1.885 ml/kg (1.54-2.31 ml/kg)

LD₅₀ (cats) = ~ 1.0 ml/kg

Symptoms of exposure and other details are given in the report.

Acute Dermal Toxicity

LD₅₀ (male rats) = >0.5 ml/kg
(4 hour exposure)

Other details are given in the report.

Ophthalmic and Dermal Irritation

Application to rabbit ears of 0.5 ml of HDI for one-half hour resulted in severe reddening and swelling with subsequent cauterization of the skin which was still visible at seven days post-exposure. Instillation of 0.05 ml of HDI into the conjunctival sac of rabbit eyes resulted in severe irritation of the conjunctiva and sclera. No corneal opacities were noted.

Dermal Sensitization

Dermal sensitization to HDI was not detected in guinea pigs either through the Landsteiner technique or through skin painting.

Other Inhalation Studies

A report from Younger Laboratories (June 9, 1966) describes the effects of exposure of male rats to Mondur HX vapor for 6 hours. (A copy of this report is attached as Attachment 10.)

A report on HDI Sensory Irritation is in progress in our toxicology laboratory.

The LC₅₀ determinations of HDI aerosol in male and female rats contained in Report 6200, June, 1976, of the Bayer Institute for Toxicology (Kimmerle, G: Acute Inhalation Toxicity of Diisocyanates, Polymeric Isocyanates and Varnish Systems in Rats) are published in: Bunge, W., H. Ehrlicher and G. Kimmerle, Medical Aspects of Work with Surface Coating Systems Using the Spraying Technique, Special Edition Zentralblatt fuer Arbeitsmedizin, Arbeitsschutz und Prophylaxe, Vol. 4, Verlag fuer Medizin Dr. Ewald Fisher GmbH, Heidelberg, 1977. (See Attachment 11)

Another unpublished report [Henschler, D.: Comparative Studies on the Inhalation Toxicity of Toluene Diisocyanate (TDI) and Hexamethylene Diisocyanate

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(HDI). University Institute for Toxicology, Wuerzburgh, West Germany, January 21, 1971] compared the acute and subchronic inhalation toxicity of HDI and TDI. The purpose of the studies reported was to furnish documentation for the establishment of a MAK for HDI. (A copy of this report is attached as Attachment 12.)

One study in the Henschler report compared the inhalation toxicity of HDI and TDI at 4, 2, 1 and 0.3 ppm on five successive days, six hours daily, in mice, rats and guinea pigs. Briefly, HDI and TDI were found to be similar with respect to observed effects on mortality, general behavior, body weight gain and survival time.

Another study in the Henschler report compared changes in respiratory rate and depth in guinea pigs exposed to 0.2, 0.1 and 0.02 ppm TDI and HDI for, in most cases, two hours. Briefly, at the 0.2 ppm level HDI was felt to have a greater effect on respiratory rate and depth than TDI. At 0.1 ppm the effects of exposure were reduced but similar for the two materials and at 0.02 ppm no effects of exposure were clear.

Diffusion capacity and oxygen consumption were measured in mice exposed to 0.5 or 1.0 ppm HDI and TDI. At these concentrations no significant differences were seen between TDI and HDI; both compounds produced reductions in diffusion capacity and oxygen consumption.

D. Subchronic and Other Toxic Effects

The reports cited above (Bayer #2146 and Henschler, 1971) report on animal studies involving subchronic inhalation of HDI. In the studies reported in Bayer Report #2146 groups of 20 male rats were exposed to HDI at average levels of 2.0 or 0.2 ppm four hours per day five days per week for four weeks. At the 2.0 ppm level some adverse effects were observed (depressed general well being, labored breathing, depressed weight gain, gross pathological findings in the lungs, relative organ weight increases in adrenals and testicles) as compared to the controls. Rats exposed to 0.2 ppm HDI demonstrated no adverse effects as compared to control animals.

In the Henschler studies, groups of rats, mice and guinea pigs were exposed to HDI or TDI at levels of 0.1 and 0.03 ppm six hours/day five days/week for four

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weeks. Untreated control groups were included for the .03 ppm exposure studies. Interim sacrifices occurred at days 2, 16 and 30 of a 30 day observation period following exposure to the test materials. At the 0.1 ppm exposure level body weight gain was comparable between the groups of rats exposed to the two materials. In mice, TDI at 0.1 ppm depressed body weight gain. No comparison of TDI and HDI could be made in guinea pigs due to concurrent infections in the TDI animals. At the 0.03 ppm level no depression of body weight gain was noted following exposure to TDI or HDI. Increased relative lung weights were noted in mice exposed to both materials. The effect was more noticeable in the TDI exposed animals, particularly at the 0.1 ppm level. In exposed rats less pronounced increases in relative lung weights were seen; again, as in the mice, the increases were relatively greater in the TDI exposed animals. No increased relative lung weights were noted in guinea pigs. Histopathological examination of the lungs from the HDI and TDI exposed animals indicate only minor deviations from normal. No pathology was noted which could be attributed to HDI or TDI exposure.

Copies of the protocols for 21 and 90 day inhalation studies on HDI conducted by Mobay are enclosed as Attachments 13 and 14. Reports of these studies are being prepared.

E. Ecological Effects

No internal or published reports relevant to the possible ecological effects of HDI were located.

REFERENCES

1. Cooper, W., R. W. Pearson and S. Darke, Ind. Chemist, 36:121 (1960).
2. Personal communication: U.S. Department of Labor, Bureau of Labor Statistics, Pittsburgh, PA. (June, 1982)
3. Supplement No. 12 for Compilation of Air Pollutant Emission Factors, Third Edition, U.S.E.P.A., Office of Air Quality Planning and Standards, Research Triangle Park, North Carolina. (1981)
4. Anderson, M., M. Binderup, P. Kiel, H. Larsen and J. Maxild, "Mutagenic Action of Isocyanates Used in the Production of Polyurethanes," Scand. J. Work Environmental Health 6: 221-226 (1980).
5. NIOSH (1978) National Institute for Occupational Safety and Health Criteria for a Recommended Standard...Occupational Exposure to Diisocyanates, Cincinnati, Ohio.

LIST OF ATTACHMENTS

1. Material Safety Data Sheet for Mondur HX (No. 0213, 3-1-82)
2. Chemistry for Coatings (C1-1-2)
3. Product Data Sheet for Desmodur N-75 (C2-10-B)
4. Material Safety Data Sheet for Desmodur N-75 (No. 1016, 10-7-81)
5. Label for Desmodur N-75 (1-22-81)
6. Safety Considerations Relating to Urethane Coatings (C2-17-1)
7. HDI Production Block Diagram
8. Biuret Polyisocyanate Production Block Diagram
9. Kimmerle, G., A. Eben and B. Solmecke: Desmodur H Toxicological Experiments, Report No. 2146, July 8, 1970
10. Toxicological Investigation of Mondur HX, Younger Laboratories, June 9, 1966
11. Bunge, W., H. Ehrlicher and G. Kimmerle, Medical Aspects of Work with Surface Coating Systems Using the Spraying Technique, Special Edition Zentralblatt fuer Arbeitsmedizin, Arbeitsschutz und Prophylaxe, Vol. 4, Verlag fuer Medizin Dr. Ewald Fisher GmbH, Heidelberg, 1977
12. Henschler, D., Comparative Studies on the Inhalation Toxicity of Toluene Diisocyanate (TDI) and Hexamethylene Diisocyanate (HDI). University Institute for Toxicology, Wuerzburg, West Germany, January 21, 1971.
13. Mobay Protocol for 21-Day Inhalation Toxicity Study with HDI.
14. Mobay Protocol for Subchronic Inhalation Toxicity Study with HDI.

Attachment 9

FABRIK FARMEN BAYER AG
INSTITUTE FOR TOXICOLOGY
WUPPERTAL-ELBERFELD
Report No. 2146

Wuppertal-Elberfeld, July 8, 1970

Copy No. 3

DESMODUR II

TOXICOLOGICAL EXPERIMENTS

by

Dr. med. Georg Kimmeler
Dipl.-Chem. Anneliese Eben
Dr. vet. med. Brigitte Solmecke

This report or excerpts of it are not to be copied. If required,
further copies can be provided by the authors.

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METHOD

Preparation

Chemically pure hexamethylenediisocyanate (Desmodur H) was used for the experiment. The liquid boils at 248°C and has a vapor pressure of 5 torr at 112°C.

Animals

Male NMRI-mice (weight 18-22 g), male Wistar-II-rats (weight 160-200g), male Wistar-II-SPF rats (weight 130-150g), female Pirbright guinea pigs (weight 300-500 g) by breeder Winkelmann, Kirchborchen; rabbits and cats of both sexes, not pure-bred.

After the respective experiments, the animals were kept individually in cages at a room temperature of $21 \pm 2^\circ\text{C}$. The cages were lighted daily from 7 AM to 7 PM. Mice, rats, guinea pigs and rabbits were given pelletized Altromine feed and water, the cats received Whiskas and milk ad libitum.

Experiment Design

The compound was administered in oil in the oral and cutaneous experiments. It was given at such a concentration that the animals received each time 0.5 ml/100 g body weight.

The inhalation tests were performed with solvent-free aerosols or vapors of Desmodur H with the dynamic inhalation apparatus for rats developed by us (NIESSEN et al., 1963). The following equipment was used as aerosol or vapor generators:

- 1) Cyclon: modified according to GAGE (1968)

The created mist was diluted by adding dry air. At higher air amounts (30 l/min.) part of it was rejected before through a branch line. The theoretic concentrations were calculated from the consumption of isocyanate and the applied amount of air. Air samples were removed from the interior of the inhalation room and analyzed. To receive still lower concentrations, the mists were first fed into a separator and diluted with air.

2) Small Atomizer:

A small atomizer was used in some of the experiments. The aerosols were also diluted with air and then fed into the apparatus.

3.) Vaporizer

In the experiments with very low concentrations, 3 liter air/minute flowed through about 40 g Desmodur H in a Fritten wash bottle, was diluted with additional air and fed into the rat apparatus. The wash bottle was standing in a water bath, in order to keep the temperature constant.

The air was analyzed as follows: Hexamethylenediisocyanate can be determined according to a method developed by VON EICKEN (1958) which was somewhat modified by us. It is based on the saponification of the substance into diamine, which is subsequently reacted with 2,4-dinitrofluorobenzene into the respective 2,4-dinitrophenylamine. The extinction of the yellow solution is measured at 355 m μ and 10 mm layer thickness in the Zeiss Spectrophotometer. For the absorption of the substance from the inhalation air, we used 3 absorption containers arranged in tandem, which were fed with dimethylsulfoxide / 0.1 n HCL (1:1) (see EBEN, Report No. 1994 of April 7, 1970).

The Tables of the experiment results under "Toxicological Result" mean:

1. Number = number of animals
2. Number = number of animals with symptoms
3. Number = number of animals used.

The observation time after the completion of the acute and subacute inhalation tests was each 14 days.

The calculation of the average lethal dosage (DL_{50} or LC_{50}) was done according to the LITCHFIELD and WILCOXON method (1949).

The following evaluations were made in the subchronic experiments:

- a) Daily observation of the rats
- b) Weekly measurement of body weight
- c) Clinical laboratory evaluations at the end of the exposures
 - a) Blood evaluation at each 5 rats per concentration

Definition of the hemoglobine content as cyano-methamoglobine (BETKE and SAVELSPERG, 1952),
Definition of hematocrite according to HEDIN;
Count of the erythrocytes and leucocytes in the Coulter Counter, Model D;
Calculation in the color coefficient (Hb_E -value) and the average erythrocyte volume (MCV);
Count of the thrombocytes (phase contrast method according to FEISSLY and LUEDIN, 1949);
Evaluation of the differential blood count (smears dyed according to PAPPENHEIM);
Definition of the prothrombine time (quick test);

- b) Liver function tests on each 10 rats per concentration

Definition of the activity of the glutamic acid - pyruvic acid - transaminase (GPT) and the glutamic acid - oxalacetic acid - transaminase (GOT, REITMAN and FRANKEL, 1957);

Definition of the activity of the sorbit-dehydrogenase (SDH) in the serum (HOLKER et al., 1955);

Definition of the activity of the alkaline phosphatase (ALP) in the serum (BESSEY, 1946);

Definition of the activity of the leucin-amino-peptidase (LAP) in the serum (TUPPY, 1962);

- c) Urine tests and kidney function tests at each 10 rats per concentration.

Urine tests for glucose, albumen and blood with Combi-Uristix test rods (Merck),

**Gile pigments: Ehrlich reagent, Schlesinger reagent,
microscopic sediment evaluations**

**Kidney function tests: definition of the urine
concentration in the serum according to HENRY (1964),
definition of the creatinine concentration in the serum
(POPPER et al, 1937).**

24 hours after the last exposure, all rats were anesthetized with ether and exsanguinated. The subsequently dissected animals were checked for macroscopically visible changes. The weights of the liver, spleen, kidneys, adrenals, thyroids, testicles and lungs were measured.

The calculation of average values of the animal weights, the absolute and relative organ weights as well as the average value comparison was done on the IBM computer Type 360/44. For the average value comparison we used the test according to WILCOXON (1947).

The evaluation of primary skin-damaging effect was done by fixing a piece of cotton saturated with Desmodur H on the skin of the rabbit ear. The effect on the mucuous skins was checked on the rabbit eye.

In guinea pigs, the test for skin allergizing effect was performed according to the recommendations of US-FDA (see enclosure).

RESULTS

I. TOXICITY AT ONE-TIME APPLICATION

1. Oral Application

The material was administered via a stomach tube.

Male Rats

Desmodur II Dosage ml/kg	Toxic. Results after 14 days	Intoxication Symptoms		Death after No. of days
		Start	End	
0,1	0/0/15	-	-	-
0,25	0/15/15	2h	3d	-
0,5	0/15/15	1h	7d	-
0,75	5/15/15	50'	7d	1
1,0	8/11/15/15	40'	7d	1
1,5	14/15/15	35'	-	1
2,0	15/15/15	30'	-	1

Result: DL₅₀ (24 days) 0.913 ml/kg (0.797 - 1.05 ml/kg)

S = 1.3

Highest dosage without any finding: 0.1 ml/kg

Lowest lethal dosage: 0.75 ml/kg

Male Mice

Desmodur H Dosage ml/kg	Toxicol. Results after 14 days	Intoxication Symptoms		Death After No. Days
		Start	End	
0,25	0/0/15	-	-	-
0,5	0/15/15	60'	3d	-
1,0	3/15/15	30'	5d	1
1,5	6/15/15	20'	6d	1
2,0	7/15/15	18'	7d	1
2,5	10/15/15	12'	7d	1

Result: DL₅₀ (14 days) 1.885 ml/kg (1.54-2.31 ml/kg)
 S = 1.8
 Highest dosage without any finding: 0.25 ml/kg
 Lowest lethal dosage: 1.0 ml/kg

Cats

Desmodur H ml/kg	Toxicol. Results after 14 days	Intoxication Symptoms		Death After No. Days
		Start	End	
0,1	0/2/2	2,5h	1d	-
0,25	0/2/2	2h	2d	-
0,5	0/2/2	1h	4d	-
1,0	1/2/2	20'	7d	1

Result: DL₅₀ about 1.0 ml Desmodur H/lg
 Highest dosage without any finding: < 0.1 ml/kg
 Lowest lethal dosage: 1.0 ml/kg

Intoxication Phenomena:

Depending on the dosage, after 10 minutes up to 2 hours the general well-being was severely reduced and had a slight sedative effect in rats, mice and cats. These intoxication symptoms remained up to one week. Death always occurred within 24 hours.

0.0.5.5

2. Cutane Application

Desmodur H was applied as 25% solution in oil to the peritoneum of male rats, which was shaved the day before. Because of the inhalation danger through evaporation, the animals were breathing fresh air through a mask. After 24 hours the skin was cleaned with soap and water and the animals were observed for 14 more days.

Desmodur H Dosage ml/kg	Toxicol. Result after 14 days	Intoxication Symptoms		Death occurred after No. Days
		Start	End	
0,1	0/5/5	4h	5d	-
0,25	0/10/10	4h	4d	-
0,5	2/10/10	4h	7d	1

Result: DL_{50} 4-hour exposure > 0.5 ml Desmodur H/kg.

First an edema formed at the treated area, and then after 3 to 4 hours necrosis and finally a scab formed.

II. INHALATION TOXICITY

One-Time Exposure of Male Rats

The results of the 1-hour, 4-hour or 5-times 4-hour exposures of rats to Desmodur H are shown in the following Table:

Desmodur H Concentration mg/l air (analytically)	Duration of Test (hours)	Toxicol. Result after 14 days
1,960	1	17/20/20
0,975	1	14/20/20
0,684	1	15/20/20
0,590	1	19/20/20
0,415	1	9/20/20
0,345	1	8/20/20
0,316	1	6/20/20
0,260	1	14/20/20
0,245	1	6/20/20
0,238	1	11/20/20
0,079	1	3/20/20
0,071	1	1/20/20
0,495	4	19/20/20
0,432	4	20/20/20
0,393	4	19/20/20
0,136	4	2/20/20
0,0905	4	6/20/20
0,0567	4	1/20/20
0,0358	4	0/20/20
0,0319	4	0/20/20
0,0163	5x4	0/20/20
0,0285	5x4	0/20/20

Result: LC₅₀ 1-hour exposure 0.29 mg Desmodur H/l air (40 ppm)
 4-hour exposure 0.15 mg Desmodur H/l air (22 ppm)
 5x4-hour exposure > 0.0285 mg Desmodur H/l air (>4.2 ppm)

After the exposures were complete, the rats were severely damaged, had labored breathing and were lying on their stomach. During the observation time the breathing continued to be labored and the general well-being was severely reduced. Some lost body weight. Death occurred in most cases within 24 hours. A few deaths, however, occurred later. The dissection

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showed that cause of death was toxic pneumonitis with bronchopneumony.

Repeated Exposures

In the following two tests each 20 male rats were exposed for 4 weeks on 5 subsequent days per week daily for 4 hours to average concentrations of 2 ppm or 0.2 ppm Desmodur H. 20 Each rats served as control group. They were only exposed to air.

4-week Exposure of Rats at 2.0 ppm Desmodur H

During the exposure the rats exposed to Desmodur H had a lower general well-being, and partially labored breathing. The following Table lists the average body weights of the rats at different times.

Test Group	Body Weight in g after Weeks					Difference between Initial and Final Weight g
	At Start	1	2	3	4	
Kontrol	140	170	191	210	224	84
Desmodur	140	158	171	181	189	49

The body weight increase of the rats exposed to Desmodur H was significantly lower ($p < 0.01$) than that of the control group.

Hematological Evaluation:

The average values are listed in the following Table:

Test Group	Hemo- glob. g%	Ery. 10 ⁶	Hb _E Y _Y	Leuko. 10 ³	Throm- boz. 10 ³	Häma- tokr. %	MCV ₃ μm	Prothrom- bine Time (sec.)
Control	15,7	8,46	19	10,3	819	50	60	13,2
Desmodur	15,2	8,58	18	13,1	738	50	59	12,9

The results of the evaluation of the white blood count are summarized in the following Table:

Test Group	Differential Blood Count in %								
	Bas.	Jgd1.	Stab.	Segm.	small Lymph.	large Lymph.	Eos.	Mono.	Other
Control	0	0	0	21,9	72,3	5,0	0,6	0,2	0
Desmodur	0	0	0,1	25,2	66,5	6,9	0,6	0,7	0

The hematological values were all normal.

Liver Function Tests:

The average activities of the serum enzymes of both rat groups are compiled in the following Table:

Test Group	G O T	G P T	S D H	A L P	L A P
	mU/ml				
Control	34,1	19,7	1,5	164,7	15,4
Desmodur	35,3	17,3	1,6	153,1	14,4

No difference resulted. All activity values were within the normal range.

Urine Tests and Kidney Function Tests;

The findings of the urine tests resulted in to pathological values in the rats of the individual concentrations. None of the tested rats had sugar, albumen or urobilinogen in the urine.

The average urea- and creatinine concentrations are listed in the following Table:

Test Group	UREA	CREATINENE
	mg/100 ml	
Kontrol	37,3	0,88
Desmodur	35,2	0,99

These values also were within the physiological range.

Dissections and Organ Weights:

The lungs of rats exposed to Desmodur H had macroscopically changed.

The average organ weights of the liver, spleen, kidneys, adrenals, thyroids, testicles and lungs determined in the dissection, are listed in the following Table:

Test Group	Body Weight g	Average absolute organ weights in mg						
		Thyroid	Lung	Liver	Spleen	Kidneys	Adrenals	Testicle
Kontrol	224	13,4	1081	9316	436	1592	35,3	279:
Desmodur	189*	12,0	949*	7588*	363*	1403*	32,5 ^o	274:

* significant difference in control group (p < 0.01)

^o significant difference in control group (p < 0.05)

The average relative organ weights (each 100 g body weight) are listed in the following Table:

Test Group	Average relative organ weights in mg						
	Thyroid	Lung	Liver	Spleen	Both Kidneys	Adrenals	Testicles
Control	6,0	483	4163	195	710	15,8	1253
Desmodur	6,4	501	4015	191	742	17,20	1458*

- * Difference to control group significant (p 0.01)
- o Difference to control group significant (p 0.05)

The absolute weights of lung, liver, spleen, and kidneys of the rats exposed to Desmodur H were significantly lower than those of the control groups. This, however, was only a false effect, because due to the low body weight, these organs were relatively insignificant toward the control groups. The adrenal weight was reduced and relatively enlarged in the exposed rats. The relative testicle weight were also increased.

4-week- Exposure of Rats at 0.2 ppm Desmodur H

Neither intoxication nor irritation effects were found during the 4-week test. The following Table shows the average body weights of the rats in relationship to the test period:

Test Group	Body weight in g after weeks					Weight Difference Start/End g
	At Start	1	2	3	4	
Control	140	170	191	215	234	94
Desmodur	141	161	194	217	240	99

No significant difference resulted in the body weight increase of the two rat groups.

Hematological Tests:

Test Group	Hemo- glob. g%	Ery. 10 ⁶	Hb g	Leuko. 10 ³	Throm- boz. 10 ³	Hema- tokr. %	MCV μm	Prothrom- bine Time (sec.)
Control	15,6	8,43	19	6,6	800	50	59	13,0
Desmodur	16,2	8,83	19	6,9	826	52	59	12,7

Test Group	Differential Blood Count in %								
	Bas.	Jgd1.	Stab.	Segm.	small Lymph.	large Lymph.	Eos.	Mono.	Other
Control	0	0	0	22,8	71,0	5,2	0,6	0,4	0
Desmodur	0	0	0	12,8	83,2	3,2	0,8	0	0

The hematological values of both groups were within the normal range.

Liver Function Tests:

Test Group	GOT	GPT	SDH	ALP	LAP
	mU/ml				
Control	24,8	14,6	1,1	93,1	14,0
Desmodur	23,6	13,6	1,4	100,8	13,9

All activity values were within the normal physiological range.

Urine Tests and Kidney Function Tests:

The urine tests did not result in any pathological findings. The average urine and creatinine concentrations are listed in the following Table:

Test Group	UREA	CREATININE
	mg/100 ml	
Control	37,5	1,18
Desmodur	40,4	1,20

All values were within the physiological range.

Dissections and Organ Weights: The dissections of all rats showed no macroscopical changes of the internal organs which could be caused by the exposure to DesmodurH.

The organ weights found in the dissection are listed in the following Table as average values:

Test Group	Body Weight g	Average absolute organ weights in mg							
		Thyroid	Heart	Lung	Liver	Spleen	Both Kidneys	Adrenal	Testicles
Control	234	9,7	670	977	10040	545	1460	43,6	277
Desmodur	240	10,3	674	982	9964	540	1497	43,7	271

The average relative organ weights (each 100 g body weight) are listed in the following Table:

Test Group	Average relative organ weight in mg							
	Thyroid	Heart	Lung	Liver	Spleen	Both Kidneys	Adrenals	Testicles
Control	4,2	285	417	4274	232	622	18,7	1185
Desmodur	4,3	282	412	4152	225	624	18,2	1132

The absolute relative organ weights of the rats exposed to Desmodur H were not significantly different from those of the control group.

Short-Time Exposures of Experimental Person

In these tests each 3 men were exposed. We obtained the following results:

- 0.001 ppm: No detection.
- 0.005 ppm: Odor was barely detected by one person.
- 0.01 ppm: Odor was detected by all three persons.
- 0.02 ppm: Odor was easily detected, 2 persons had slight eye irritations.
- 0.1 ppm: Very strong odor, significant eye and throat irritation, uncomfortable after longer exposure.

0.074

III. SKIN FUNCTION TESTS

1. Primary Skin-Damaging Effect

a) Experiment on the Skin of Rabbits

About 0.5 ml Desmodur H was placed drop by drop on a piece of cotton and bandaged into the inner side of the outer ear of rabbits for ½ hour. This caused a severe reddening and swelling with subsequent cauterization of the treated skin areas. This effect was clearly visible up to 7 days.

b) Experiments on the Ocular Mucuous Skin

About 0.05 ml Desmodur H were applied to the conjunctiva sack of a rabbit eye (2 animals). This caused severe damage of the conjunctiva and the scleren (reddening, swelling and cauterization). No changes were observed on the cornea.

2. Secondary (allergizing) Effect on Guinea Pigs

Results at intracutane injection:

Animal No. ??	Pre-Injection		Re-Injection	
	Degree of Reddening	Swelling β cm	Degree of Reddening	Swelling β cm
1	1-2	1,7	1	0,9
2	1-2	1,6	1	0,8
3	1-2	1,5	1	0,9
4	2	1,6	1	0,5
5	2	1,4	1	0,9
6	1-2	1,5	1	0,8
7	1-2	1,5	1	0,5
8	1-2	1,6	1	0,7
9	1	1,8	1	0,4
10	1-2	1,4	1	0,4
11	1-2	1,8	1	0,6
12	2	1,5	1	0,4
13	2	1,5	1	0,7
14	1-2	1,4	1	0,7
Average	1,6	1,6	1,0	0,7

Results of the skin painting:

Animal No.	Pre-Treatment Degree of Reddening	Post-Treatment Degree of Reddening
16	1-2	1
17	1	0
18	1	0
19	1	0
20	1	0
21	1	0
22	1	0
23	2	0
24	1-2	0
25	1-2	1
26	1	0
27	1	0
28	1	0
29	1	0
30	1	0
Average	1,2	0,1

These tests show that in none of the cases a higher irritating came about due to the re-injection or post-treatment than in the pre-treatments. A skin-sensitization through Desmodur H was not found in guinea pigs

DISCUSSION

As an aliphatic isocyanate, Desmodur H has the typical toxicological properties of the isocyanate residue.

The acute oral toxicity of Desmodur H is low and the compound is absorbed through the skin only at a low rate. The acute inhalation toxicity of Desmodur H can be considered medium high. At lower concentrations, however, it should have no practical importance at high irritations in the respiratory system. After longer exposure a concentration of 2 ppm was found damaging in rats; but 0.2 ppm was tolerated.

Desmodur H has very high primary skin and mucuous skin damaging properties. A skin sensitizing effect in the guinea pig could not be found through the intracutane injection or through brushing the skin with the solution. Such effects, however, have occurred in workers.

SUMMARY

1. At a single application of Desmodur H the DL₅₀-values were as follows:

male rat	p.o.	0.913 ml/kg
male mouse	p.o.	0.885 ml/kg
male rat	cutaneously	>0.5 ml/kg (4 hours)

2. The LC₅₀-values at inhalation of Desmodur H were as follows:

male rat	1-hour exposure	0.29 mg/l (40 ppm)
male rat	4-hour exposure	0.15 mg/l (22 ppm)
male rat	5x4-hour exposure	>0.02585 mg/l (>4.2 ppm)

Male rats could tolerate 0.2 ppm of Desmodur H, during a 4-week exposure (20 times 4 hours). A concentration of 2 ppm was harmful to the animals, but caused no hematological changes and damages of the liver and kidney function.

3. Desmodur H has a severe primary skin and mucuous skin damaging effect in rabbits. A skin sensitizing effect could not be found in guinea pigs.

Dr. Kimmerle

Dipl.-Chem. Eben

Dr. Solmecke

Dr. Lorke

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Excerpt from

APPRAISAL OF THE SAFETY OF CHEMICALS IN FOODS, DRUGS
AND COSMETICS

Assoc. of Food and Drug Officials of the United States
1959

White male guinea pigs weighing 300-500 grams and subsisting on a commercial rabbit pellet ration supplemented with greens (kale or lettuce) are identified, and hair removed from back and flanks by close clipping. A 0.1 per cent solution or suspension in physiological saline of the material to be tested is injected intracutaneously, using a 26-gauge hypodermic needle. The injections are made every other day or three times weekly, until a total of ten have been made. The ten sensitizing injections are made at random in an area of the back and upper flanks. This area measures three or four centimeters square. The retest injection is made in an area just below the region or sites of the sensitizing injections. The first injection consists of 0.05 ml., while the remaining nine injections consist of 0.1 ml. each. Two weeks after the tenth injection, a retest injection is made, using 0.05 ml. of a freshly prepared solution or suspension as before. Twenty-four hours following injections, readings are made of the diameter, height, and color of reaction. A comparison of the reaction following retest is made with the average of the readings taken after each of the original ten injections. If the value for the retest reading is substantially higher than for the average of the ten original readings, the substance can be considered to have produced sensitization. The degree of sensitization is proportional to the increase in the final reading compared to the average of the readings following the ten original doses.

Code for Degree of Reddening

Zeichenerklärung Rötungsgrad:

0	=	o.B.	without finding
1	=	ganz gering	very low
2	=	gering	slight
3	=	mässig	medium
4	=	stark	severe

YOUNGER LABORATORIES

Biochemists... Pharmacologists... Analysts

125 CLIFF CAVE ROAD
SAINT LOUIS, MO., 63129

PHONE: TILDEN 6-2640

Certificate of Analysis

June 9th, 1966

SUBJECT -

Toxicological Investigation Of: **MORDUR IX**
Monsanto Sample Number 128
Monsanto Project Number Y-66-113

STUDY CONDUCTED FOR -

Monsanto Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

Vapor Inhalation (Male Rats)

Four mature male rats were placed in a chamber of 35 liters capacity and exposed for six hours to a concentrated exposure of vapors produced by passing a stream of air through 100 milliliters of the compound contained in a 250-milliliter Erlenmeyer flask.

Vapors from the flask passed into a 500-liter bottle to remove droplets and then into the chamber.

Air flow through the sample was four liters per minute as measured by a calibrated rotameter. This was sufficient to violently agitate the liquid. No supplementary air was introduced inasmuch as the above supply was ample for the animals oxygen requirements.

The animals were observed for behavior and, since there were no deaths, all were held for ten days observation.

The data are shown in Table I.

SUMMARY -

Vapor Inhalation (Male Rats)

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were mildly toxic under conditions of the test.

YOUNGER LABORATORIES

Fred H. Younger
BY: FRED H. YOUNGER

T A B L E I

INHALATION OF 'MONDUR NX' VAPORS BY RATS

Average Temperature Inside Chamber 75° F.
Average Relative Humidity Inside Chamber 57 %

Amount Of Sample -- To Start 100.0 cc
Recovered 99.6 cc
Vaporized or left
in equipment 0.4 cc (0.4%)

<u>Animal No. - Sex</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1 - Male	Survived	Considerable discomfort in five minutes ...
2 - Male	Survived	
3 - Male	Survived	Eyes partially closed ...
4 - Male	Survived	Nasal discharge ... Occasional dyspnea ... Mild weakness ... Reduced activity.

DISCUSSION -

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were mildly toxic under conditions of the test.

The fumes were moderately irritating to the nasal and ocular mucosae soon after start of exposure. Activity reduced considerably and some weakness was detected. Bronchial rales did not develop. Normal activity and appetite returned in forty-eight to seventy-two hours.

The material in this report is to be used in development of the product and may be given to responsible sales contacts, but it is not to be used by them in advertising copy. The source of this material is not to be disclosed until it appears in formal publications. No export or importable rule may be made without the approval of the Medical Department in St. Louis. Customer's inquiries regarding matters of toxicity are to be referred as a matter to the Medical Department in St. Louis for reply.

— Monsanto Chemical Company

Attachment 12

PRELIMINARY REPORT FROM THE UNIVERSITY INSTITUTE FOR TOXICOLOGY
WUERZBURG, WEST GERMANY
January 21, 1971

Title: Comparative Studies on the Inhalation Toxicity of Toluene
Diisocyanate (TDI) and Hexamethylene Diisocyanate (HDI)

Toxicity studies with TDI and HDI were done in the Institute for Pharmacology and Toxicology between 1968 and 1970. The goal of these studies was to furnish documentation for the establishment for an MAC value for HDI. These studies were based on the assumption that the MAC value for TDI (2,4- and 2,6- isomers) of 0.02 ppm is well founded through experimental toxicological studies and field experience on workers. Based on the assumption that HDI corresponds completely qualitatively to TDI and that its effect quantitatively at least approaches that of TDI, an experimental toxicological comparative study limited to acute and subchronic tests could supply adequate documentation for the evaluation of HDI.

Proceeding on this basis , the following tests were done:

- A. Acute and subchronic toxicity on three types of animals (rats, mice and guinea pigs) during repeated 6-hour inhalation.
- B. Change in the respiration of guinea pigs under the influence of low concentrations (at the MAC value and above).
- C. Effect on the diffusion capacity and the oxygen intake in the lungs of test mice following single 6-hour exposure at non-fatal concentrations.

1. Production of defined TDI and HDI concentrations in air for the dynamic exposure of test animals.

Diisocyanates were released in a sintering bottle (see Illustration 1) with flowing, pre-dried nitrogen (rotometrically measured), and entrained isocyanate droplets were precipitated in a separator, the primary mixture was mixed with air (rotometrically metered) in a mixing bell, and was conducted to an exposure chamber with three grid plates. The exposure chamber was a hexahedral column with a diameter of approximately 60 cm and 1.80 m high. After running through the exposure chamber (approximately 15-fold air exchange) the isocyanate-air mixture was let off through a roof exhaust.

2. Determination of the isocyanate concentrations predominant in the test room.

- a. TDI: Method by PILZ (1956).
- b. HDI: Method by PILZ and Johann (1970).

3. Test Animals

- a. Mice: M:RI, Female, 24.5 (19.5-34.4) g, (minimum initial, maximum final weight). Central Institute for Test Animal Breeding, Hannover.
- b. Rats: WISTAR, Female, 167.5 (127-207) g, Central Institute for Test Animal Breeding, Hannover.
- c. Guinea Pigs: PIRSBRIGHT, 276.3 (187-429) g, E. Spock, Gelnhausen.

4. Acute Inhalation Toxicity (One-Week Tests)

In each test series, ten mice, ten rats, and five guinea pigs were used with one concentration of one of the diisocyanates. On five successive days (Monday thru Friday) the animals were exposed for six hours each (10:00 A.M. to 2:00 P.M.), alternating by week to HDI or TDI. Target concentrations were 4 ppm, 2 ppm, 1 ppm, and 0.3 ppm (achieved analytical average concentrations, see Illustrations 2-5). The mortality, the general behavior, the survival time and the body weight progression were recorded.

a. Mortality

		<u>MICE</u>		<u>RATS</u>		<u>GUINEA PIGS</u>	
		<u>Mortality</u>	<u>Survival Time (First & Last Date) In Days</u>	<u>Mortality</u>	<u>Survival Time (First & Last Date) In Days</u>	<u>Mortality</u>	<u>Survival Time (First & Last Date) In Days</u>
4 ppm	HDI	10/10	1-7	9/10	2-9	3/5	11-27
	TDI	10/10	3-6	6/10	2-23	5/5	5-8
2 ppm	HDI	6/10	5-7	1/10	6	4/5	8-10
	TDI	4/10	4-11	1/10	13	1/5	16
1 ppm	HDI	8/10	5-11	0/10		1/5	14
	TDI	6/10	6-14	0/10		3/5	4-13
0.3 ppm	HDI	1/10	7	0/10		0/5	
	TDI	0/10		0/10		0/5	

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According to the mortality figures, HDI has a slightly higher toxicity than TDI; the difference is clearest with mice, and less clear with rats. No such trend results with the guinea pigs. None of the differences are, however, significant.

b. Body Weights

The mortality was the highest with the mice and lowest with the rats, with the guinea pigs falling in the middle. This sequence results at all concentrations (See Illustrations 2, 3, 4 and 5). At the lowest concentration (0.3 ppm), where the mortality was close to zero (i.e., with the exception of one mouse all of the animals survived the exposure series), only the mice still suffered losses in body weight. Overall, the comparison of the weight curves at corresponding concentrations does not result in any relevant difference between HDI and TDI.

c. General Behavior and Survival Time

The observation did not produce any basis to conclude that there is a difference between the effects of TDI and HDI.

5. Change in Respiratory Frequency and Respiratory Depth Under the Influence of HDI and TDI.

a. Methodology

The tests were done on guinea pigs. There were two animals in each air-tight, elongated box, through which the isocyanate air mixture was conducted (EK, see Illustration 6). Each animal was placed in a body plethysmograph (P). The volume forced with each respiration in the plethysmograph was cumulatively conducted through two respiration valves (V_1 and V_2) under a spirometer (Sp). The spirometer excursions are kymographically (Sky) recorded. At the upper and lower end of the spirometer excursion, electrical contacts (K_1 and K_2), which are hit by the recorder indicator, provide a complete discharge of the filled spirometer, or a return to zero. The suction of the spirometer content was accomplished with a water jet pump (WSP); the suction of the pump was released through a valve (EMF) controlled by a relay switch (R).

b. Normal Values

Illustration 7 shows the normal values of two guinea pigs during recording over a three-hour period. There was a slight decrease of respiratory dimensions over time, caused by a dissipation in an initial slight excitability on the part of the animals (acoustic and optical stimulation).

- c. Under the inhalation of isocyanate vapors (Illustrations 8 thru 10), there occurs a decrease in respiratory frequency with a simultaneous deepening of respiration (compare the values under normal conditions with the measured values following conversion to irritant inhalation: 45, 75, 105 etc. min.). Illustration 8 shows the ratios at 0.2 ppm TDI (1 1/2 hours) and HDI (2 hours). At 0.1 ppm over two hours, the change in respiratory dimensions is slighter (Illustration 9), it occurs later, and the values return faster and closer to the norm following the end of exposure. At 0.02 ppm (Illustration 10) a deviation from the normal progression is no longer clear.

At 0.2 ppm HDI seems to have a stronger effect than TDI but this difference is no longer certain at 0.1 ppm.

6. Diffusion Capacity and Oxygen Consumption

a. Methodology (compare Illustration 11)

Mice (groups of five animals) are transferred into a thermostatically maintained receptacle (R) where the air is continually circulated with a membrane pump (MP₁); CO₂ is removed from this air with lime (?), and water vapor with CaCl₂ (WT = Heat Exchanger). The oxygen consumed is replaced through a spirometer and the replacement is kymographically recorded. At the beginning of the test period (15 minutes) a small amount of CO is injected into the system so that a CO concentration of just below 200 ppm builds up. A membrane pump (MP₂) forces the CO air mixture in the bypass through the graduated curvette (MK) of an infrared printer (Model URAS 1). The actual CO concentration is recorded on a line printer (MLS) following intensification of the signal current (V). The inhalation (?Abatmung?) curve (logarithmic profile) is linearized on double logarithm paper, and the slope (?Anstiegswinkel?) of the line is a criterion for the diffusion capacity of the lungs.

- b. The CO diffusion (expressed as a percent of the initial values before isocyanate exposure) fluctuates a maximum of 8% at the normal value (Illustration 12), and the oxygen consumption a maximum of 12% with normal animals on repeated exposure on two successive days. If mice are subjected to isocyanate concentrations of 1 or 0.5 ppm for six hours each and the diffusion capacity is repeatedly determined on six successive days, then the values drop significantly below the norm both for the CO inhalation and for the oxygen consumption. At 1 ppm, the decrease is sharper (Illustration 13) than at 0.5 ppm (Illustration 14). The deficit is still not completely equalized at 1 ppm after six days.

The individual values fluctuate considerably; this can be attributed to external conditions (excitement, noise and light stimulation). It can be seen that there is no significant difference between the effect of HDI and that of TDI in the concentration ranges tested.

7. Subchronic Toxicity (Four Weeks Test)

TDI and HDI were tested on animals for four weeks, six hours daily, five times a week (Monday thru Friday) in concentrations of 0.1 ppm or 0.03 ppm. For each concentration of each material, ten rats, twenty mice and ten guinea pigs were used. In a four-week series (0.03 ppm TDI), the same animal types and numbers were run as controls.

INITIAL WEIGHTS (GROUP AVERAGES OF THE ANIMALS) IN GRAMS:

	<u>GUINEA PIGS</u>	<u>RATS</u>	<u>MICE</u>
TDI 0.1 ppm	-	151.4	23.0
0.03 ppm	289.7	183.5	26.3
HDI 0.1 ppm	336.8	163.7	22.0
0.03 ppm	328.8	159.4	21.9
Controls	340.7	183.5	25.0

The following were tested: body weights, the lung weights (2) sixteen and thirty days subsequent to the last six-hour exposure, the pathohistology of the lungs and tracheae each on one-third of the animals on the same dates.

a. TDI and HDI Concentrations Measured in the Test Room

The target concentrations (0.1 and 0.03 ppm) were usually not yet reached at beginning of testing since a certain amount of caution was taken to avoid extreme overdoses. The results of the six-hour averages and of the given minimum and maximum values are listed in Illustrations 15 and 16. The overall averages are about at the ideal level; with HDI there were occasional difficulties (interference from the formation of the red coloring material, cause unknown), so that the deviations from the ideal value are greater here.

b. 0.1 ppm TDI and HDI

The guinea pigs used at 0.1 ppm TDI died of an infection (staph ?). Otherwise, there were no instances of death. The body weights are separated by animal type but they are

summarized in one illustration for both of the diisocyanates. With rats (Illustration 17), there is no difference between the two materials. In the course of one test week, however, a significant slowdown of the body weight increase can be seen among the effects of the isocyanate. During the weekends when there is no exposure, a certain recovery occurs. In mice (Illustration 18), a clear difference is observed: TDI impairs growth much more than HDI; in the TDI group actual weight losses occur of up to 8% of the initial weight. The weekly rhythm is even more apparent here. In the guinea pigs, the TDI group was eliminated because of an infection (staph ?); the body weights at 0.1 ppm HDI are shown in Illustration 19. Compared with the control animals (Illustration 22), the body weight increase is somewhat less significant.

c. 0.03 ppm TDI and HDI

No deaths occurred. With the rats (Illustration 20), the body weights increased both with TDI and HDI somewhat faster than was the case with the control animals, but the difference was not significant. The slightly higher values with HDI could be attributed to the lower initial weight. With mice (Illustration 21) the weights of the TDI group are slightly below the control animals, and the weights of the HDI group were clearly above; the obviously lower initial weight is probably also the cause here. With guinea pigs (Illustration 22), body weight development in both test groups is significantly faster than with the control animals. Here as well, it must be taken into account, that the initial weights in the TDI group are the lowest and those in the control group are the highest.

Overall, at 0.03 ppm, there is no detectable impairment of overall condition based on observation of body weights, neither from TDI nor from HDI.

d. Relative Lung Weights

The values are listed in the following table in a systematic overview.

Time of the Last Exposure After Beginning	Animal Group	No. of Animals	TDI 0.003ppm		Animal Group	Relative Lung Weights (g/kg)		No. of Animals	Animal Group	TDI 0.1 ppm	Animal Group	No. of Animals	NDI 0.1ppm
			TDI 0.003ppm	Animal Group		MDI 0.03ppm	Animal Group						
2 Days (20 Expos.) 16 Days (20 Expos.) 30 Days (20 Expos.) 30 days (20 Expos.)	I	7	7.6 ± 0.86	I	7	6.8 ± 0.27	I	5	I	8.6 ± 1.74	I	7	6.7 ± 0.92
	II	7	8.3 ± 1.31	II	5	7.7 ± 0.77	II	7	II	9.8 ± 0.63	II	6	7.3 ± 0.88
	III	6	7.0 ± 0.74	III	7	8.0 ± 1.35	III	7	III	9.5 ± 1.11	III	7	6.7 ± 0.31
	Control	2	6.4 ± 5.06										
2 Days (20 Expos.) 16 Days (30 Expos.) 30 Days (20 Expos.) 30 Days (20 Expos.)	I	4	5.0 ± 0.29	I	3	5.0 ± 0.81	I	4	I	5.2 ± 0.55	I	4	4.7 ± 0.12
	II	3	4.8 ± 0.54	II	3	6.2 ± 0.44	II	3	II	5.6 ± 0.07	II	3	4.8 ± 0.31
	III	3	5.2 ± 0.27	III	4	4.7 ± 0.41	III	3	III	6.6 ± 0.74	III	3	4.9 ± 0.31
	Control	2	4.7 ± 4.86										
2 Days (20 Expos.) 16 Days (20 Expos.) 30 Days (20 Expos.) 30 Days (20 Expos.)	I	3	8.3 ± 1.28	I	3	8.3 ± 0.99	I	3	I	8.3 ± 0.99	I	4	6.7 ± 0.92
	II	3	8.0 ± 1.08	II	3	8.8 ± 0.60	II	3	II	8.8 ± 0.60	II	3	8.7 ± 1.75
	III	2	9.5 ± 1.92	III	4	8.3 ± 1.06	III	4	III	8.3 ± 1.06	III	3	6.9 ± 0.41
	Control	2	8.6 ± 11.26										

MICE

RATS

GUINEA PIGS

1952 Animals Died of Infection (Staph)

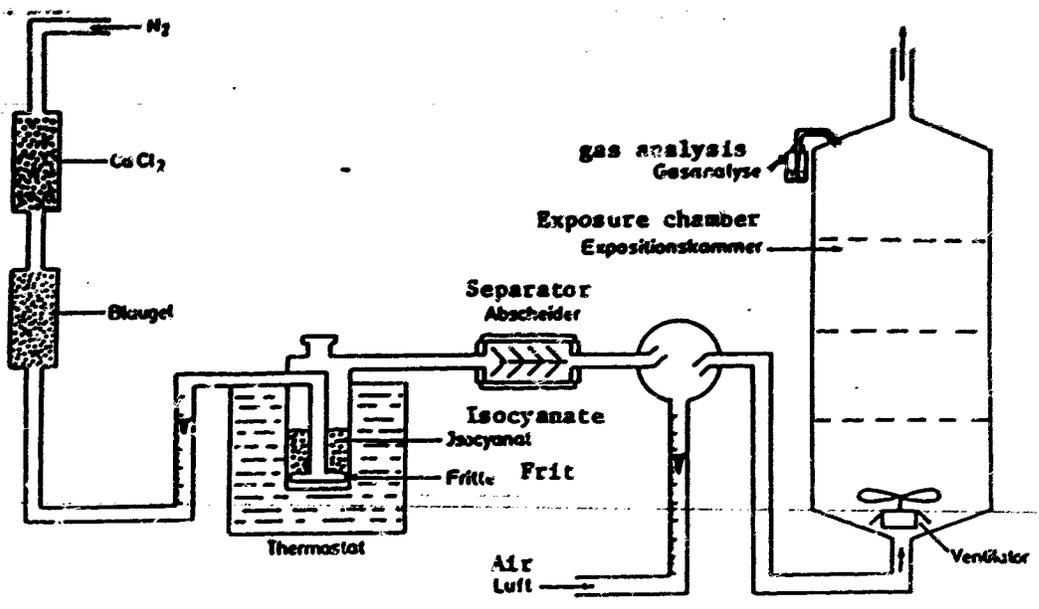
With mice the weights are always above the normal values; this is the most pronounced with TDI 0.1 ppm where the difference is statistically significant on the 16th and 30th day after the end of exposure. It is noticeable that, analogous to the development of the body weights, the deviation with TDI is stronger than that with HDI, both at the higher and at the lower concentration. With rats the increases are generally slighter, but with TDI they are again more pronounced than with HDI. There is no difference with guinea pigs.

e. Histological Findings

All lungs, the weights of which are listed in the preceding Table, were also histologically examined. The result: only insignificant deviations from the normal profile, none of which are related in any way to the type, intensity, or duration of the isocyanate exposures.

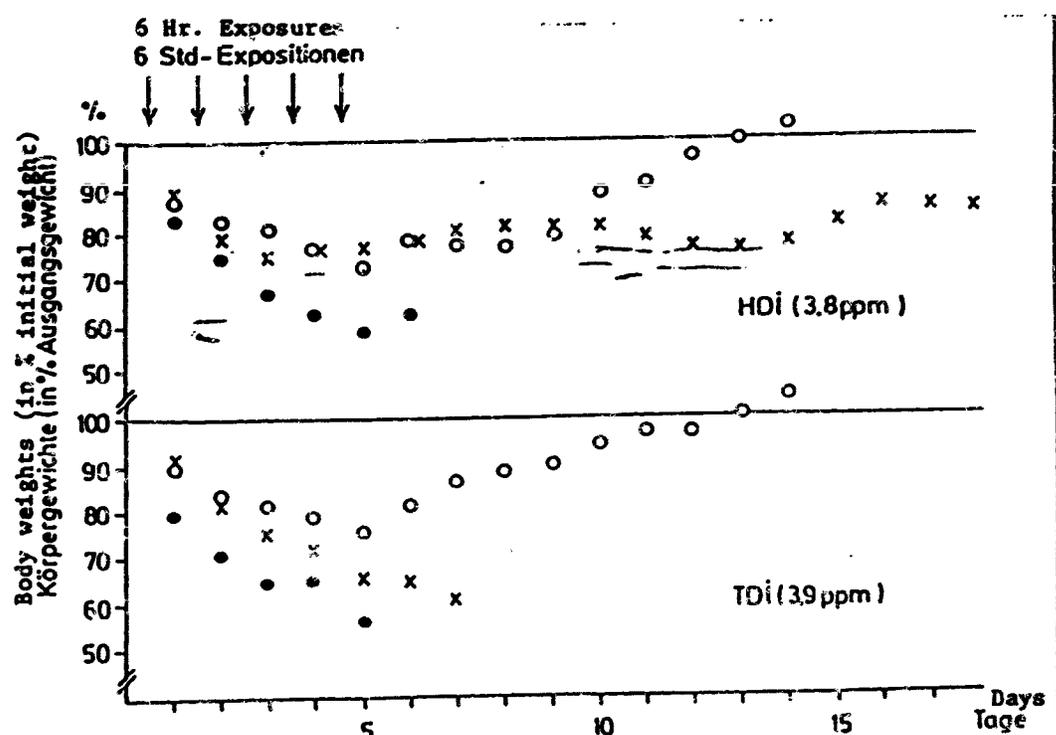
(Professor Dr. D. Henschlar)

CC:e107/026



III. 1 Diagram of the release of diisocyanate vapors for the dynamic exposure of test animals.

Abb. 1. Schema der Freisetzung von Diisocyanatdämpfen zur dynamischen Exposition von Versuchstieren.



III. 2 Relative body weights of rats (ooo), guinea pigs (xxx) and mice (ooo) exposed 5 times, 6 hours each, to ca. 4ppm HDI and TDI.

Abb. 2. Relative Körpergewichte von Ratten (o o o), Meerschweinchen (x x x) und Mäusen (o o o) unter 5 Expositionen zu ca. 4 ppm HDI und TDI.

Mobay

Attachment 12



**Mobay
Chemical Corporation**

**Environmental
Health Research**

Stanley Research Center
9801, Kansas 66085
Telephone: 913/681-2451
Cable: Kamego Kansas City

PROTOCOL FOR STUDY NO. 80-141-01

21-DAY INHALATION TOXICITY STUDY WITH HDI

STUDY DIRECTOR

G. K. SANGHA

MANAGER, CORPORATE TOXICOLOGY

D. W. LAMB

0-1-0-6

STUDY NO. 80-141-01

21-DAY INHALATION TOXICITY STUDY WITH HDI

PERSONNEL:

Study Director:	G. K. Sangha
Technician in Charge:	G. N. Elzie
Clinical Lab:	G. J. Brewer
Analytical Lab:	K. D. Moore
Pathology:	H. E. Hoss
Animal Care Supervisor:	D. R. Mallicoat
Quality Assurance:	R. S. Schroeder

TEST SUBSTANCE:

Chemical Name: 1,6, Hexamethylene diisocyanate

Chemical Formula: $O=C=N-CH_2-CH_2-CH_2-CH_2-CH_2-H_2C-N=C=O$

Batch No.:

Purity: 99.83%

Physical Appearance: Clear, yellowish liquid

Storage: At room temperature under the hood

DATES:

Initiation of Study: March 1980

Termination of Study: May 1980

Histopathology Results Available: July 1980

SPONSOR:

Mobay Chemical Corporation
Plastics and Coatings Division

TEST FACILITY:

Mobay Chemical Corporation
Corporate Toxicology Department
Stanley Research Center
Stilwell, Kansas 66085

ANIMALS:

Fischer 344, young adult, male and female rats, obtained from Charles River, and weighing between 180 to 200 grams will be used for the study.

Animal Identification - Each animal will be ear punched for identification according to the ear-notch punch code of Harkness and Wagner (1977) and corresponding to the ear punch code, each animal will be assigned a number. Both the ear punch code and the number will be displayed on their respective cages. The animals will be numbered 1 through 80 as follows:

Test Level	Exposure Concentration (ppm)	Male I.D.	Female I.D.
Control	0	1 - 10	11 - 20
1	0.002	21 - 30	31 - 40
2	0.02	41 - 50	51 - 60
3	0.2	61 - 70	71 - 80

Number of Animals/Test Level - Ten male and ten female rats will be used for control and each of the exposure levels.

Animal Care - Animals will be quarantined for one week prior to the initiation of the study for acclimatization and observation. One week prior to study and during the non-exposure period of study, animals will be housed individually in stainless steel cages. Maintenance and cleaning of cages will be according to standard operating procedures. For the purpose of recording food consumption, a weighed amount of ration (Rodent Lab Chow from Ralston Purina) will be provided and any uneaten feed will be weighed and discarded each week. Drinking water will be available ad libitum by automatic waterers for each cage.

EXPOSURE CONCENTRATIONS:

Three exposure concentration levels, in addition to the control, will be used. The exposure concentration levels used will be 0.002-(.005) ppm, 0.02 ppm and 0.2 ppm. These three levels represent the present TLV of 0.02 ppm, ten times below TLV and ten times above TLV level. These concentration levels cover the ranges of work-place environment and are also suggested on the basis of sensory irritation data by G. K. Sangha and Y. Alarie (1980).

EXPOSURE DURATION:

Animals will be exposed to HDI vapours for five hours daily, five days a week over a three-week period.

EXPOSURE CONDITIONS:

Animal Exposures - Dynamic airflow Kimmerle Chamber, based on nose-only exposure, will be used for exposing animals. The animals are individually positioned in the tubes in such a manner that only their noses are exposed to the test chemical and dermal and oral exposures are minimized.

Airflow - A minimum of 20 LPM of airflow will be maintained through the chamber to ensure proper O₂ supply. The airflow will be monitored continuously and will be maintained constant.

Temperature and Humidity - Temperature in the chamber will be maintained at $26 \pm 2^{\circ}\text{C}$ and relative humidity between 40 - 60%. Both will be monitored continuously and maintained within a narrow range.

Treatment of Exhaust Air - Outgoing air from the chamber will be filtered through a series of filters and will be exhausted through an incinerator.

GENERATION OF HDI CONCENTRATIONS:

HDI vapours will be generated by bubbling filtered dry air through an impinger containing HDI. Required concentration ranges will be obtained by varying airflow rate through the impinger or through the exposure chamber (but never less than 20 LPM). Room air of breathing quality will be used as dilution air. (Efforts are being made to compare other generation techniques. If any of the other techniques is found to be more satisfactory than the above method, the use of that technique will be considered).

CHAMBER CONCENTRATION AND ANALYSIS

In order to determine the concentration of HDI in the exposure chamber, samples will be taken near the animal breathing zone repeatedly during the exposure period to ensure that concentrations are maintained constant and results are reproducible. Analytical determinations for HDI will be made according to Dunlap et al. (1976).

OBSERVATIONS:

Daily Observation - All signs of toxicological and pharmacological effects will be recorded at the end of exposure and within one hour post-exposure period. Animals will be checked at random during exposure period by pulling the tubes out for any sign of toxicity. All observations will be recorded twice daily, including the pre-exposure observations each morning. Animal weights will be recorded at initiation of the study and at weekly intervals. Feed consumption will be monitored at weekly intervals. An external eye examination will be conducted at termination of the study.

Treatment of Animals After Three-Week Exposure - At the end of three-week exposure duration, one-half of the males and females (five males and five females) from each exposure level will be sacrificed to see the extent of injury with repeated exposures. The other half of the animals will be allowed to recover over a two-week period, blood samples will be taken for clinical laboratory tests, and then sacrificed. This will determine the amount of recovery achieved during that period. During the recovery period animals will be observed twice daily and all observations will be recorded.

CLINICAL LABORATORY TESTING:

Prior to the study and at termination of study, hematology, blood chemistry and urinalysis will be conducted on all animals. During the three-week exposure period, hematology and blood chemistry will be performed on one-half of the animals (five males and five females) from control and each of the exposure levels at weekly intervals in a manner that each one-half of the animals per test level will be used at alternate weeks. This denotes that blood samples will be drawn from one-half of the animals/test level at the end of the first and third week of exposure and this group will be sacrificed at the end of the three-week exposure period. The blood samples from the second one-half of the animals will be taken at the end of the second week of exposure and after two weeks of recovery period, i.e. fifth week, and then sacrificed. Hematological determinations will include: hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, platelet counts and if any signs of anemia are present, reticulocyte count.

Blood chemistry determination will be made for: calcium, potassium, sodium, serum lactic dehydrogenase, oxaloacetic transaminase, glucose, blood urea nitrogen, serum alkaline phosphatase, total cholesterol, albumin, globulin, total protein, bilirubin, and if any increased bilirubin is noted, direct bilirubin determination will also be conducted.

GROSS NECROPSY:

All test animals will be subjected to gross necropsy at their death during the study, when sacrificed due to becoming moribund or when sacrificed at the termination of the study. Results of gross examination will be recorded for the external surface, all orifices, the cranial cavity carcass, external and cut surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs. The following organs will be examined for gross lesions: adrenals, heart, lungs, trachea, bronchi, nasal passages and para-nasal sinuses and organs of abdominal and pelvic cavities.

Special treatment of the lung will be undertaken for morphological evaluation of emphysema. The lungs will be removed in toto, weighed and perfused intratracheally with 10% buffered formalin.

Other organs to be weighed include: liver, kidneys, heart and brain.

All tissue samples taken will be preserved in 10% buffered formalin.

HISTOPATHOLOGY:

Histopathology studies will be made on selective animals (about one-half) regardless of time of death.

Organs and tissues taken for histopathology include: brain (fore-brain, midbrain and hindbrain), eye, pituitary, salivary glands, thymus, heart, esophagus, lungs (with mainstem bronchi), trachea, nasal passages and paranasal sinuses, liver, stomach, small and large intestines, spleen, kidneys, thyroid, adrenals, pancreas, urinary bladder, aorta, testes, ovaries, corpus and cervix uteri, bone (with marrow), skeletal muscle, skin and all other tissues in which lesions are obtained.

RECORDING OF DATA:

Forms for recording of data will be used to include feed consumption with weekly means and standard deviation for each level, daily observations and pathology-necropsy. Similarly, hematology and blood chemistry records will be made on their respective forms. Sampling and analysis data for chamber concentration will also be recorded daily for each exposure level on the forms.

EVALUATION OF DATA:

Evaluation of the test results will be made on the basis of body weight, food consumption, clinical findings, gross necropsy and histopathology findings.

STORAGE OF RECORDS AND RAW DATA:

Raw data, tissues, tissue blocks and slides will be stored by the Corporate Toxicology Department, Mobay Chemical Corporation, Stanley Research Center, Stilwell, Kansas 66085.

QUALITY ASSURANCE INSPECTIONS:

An inspection using a standard checklist will be conducted periodically during the study by the Quality Assurance Unit.

REFERENCES:

Dunlap, K.L., R.L. Sandridge and Jürgen Keller. "Determination of Isocyanates in Working Atmospheres by High Speed Liquid Chromatography," 1976, Analytical Chemistry, 48, 497.

Harkness, John E. and J.E. Wagner. "The Biology and Medicine of Rabbits and Rodents," 1977. Lea and Febiger publisher.

Sangha, G.K. and Y. Alaric. "Comparative Toxicity of Some Mono- and diisocyanates as Sensory Irritants," 1980. In preparation. To be presented at Society of Toxicology in March 1980.

APPROVALS:

G. K. Sangha
Study Director

GK Sangha

G. J. Brewer
Clinical Lab

Gary J. Brewer Feb. 26, 1980

H. E. Hoss
Pathology

Herbert E. Hoss 2/26/80

R. S. Schroeder
Quality Assurance

Robert S. Schroeder 2/27/80

D. W. Lamb
Manager, Corporate Toxicology

Donald W. Lamb 2/29/80

IDENTIFICATION:

D. W. Lamb
E. D. Ziegler
E. L. Reichard
G. K. Sangha

Q. A. File
Study File

***** PROTOCOL AMENDMENT FORM *****

STUDY NUMBER 80-141-01

TYPE OF STUDY Inhalation

SPECIES Rat

DURATION 21-day

DATE OF REVISION 5/27/80

START DATE June 2, 1980

REVISION BY G. K. Sangha

TERMINATION July 3, 1980

HISTOPATHOLOGY RESULTS September 1980

AMENDMENT:

Page 3, Subheading Animal Exposures

The animals will be exposed by head only instead of nose only. The animals will be individually positioned in the tubes in such a manner that only the head will be exposed to the HDI atmosphere.

REASON:

APPROVAL BY:
Donald W Lamb Date 5/28/80
Date _____

G. K. Sangha
Study Director
Date 5/28/80

1114



MOBAY CHEMICAL CORPORATION
CORPORATE TOXICOLOGY DEPARTMENT
STANLEY RESEARCH CENTER
17745 SOUTH METCALF
STILWELL, KANSAS 66085

PROTOCOL FOR
SUBCHRONIC INHALATION TOXICITY STUDY WITH HDI
STUDY No. 81-141-01

BY G. K. SANBHA

RECEIVED
MAY 15 1981

TITLE

Subchronic Inhalation Toxicity Study with NDI

TEST SUBSTANCE

Chemical Name: 1, 6, Hexamethylene diisocyanate
Chemical Formula: $O=C-N-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-N=C-O$
Lot No.: C-021-0-602
Product Code: C-021
Purity: 99.83%
Grade: Commercial
Physical Appearance: Clear, yellowish liquid
Storage: Room temperature, under the hood

SPONSOR

Mobay Chemical Corporation
Plastics and Coatings Division

TEST FACILITY

Mobay Chemical Corporation
Environmental Health Research Institute
Corporate Toxicology Department
17745 South Matcalf
Scilwell, Kansas 66085

PERSONNEL

Laboratory Management: D. W. Lamb
Study Director: G. K. Sangha
Study Conduct: T. Espindola
H. Dejong
Analytical: R. S. Schroeder
Pathology: H. E. Hoss
Animal Care: D. R. Mallicoat
Quality Assurance: R. S. Schroeder

DATES

Exposure Initiation: January 18, 1982
Exposure Completion: April 22, 1982
Report: December 1982

TEST SYSTEM

Animals

Young adult Fischer 344 rats obtained from Charles River Breeding Labs, Wilmington, MA, and weighing between 120-150 g will be used during the study.

Quarantine and Randomization

Animals will be quarantined at least for one week prior to selection of groups. Groups will be selected in an order after randomization with a table of random numbers (Snedecor 1956).

Number of Animals/Exposure Level

Groups of 20 males and 20 females will be used for control and each of the exposure levels.

Animal Identification

Each animal will be ear punched for identification according to the ear-notch punch code of Harkness and Wagner (1977), and corresponding to the ear-punch code, each animal will be assigned a number which will be displayed on their respective cage. The number of the rack, sex of animals and range of animal numbers/rack will be displayed in front of each rack.

The animals and their exposure positions will be numbered as follows:

Exposure Level	Exposure Concentration	Chamber Position	Male ID		Female ID	
			Rack No.	Animal No.	Rack No.	Animal No.
0	Control	H-1	1-1	1-20	1-2	21-40
1	0.01 ppm	H-2	2-1	41-60	2-2	61-80
2	0.04 ppm	H-3	3-1	81-100	3-2	101-120
3	0.16 ppm	H-4	4-1	121-140	4-2	141-160

Animal Care

After quarantine and randomization, the animals will be transferred to their assigned H-1000 chambers where they will be housed individually in wire mesh cages over catch pans lined with DACB (Upjohn Co.) bedding. Animals will be kept in these chambers for one to two weeks for acclimatization and observations. Prior to and during the study, animals will remain in these chambers. Prior to the study and during the non-exposure period, food (Purina Lab Chow) and water will be available ad libitum; but during the six-hour exposure, food will be removed and only water will be available from the automatic waterers.

MAINTENANCE AND CLEANING OF CAGES AND CHAMBERS

Maintenance of the exposure chamber will be as follows:

1. Catch pans will be lined with cage boards at all times except for six-hour exposure duration. Prior to onset of the exposure, catch pans with cage boards will be removed and replaced with clean catch pans (without cage board). After the exposure period, catch pans will be lined with fresh cage board.
2. The chambers will be washed every two weeks. In case the need arises, chambers will be cleaned once a week. The animals from the chamber scheduled for washing will be transferred into the replacement chamber which will be pre-conditioned and prepared for the purpose.

EXPOSURE CONCENTRATIONS

Three exposure concentration levels in addition to the control will be used. The exposure concentration will be 0.01, 0.04 and 0.16 ppm. These concentrations are suggested on the basis of the results obtained from the 21-day inhalation study with HDI.

EXPOSURE DURATION

Animals will be exposed to HDI vapors for six hours daily, five days a week, over a 90-day period (13 weeks).

EXPOSURE CONDITIONS

Animal Exposures

Animals will be exposed in the Hazleton-1000 chambers, (based on the whole-body exposure) under dynamic airflow conditions. The animals will remain in these chambers during the entire study period. Only two tiers of the chamber will be used and racks will be rotated in an order so that any positional variation in the chamber concentration is taken into consideration.

Airflow

An airflow of 20 CFM/minute will be maintained through the chambers at all times. The airflow will be monitored and recorded continuously and will be kept within a narrow range of fluctuations. The chamber air supply (room air of breathing quality) will be filtered through a charcoal and HEPA filters and conditioned prior to its delivery into the chambers.

A negative pressure of up to 1/2" water will be maintained in the chamber so that any leaks in the chamber will have the flow in the inward direction of the chamber. The chamber pressure will be monitored continuously and recorded at hourly intervals during the six-hour exposure.

Temperature and Humidity

Temperature in the exposure chambers will be maintained at $72 \pm 2^{\circ}\text{F}$ and will be monitored and recorded continuously during the entire study period. Relative humidity in the exposure chambers will be maintained between 40 to 60%. In the control chamber, it will be monitored and recorded continuously during the study period. Due to reactivity of the test substance to the humidity probes relative humidity in the chamber with animals exposed to HDI, will be recorded during the non-exposure period only.

Treatment of Exhaust

Out-going air from the chambers will be filtered through charcoal and purafil filters prior to its exhaustion into the atmosphere.

GENERATION OF HDI ATMOSPHERE

HDI vapors will be generated by bubbling filtered, dry air through a bubbler containing HDI, and kept in a constant temperature water bath (Thermonix 1441E, Braun-Melsungen). The airflow through the bubblers will be maintained constant by mass flow meters (Matheson). The required concentrations will be obtained by either varying airflow rates through the bubbler and/or by varying temperature of the water bath. The airflow rates through the bubbler will be kept at a rate where no aerosol is produced. During the development of the system, it has been established that by keeping the temperature of the water bath, airflow rate through the bubbler and airflow rate through the chamber constant, desired concentrations are obtained with excellent reproducibility. The generation parameters will be checked frequently and recorded at hourly intervals during the six-hour exposure. New sample of HDI will be used for each exposure.

ANALYSIS OF THE CHAMBER ATMOSPHERE

Daily mean exposure concentration of HDI in each exposure chamber will be established by taking 4 to 6 samples during the six-hour exposure. Samples will be drawn alternately from the two sampling ports near the animal breathing zone at a rate of 1 lpm for 10 to 30 minutes. The frequency and duration of sampling was determined from the preliminary data obtained during the development of the system. The preliminary data also showed that only small variation (2 to 5%) in concentration existed between the twelve sampling ports (6 in the back and 6 in the front side of the chamber). In order to determine any contamination with HDI, the atmosphere of chamber with control animals and room air will be analysed twice daily on the exposure days. All samples will be analysed by an HPLC method similar to Dunlap et.al. (1976).

OBSERVATIONS

Daily Observations

All animals will be observed for signs of toxicity and mortality prior to the onset of exposure and at one hour post-exposure on the exposure days and twice daily during the non-exposure days. All observations will be recorded daily.

Body Weights

Individual animal weights will be recorded at initiation of the study, prior to first exposure, days 3 and 7 of the study and weekly thereafter until termination of the study.

Treatment of Animals After 90-Day Exposure Period

At the end of 90-day exposure, all animals will be sacrificed. The sacrifice will be staggered over four days. Five animals/sex/level will be sacrificed each day and HDI exposures will be continued on the rest of the animals until scheduled for sacrifice.

Clinical Laboratory Testing

Prior to the study (prior to onset of first exposure), at an intermediate time and at the termination of the study (prior to sacrifice), hematology and blood chemistry will be conducted on one-half of the animals (10 males and 10 females) from control and each of the exposure levels. Urinalysis will be done at the beginning and at the termination of the study only. Same groups of animals will be used for hematology, blood chemistry and urinalysis during the entire study period.

Hematological determinations will include: hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, platelet counts and, if any signs of anemia are present, reticulocyte count.

Blood chemistry determinations will be made for: calcium, phosphorous, potassium, sodium, serum lactic dehydrogenase, oxaloacetic transaminase, glucose, urea nitrogen, serum alkaline phosphatase, total cholesterol, albumin, globulin, total protein, bilirubin and, if any increased bilirubin is noted, direct bilirubin determinations will also be conducted.

Urinalysis will be performed for specific gravity, pH, ketone, glucose, bilirubin, protein, urobilinogen along with microscopic observations.

GROSS NECROPSY

All test animals will be subjected to gross necropsy at their death during the study, when sacrificed due to becoming moribund or when sacrificed at the termination of the study. Results of gross examination will be recorded for the external surface, all orifices, the cranial cavity, external and cut surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs.

Weights of lung, liver, kidneys, heart, spleen, adrenals, gonads and brain will be recorded.

A special treatment of the lung will be undertaken for morphological evaluation. The lungs will be removed in toto, weighed and perfused intratracheally with 10% buffered formalin.

GROSS NECROPSY, continued

Similarly special treatment of the nasal passages and paranasal sinuses will be undertaken. Heads of the animals (with nasal and paranasal passages intact) will be removed and fixed in 10% buffered formalin for histopathological processing and examination.

Other organs and tissue samples for histopathological examination will include: brain (forebrain, midbrain and hindbrain), eye, pituitary and salivary glands, thymus, heart, esophagus, trachea, liver, stomach, small and large intestines, spleen, kidneys, thyroid, adrenals, pancreas, urinary bladder, aorta, testes, ovaries, corpus and cervix uteri, bone (with marrow), skeletal muscles, skin and any other tissues with lesions.

All tissue samples taken will be preserved in 10% buffered formalin.

Histopathology - Histopathological studies will be made on the above-mentioned organs of all animals regardless of time of death.

For examination of nasal passages and paranasal sinuses, the heads of animals will be decalcified with Decal®. At least four transverse sections of the head at equal intervals and tracheal sections at the larynx, mid-portion and near pulmonary bifurcation will be taken and then processed for microscopic examination. The results from the 21-day inhalation toxicity study with HDI showed this practice to be very useful in evaluating the effect of HDI at various levels of the upper respiratory tract.

RECORDING OF DATA

Forms for recording of data will include daily observations, body weights with weekly means and standard deviation for each level and pathology-necropsy. Similarly, hematology and blood chemistry records will be made on respective forms. Sampling and analysis data for chamber concentration for each exposure level will also be recorded daily on the forms. A master schedule will be available to all personnel involved in the study.

EVALUATION OF DATA

Evaluation of the test results will be made on the basis of body weight, clinical findings, gross necropsy and histopathology findings.

STORAGE OF RECORDS AND RAW DATA

Raw data, tissues, tissue blocks, slides and a sample of the test material will be stored according to Toxicology Report Number by the Corporate Toxicology Department, Mobay Chemical Corporation, Environmental Health Research Institute, 17745 South Metcalf, Stilwell, Kansas 66085.

QUALITY ASSURANCE INSPECTIONS

An inspection using a standard checklist will be conducted periodically during the study by the Quality Assurance Unit.

REFERENCES

Dunlap, K.L., R.L. Sandridge and Jürgen Keller. "Determination of Isocyanates in Working Atmospheres by High Speed Liquid Chromatography," 1976, Analytical Chemistry, 48, 497.

Harkness, John. E. and J.E. Wagner. "The Biology and Medicine of Rabbits and Rodents," 1977. Lea and Febigee publisher.

Snedecor, G. W., Section 1.5 of "Statistical Methods to Experiments in Agriculture and Biology" 5th Edition, Iowa State University Press 1956.

Contains No ORI

SIGNATURES:

Study Director:

G. K. Sangha

G.K. Sangha

Laboratory Management:

D. W. Lamb

D.W. Lamb

Analytical:

R. S. Schroeder

R.S. Schroeder

Pathology:

H. E. Hoss

Herbert E. Hoss

Quality Assurance:

R. S. Schroeder

R.S. Schroeder

G. Minicale
P. D. Eagles
H. Moss
R. Schroeder

***** **PROTOCOL AMENDMENT FORM** *****

STUDY NUMBER 81-141-01 **TYPE OF STUDY** Subchronic Inhalation
SPECIES Rat (F - 344) **DURATION** 13 Week
DATE OF REVISION 1/20/82 **START DATE** 1/18/82
REVISION BY G. K. Sangha **TERMINATION** 4/22/82

AMENDMENT:

1. Page 2 of 8
Exposure initiation will be January 25, 1982.
Exposure completion will be April 29, 1982
2. Page 6 of 8
Clinical Laboratory Testing, 3rd paragraph, 2nd line.
ADD "Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase"
DELETE "oxaloacetic transaminase"

4th paragraph
In urinalysis "blood" will also be determined.

REASON:

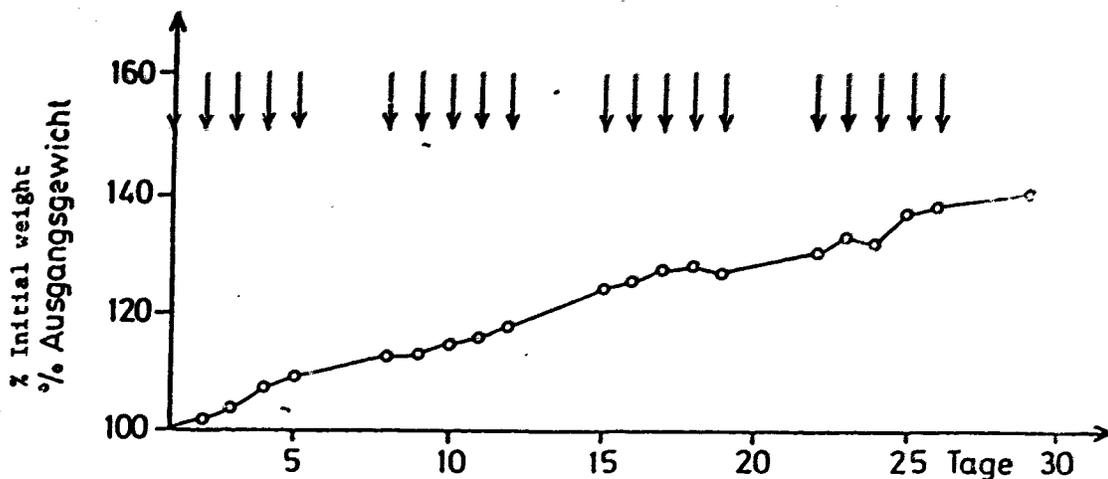
1. The animal shipment of females arrived one week later than the scheduled arrival.
- 2 & 3. To comply with the standard determinations in this laboratory.

APPROVAL BY:
Guarant 1/20/82 Date 1/20/82

Date _____

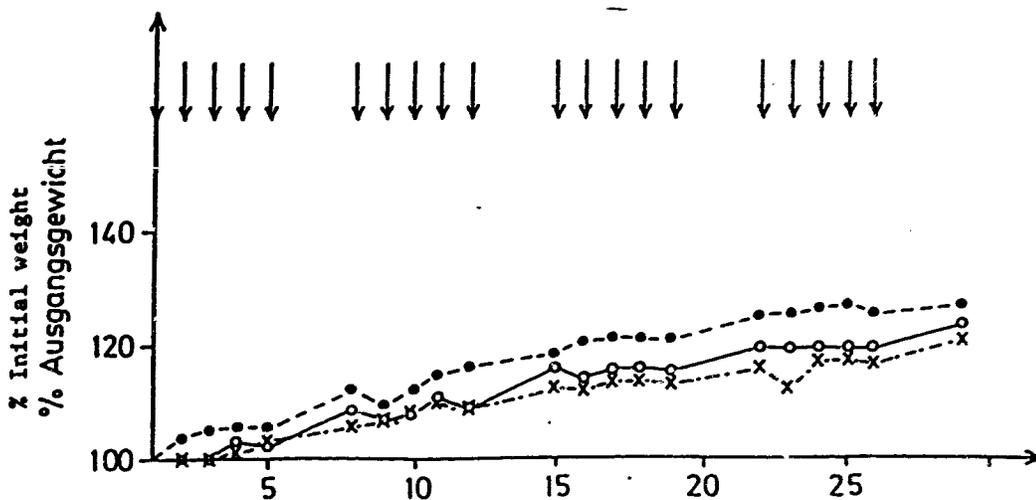
G. K. Sangha
Study Director
Date 1-20-82

0 1 2 4



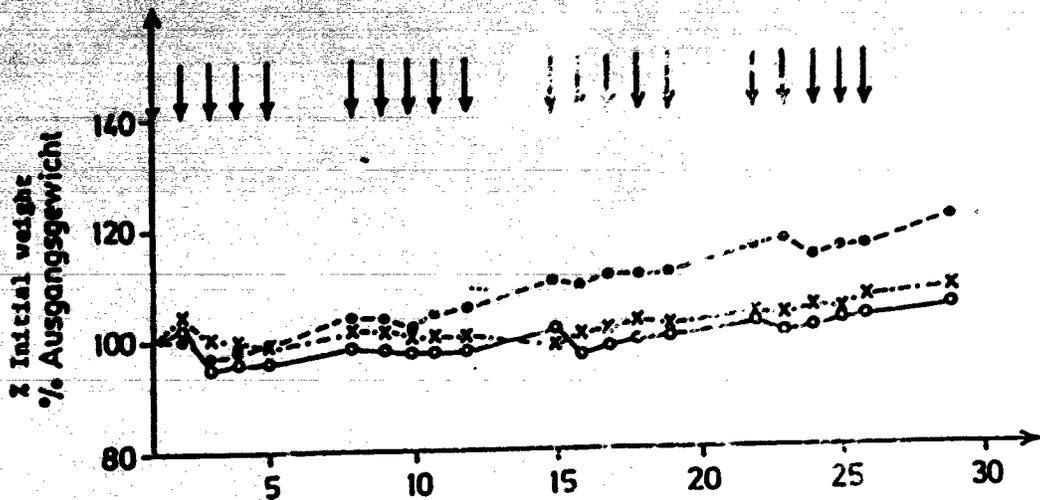
III. 19 Body weights of guinea pigs under 0.1 ppm HDI exposure. Days
 Arrows: 6 hour exposures.

Abb. 19. Körpergewichte von Meerschweinchen unter 0,1 ppm HDI. Pfeile: 6-Std-Expositionen.



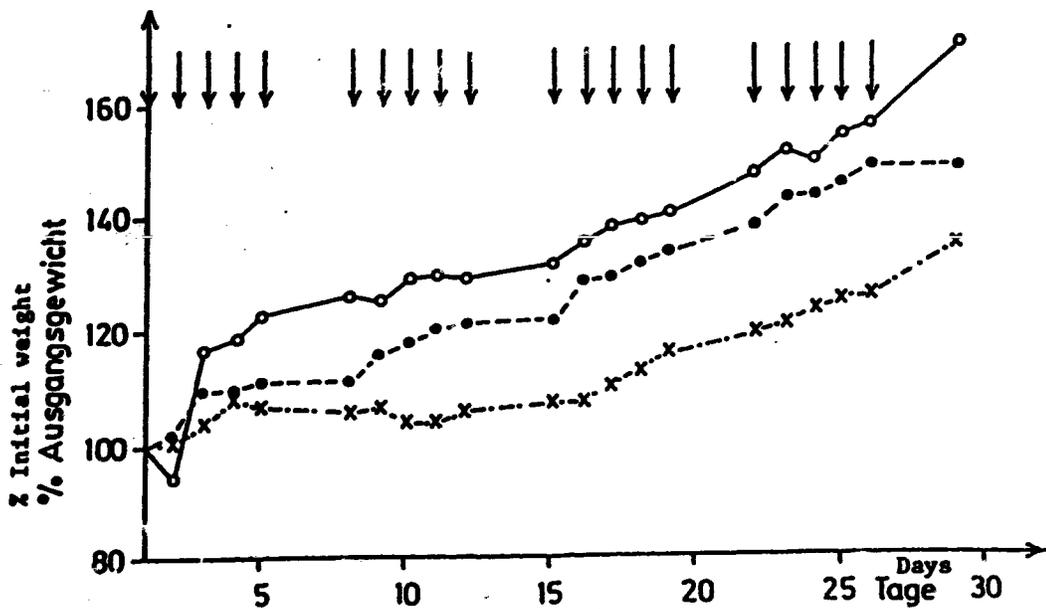
III. 20 Body weights of rats under 0.03 ppm TDI (o—o) and 0.03 ppm HDI (●---●) exposure. Control animals: X---X
 Arrows: 6 hour exposures.

Abb. 20. Körpergewichte von Ratten unter 0,03 ppm TDI (o—o) und 0,03 ppm HDI (●---●). Kontrolltiere: x:---x,
 Pfeile: 6-Std-Expositionen.



III. 21 Body weights of mice under 0.03 ppm TDI (o—o) and 0.03 ppm HDI (o---o) exposure; Control Animals; X---X; arrows: 6 hour exposures.

Abb. 21. Körpergewichte von Mäusen unter 0,03 ppm TDI (o—o) und 0,03 ppm HDI (o---o). Kontrolltiere: x---x. Pfeile: 6-Std-Expositionen.



III. 22 Body weights of guinea pigs under 0.03 ppm TDI (o—o) and 0.03 ppm HDI (o---o) exposure.

Control animals: X---X; arrows: 6 hour exposures.

Abb. 22. Körpergewichte von Meerschweinchen unter 0,03 ppm TDI (o—o) und 0,03 ppm HDI (o---o). Kontrolltiere: x---x. Pfeile: 6-Std-Expositionen.

Attachment 11

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Vol. 4

Dr. Curt Haefner Verlag GmbH

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1.Auflage 1977
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0 1 3 0

Summary

The technique of spray applying surface coatings, on account of its economic and technical advantages, is replacing to an increasing extent the conventional methods of painting surfaces of many different materials. With the spraying of coatings, aerosols containing the whole spectrum of the coating ingredients are discharged into the ambient air, and new problems become prominent in occupational medicine. Animal inhalation studies using aerosols of various coating systems with different chemical compositions (polyurethane, polyacrylic and polyalkyd types) show in terms of the LC₅₀ values a degree of acute toxicity that is not fully explainable by the solvent-content or the proportion of free monomer precursors of the characteristic polymer. The test results indicate no obvious correlation between the level of acute toxicity and the relationship of the tested coatings to the known principles of chemical reaction. The inhalation test method used does not provide toxicological data that might be related to absorption of the materials into the body.

A field study comprising 341 males who had worked with di- and polyisocyanates for periods up to 25 years has shown that long-term exposure to isocyanates at relatively low concentrations, usually below the MAK values, does not result in toxic impairment of pulmonary function of a vitalographic degree. Toxic effects due to absorption of substances of this class were also not evident in a large-scale investigational program.

Health problems associated with inhalation of spray-applied coatings of different types have been reported in the literature. These problems have indicated the need for improved protective measures for personnel involved in spray operations. One means of personnel protection is by the use of respirators. An evaluation of masks was made in a manner that simulated typical spray operations. It was concluded that the effectiveness of the masks and comfort considerations would only permit the use of the masks for short time periods. Work requiring masks for a full-time job or for extended time periods will require the use of a fresh-air mask in order to assure protection against health hazards.

It is apparent that the standard industrial procedures should be established for the characterization of the effectiveness of protective masks, and standards should be established in regard to the use of the masks for assuring protection of workers involved with the spray application of coatings.